

Figure S1. Phylogenetic analysis of blue light receptor proteins of *Medicago* and *Arabidopsis*. A phylogenetic tree was generated using the maximum likelihood method. FKF1 showed greater similarity to ZTL and LKP2 compared to the other four blue-light receptors (CRY1, CRY2, PHOT1, and PHOT2).

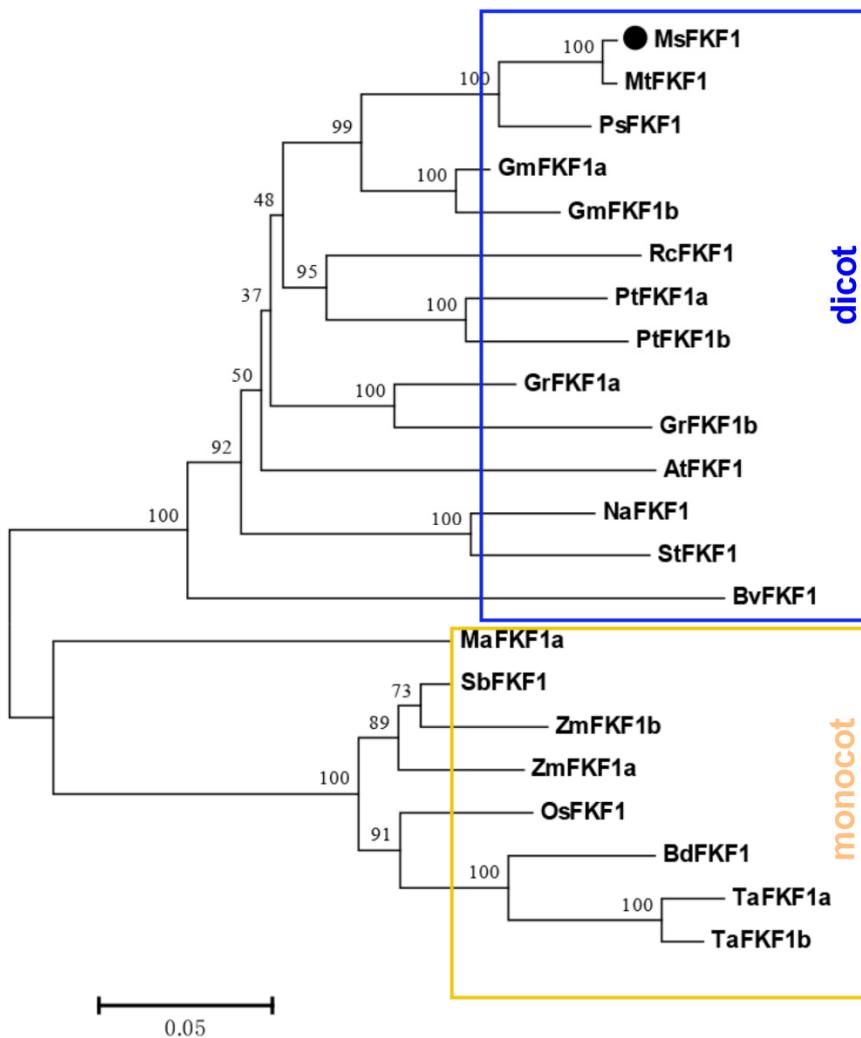


Figure S2. Phylogenetic analysis of MsFKF1 and its orthologs.

The phylogenetic tree was generated through the utilization of the neighbor-joining method, following sequence alignment conducted with the Clustal W program. The branch numbers present on the tree represent the percentage of replicates that provide support for each specific branch, as determined by implementing the bootstrap method with 1000 replicates. The scale bar depicted on the tree corresponds to a measurement of 0.05 amino acid substitutions per residue. The accession numbers of the proteins employed in constructing the phylogenetic tree can be found in Table S1.

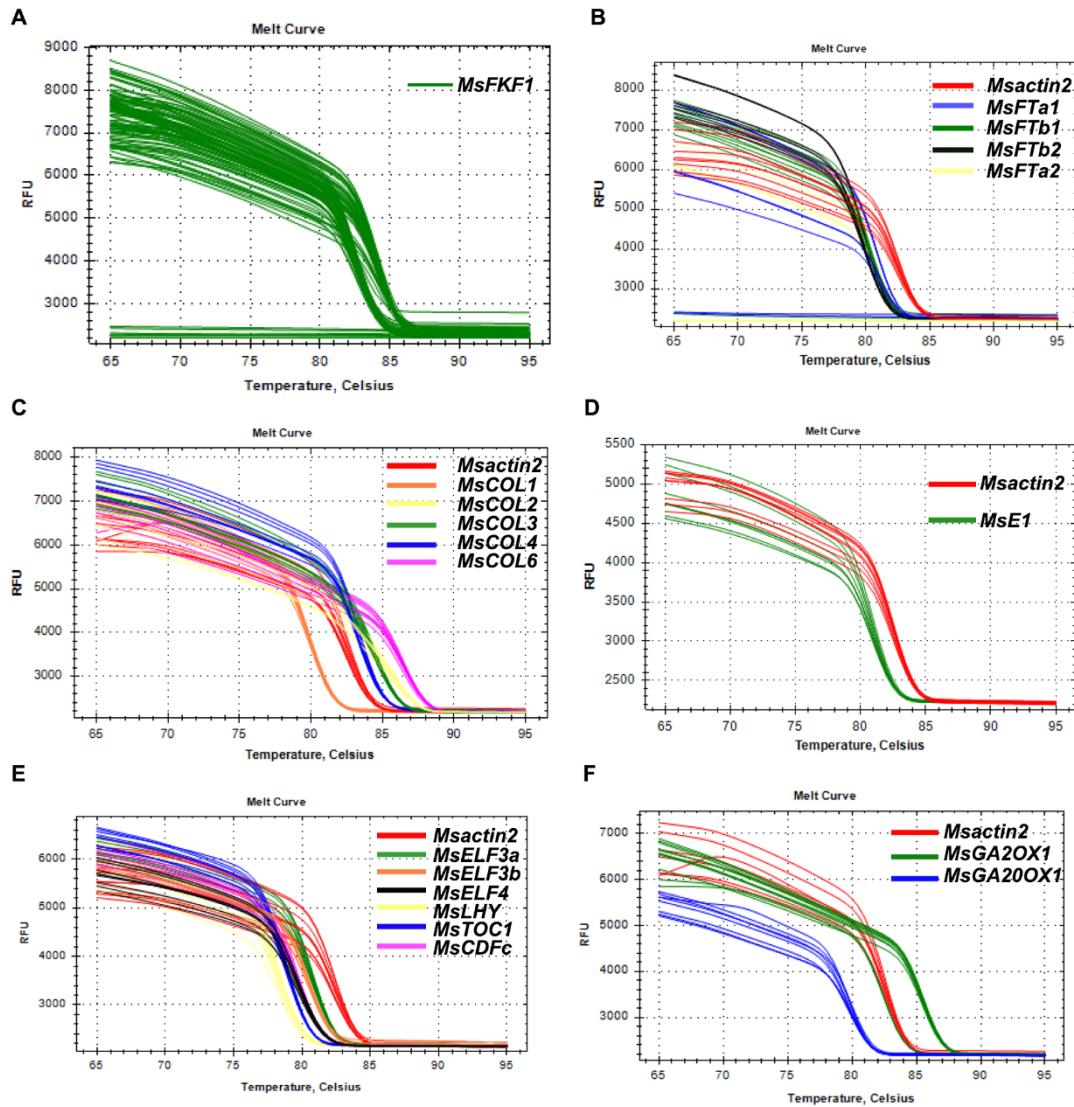


Figure S3. The melting curve diagram of RT-qPCR primers used in this study.

(A) The melting curve diagram of the MsFKF1 primer is used to analyze the effect of photoperiod on the expression level of MsFKF1. (B) The melting curve diagram of the RT-qPCR for alfalfa flower locus T gene primers. (C-E) The melting curve diagram of the RT-qPCR for Flowering time related genes primers. (F) the melting curve diagram of the RT-qPCR for GA biosynthesis related genes primers.

Table S1. Sequence homology of FKF1 proteins in the indicated species.

Gene	Homology matrix
MsFKF1	100%
MtFKF1	99.3% 100%
GmFKF1a	90.4% 90.2% 100%
AtFKF1	81.5% 81.5% 82.8% 100%
ZmAGO3	72.9% 72.7% 73.2% 71.2% 100%

Table S2. Comparison of the exon/intron composition of FKF1 in the indicated species.

Species	Gene symbol	Accession numbers	CDS (bp)	amino acid (a.a.)	extron 1 length (bp)	intron length (bp)	extron 2 length (bp)
<i>Medicago sativa</i>	<i>MsFKF1</i>	<i>Msa0930760</i>	1911	636	384	677	1942
<i>Medicago truncatula</i>	<i>MtFKF1</i>	<i>MTR_8g105590</i>	2328	635	384	677	1942
<i>Pisum sativum</i>	<i>PsFKF1</i>	<i>Psat7g007600</i>	2453	610	447	359	2004
<i>Glycine max</i>	<i>GmFKF1a</i>	<i>Glyma05g239400</i>	2400	620	517	992	1883
	<i>GmFKF1b</i>	<i>Glyma08g046500</i>	2252	647	377	1040	1875
<i>Rosa chinensis</i>	<i>RcFKF1</i>	<i>RchiOBHm_Chr4g0442801</i>	2354	631	464	822	1888
<i>Nicotiana attenuata</i>	<i>NaFKF1</i>	<i>A4A49_16690</i>	2307	634	326	4548	1981
<i>Gossypium raimondii</i>	<i>GrFKF1a</i>	<i>B456_002G166800</i>	2392	629	416	1892	1974
	<i>GrFKF1b</i>	<i>B456_008G168500</i>	2301	627	376	704	1923
<i>Arabidopsis thaliana</i>	<i>AtFKF1</i>	<i>At1g68050.1</i>	2168	619	369	100	1797
<i>Beta vulgaris</i>	<i>BvFKF1</i>	<i>BVRB_6g154770</i>	2322	633	490	5653	1832
<i>Populus trichocarpa</i>	<i>PtFKF1a</i>	<i>Potri.010G105700.v4.1</i>	2442	627	300	411	3240
	<i>PtFKF1b</i>	<i>Potri.008G135200.v4.1</i>	2532	637	454	1147	2076
<i>Solanum tuberosum</i>	<i>StFKF1</i>	<i>PGSC0003DMT400051416</i>	2239	634	343	2358	1896
<i>Sorghum bicolor</i>	<i>SbFKF1</i>	<i>SORBI_3005G145300</i>	2111	594	197	1785	1914
<i>Zea mays</i>	<i>ZmFKF1a</i>	<i>GRMZM2G107945</i>	2582	629	494	8694	2086
	<i>ZmFKF1b</i>	<i>GRMZM2G106363</i>	2367	618	422	743	1945
<i>Triticum aestivum</i>	<i>TaFKF1a</i>	<i>TraesCS4A02G164000</i>	2320	626	437	862	1883
	<i>TaFKF1b</i>	<i>TraesCS4B02G157500</i>	2191	620	300	843	1891
<i>Brachypodium distachyon</i>	<i>BdFKF1</i>	<i>KQJ88263</i>	2330	621	423	1529	1907
<i>Oryza sativa</i>	<i>OsFKF1</i>	<i>Os11g34460</i>	2190	630	397	2651	1791
<i>Musa acuminata</i>	<i>MaFKF1a</i>	<i>Ma07_g10990</i>	2358	616	519	1609	1839

Table S3. Cis-element of the MsFKF1 promoter.

Name	Cis element	Number	Description
circadian	CAAAGATATC	1	circadian control
GT1-motif	GGTTAA	2	light responsiveness
G-box	CACGA/TC	3	light responsiveness
TCT-motif	TCTTAC	1	light responsiveness
ATCT-motif	AATCTAATCC	2	light responsiveness
Box 4	ATTAAT	1	light responsiveness
GATA-motif	GATAGGA	1	light responsiveness
ABRE	ACGTG	2	abscisic acid responsiveness
CGTCA-motif	CGTCA	3	MeJA-responsiveness
TGACG-motif	TGACG	3	MeJA-responsiveness
CAT-box	GCCACT	2	related to meristem expression

Note: The *MsFKF1* promoter sequence was extracted from 2000 bp upstream of the start codon (ATG) in the assembled genome of Zhongmu

No. 4.

Table S4. Primers used in this study.

Name	Popups	Forward (5' → 3')	Reverse (5' → 3')
MsFKF1	clone	GAAAATTATTGTACACTCCACGG	CGGTTCAAGTTGATTTCATAAGT
Homo-FKF1	Overexpression and GFP	CTCTAGAGTTAACCGGGCTCAG	CCTCAGAAATCAACTTTGCTC
BD-FKF1	Assemble clone to PGBKT7	ATGTGTGGCATTCTTCAGCT	CATGTCAGAGTCTTGTGCG
qMsFKF1	qPCR	atatggccatggaggccgaattcATGTGTGG	aggtcgacggatccccggaaattTCACATG
		CATTCTTCAGCT	TCAGAGTCTT
		CAGGTCTTCATTCACCGTCTC	ACTCATTCAAGCACCCATTCC
qMsFTa1	qPCR	TCAGAAACCTCAACACCCTAGTT	CCTGGAATATCAGTCACCAACC
		A	AG
qMsFTa2	qPCR	ACCCAACAACCTCTACACCCCTG	AATCCCTAAATTGGGTCGTGGCT
qMsFTb1	qPCR	GACTACAGGGACAACCTTCGGAG	GCCATCCAGGAGCTAATATAGTG
		A	A
qMsFTb2	qPCR	GGGGACAACGTTGGAC	CGCCATCCTGGAGCTAATATATT
			G
qMsE1	qPCR	TCTTGTGATGAAGCCTCTAC	TATCCCTTGTGTCGTGTTG
qMsCOL1	qPCR	CTTGGGTGGAGTTGAGTC	TGTTCCCTTGGAGGTCTTG
qMsCOL2	qPCR	TCTCCAAACTCCTCAAACC	TACAACCTCAACCTCCATCG
qMsCOL3	qPCR	GAATCCAACACTCTCACTCTG	TCCTCTTCTCCCTATAACCTCA
qMsCOL4	qPCR	GGTGTGAATAGTAACCAAGGGAT	CATAGATGAAGGAACTGCGAGA
qMsCOL6	qPCR	TTTCCGTGTGACTATTGCG	AAAGATGAAGAGGTGGGTT
qMsELF4	qPCR	AGTTTCAGGCAGGTTCAAGTCG	CTTCACCATATTATCAGGCATTCT
qMsELF3a	qPCR	AAAGGGATCTAACAAATCTGCG	TTGGCTTGTCTTACTGAGGTGTC
qMsELF3b	qPCR	CCAGCGAGGCAAGCTAAGAA	CCATTGTAGTGTCAAGGAGGC
qMsLHY	qPCR	GAGGAGCATAAAGATGAGGAAA	CCGAAGATAACAGATGAACAAGG
		G	G
qMsTOC1	qPCR	AGCAAGAGTGGTGTGGATTCA	TGCCGTGGGATTTCACAGA
qMsCDFc	qPCR	CTTCATCTCCCTCTTCTTCTCA	AGGCTCCATCGTCTTGTGCT
qMsGA20ox1	qPCR	GAATGAGCCTGGAGTTGG	TGGAATGCCATTGATCGTC
qMsGA2ox1	qPCR	TCCCCCTGCTTTGGTAAAGAT	GGACAAGGGGATAATGGTTG