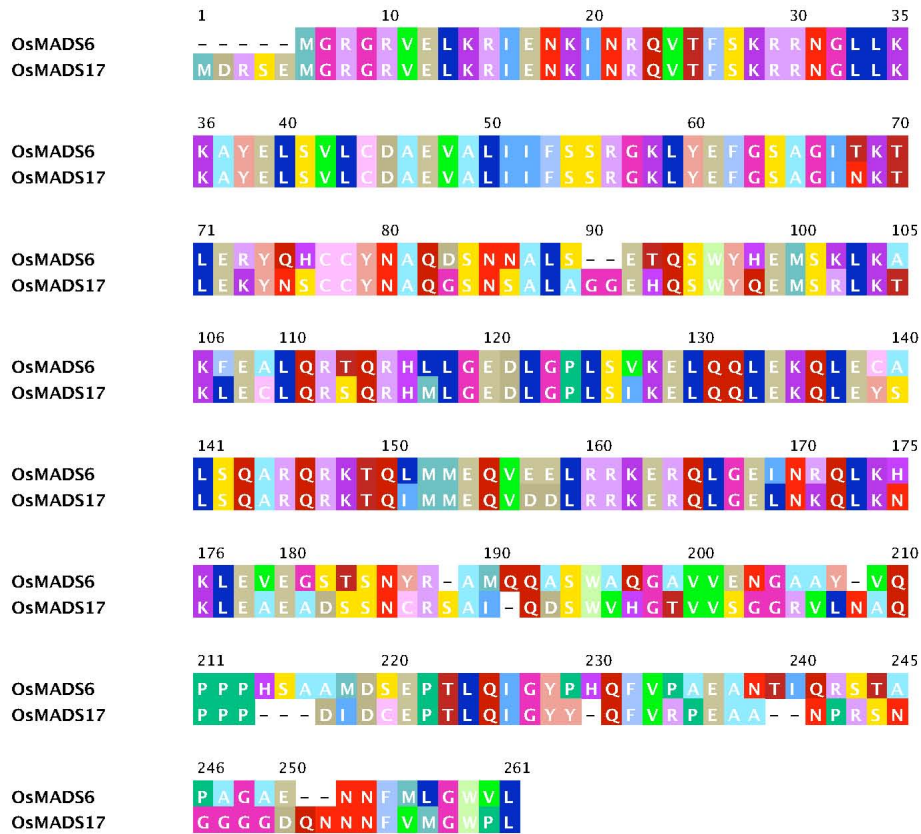
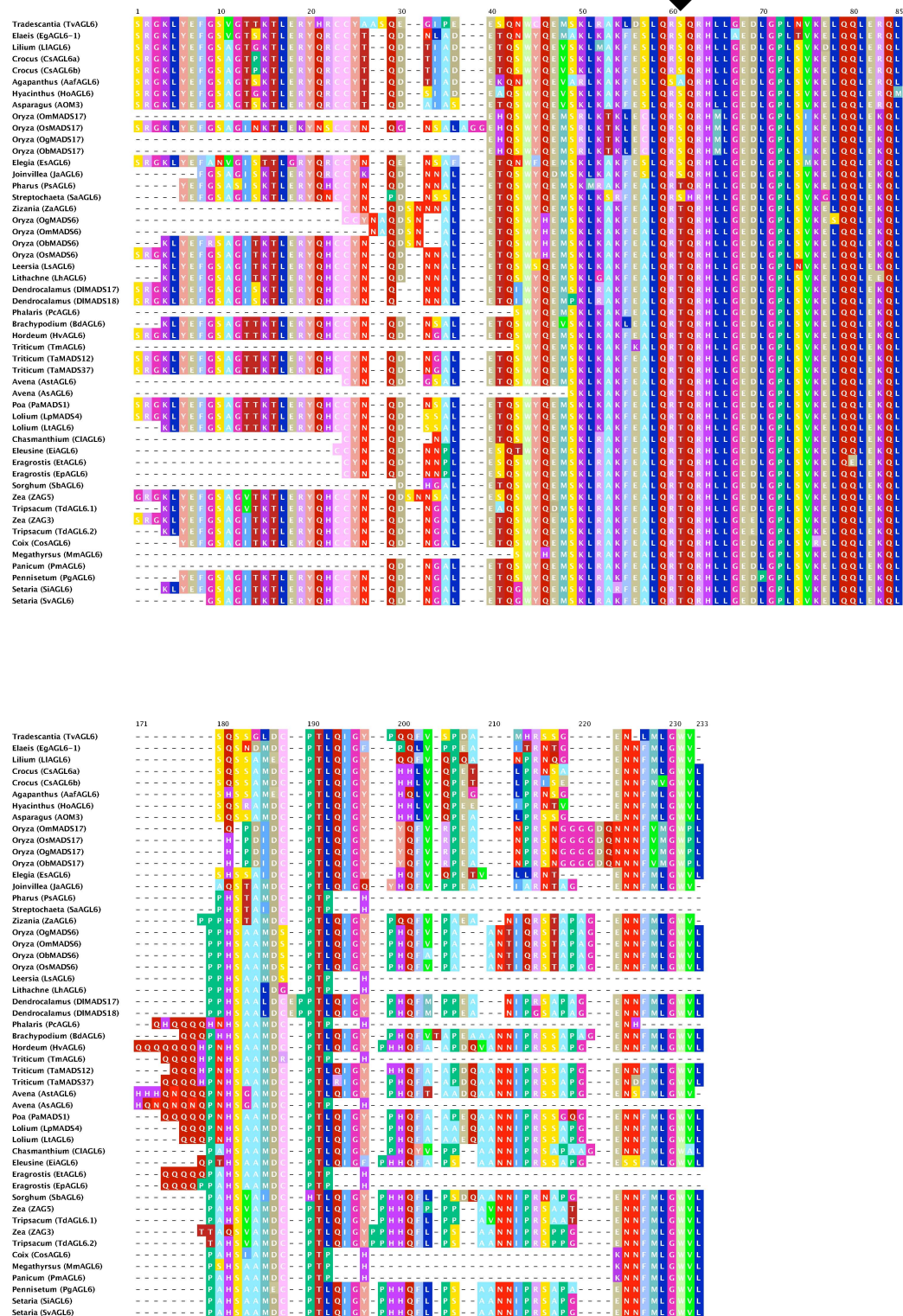


Supplemental Data. Reinheimer and Kellogg (2009). Evolution of *AGL6-like* MADS-box genes in grasses (Poaceae): ovule expression is ancient and palea expression is new

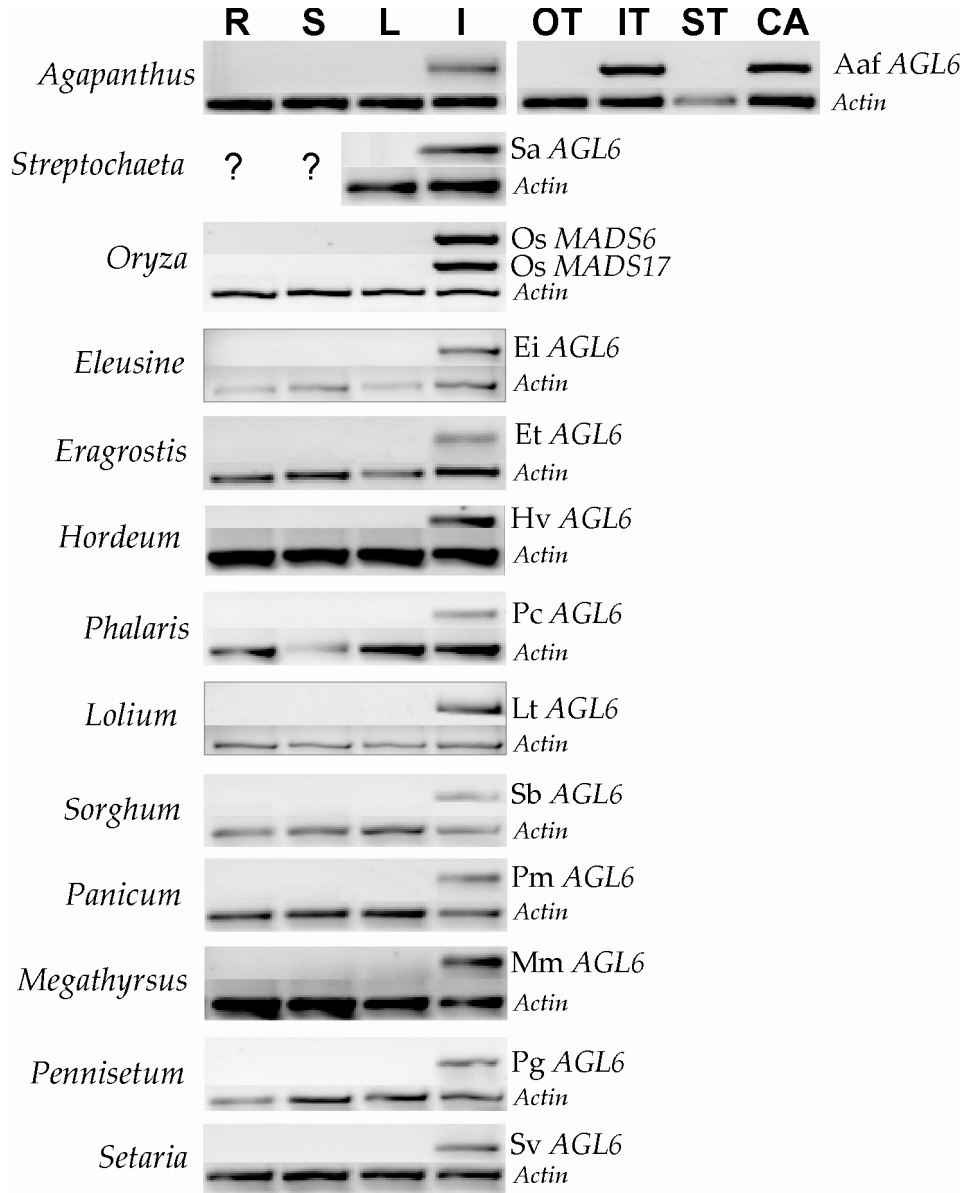
Supplemental Figure 1. Differences in amino acid composition between the paralogous copies *OsMADS17* and *OsMADS6*.



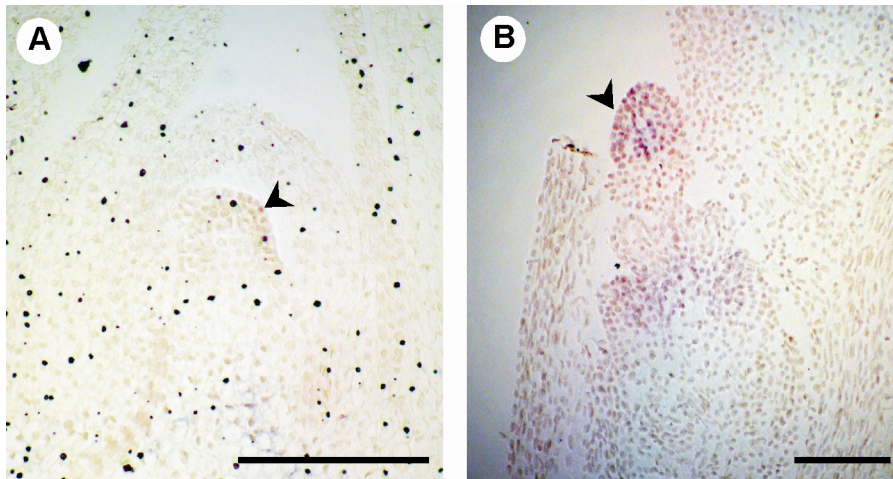
Supplemental Figure 2. Alignment of the predicted amino acid sequences of *AGL6*-like genes included in this study (only the IKC-terminus domains are shown). The black arrow indicates the single amino acid in the K-domain changed before the divergence of *Pharus*, which correlates with the origin of the grass spikelet.



Supplemental Figure 3. *AGL6*-like RT-PCR products from RNA of roots (**R**), stems (**S**), leaves (**L**), and inflorescence (**I**) in *Agapanthus africanus* and grasses, and outer tepals (**OT**), inner tepals (**IT**), stamens (**ST**) and carpels (**CA**) in *Agapanthus africanus*. *ACTIN* was used as a positive control. The question mark indicates missing data.



Supplemental Figure 4. *AGL6*-like expression in floral meristems of *Streptochaeta angustifolia* (Anomochlooideae), and *Triticum monococcum* (Pooideae). **(A)** Sa *AGL6* in *S. angustifolia*. **(B)** Tm *AGL6* in *T. monococcum*. The arrowhead indicates the floral meristem. Bar = 100 μ m.



Supplemental Table 1. Expression patterns reported for *AGL6-like* homologs in gymnosperm species. Abbreviations: IH, in situ hybridization; RT-PCR, Reverse transcriptase, polymerase chain reaction.

Species	Gene	Expression				Technique	References
		Female cones	Male cones	Leaves	Vegetativ e shoot		
<i>Gnetum gnemon</i>	<i>GMM9</i>	+	+	-	?	RNA gel blot	Winter et al. (1999) Becker et al. (2003)
	<i>GMM11</i>	+	+	-	?	IH	
<i>Picea abies</i>	<i>DAL1</i>	+	+	?	+	RNA gel blot	Tandre et al. (1995)
<i>Pinus radiata</i>	<i>PrMADS2</i>	+	+	?	-	RNA gel blot, IH	Mouradov et al. (1998)
	<i>PrMADS3</i>	+	+	?	+		
<i>Pinus resinosa</i>	<i>PMADS1</i>	+	+	?	?	RNA gel blot	Liu and Podila (1997)

Supplemental Table 2. *AGL6-like* gene expression patterns reported in eudicots and monocots. Abbreviations: IH, in situ hybridization; MA, microarrays; RQ RT-PCR, Relative Quantitative RT-PCR; RT-PCR, Reverse transcriptase - polymerase chain reaction; DNA gel blot; Veg., vegetative tissues; W1, first whorl or sepals in eudicots, outer tepals in non-grass monocots, and lemma and palea in Grasses; W2, second whorl or petals in eudicots, inner tepals in Non-grasses monocots and lodicules in grasses; W3, third whorl or stamens; W4, fourth whorl or carpels.

Species	Gene	Expression						Technique	References
		W1	W2	W3	W4	Fruit	Veg. tissues		
Dicots									
<i>Arabidopsis</i>	<i>AGL6</i>	+	+	+	+	-	+	RNA gel blot	Ma et al. (1990)
	<i>AGL13</i>	-	-	+	+	-	+	RNA gel blot, IH	Rounsley et al. (1995)
<i>Vitis vinifera</i>	<i>VvMADS3</i>	-	+	+	+	+	-	RNA gel blot	Boss et al. (2002)
<i>Petunia</i>	<i>pMADS4</i>	+	+	-	+	?	?	RNA gel blot	Tsuchimoto et al. (2000)
Amborellales									
<i>Amborella trichopoda</i>	<i>Am.tr.AGL6</i>	+	+	+	+	?	-	RQ RT-PCR	Kim et al. (2005)
Magnoliales									
<i>Magnolia grandiflora</i>	<i>Ma.gr.AGL6</i>	+	+	-	-	?	-	RQ RT-PCR	Kim et al. (2005)
<i>Persea americana</i>	<i>AGL6.1</i>	+	+	-	-	?	-	RQ RT-PCR	Chanderbali et al. (2006)
<i>Persea borbonia</i>	<i>AGL6.2</i>	+	+	-	-	?	-		
Non-grass monocots									
<i>Asparagus officinalis</i>	<i>AOM3</i>	+	+	+	+	?	-	RNA gel blot, IH	Losa et al. (2004)

<i>Oncidium Gower Ramsey</i>	O <i>MADS1</i>	-	+	-	+	?	-	RT-PCR, DNA gel blot	Hsu et al. (2003)
<i>Hyacinthus orientalis</i>	Ho <i>AGL6</i>	+	+	-	+	?	-	RNA gel blot	Fan et al. (2007)
Grasses									
<i>Oryza sativa</i>	Os <i>MADS6</i>	+	+	-	+	?	-	RNA gel	Moon et al. (1999)
		-	-	-	+	?	?	blot	Pelucchi et al. (2002)
	Os <i>MADS17</i>	?	?	?	?	?	-	IH MA	Arora et al. (2007)
<i>Lolium perenne</i>	Lp <i>MADS4</i>	-	?	?	?	?	-	RQ RT- PCR	Petersen et al. (2004)
<i>Triticum aestivum</i>	Ta <i>MADS37</i>	?	?	?	?	+	-	RT-PCR	Zhao et al. (2006)
<i>Poa annua</i>	Pa <i>MADS1</i>	-	?	?	?	?	-	RNA gel blot	Aiguo and Griffin (2002)
<i>Zea mays</i>	<i>zag3</i>	+	+	-	+	?	-	RNA gel	Mena et al. (1995)
		+	+	-	+	?	?	blot	Thompson et al (submitted)
	<i>zag5</i>	-	-	-	+	?	-	IH RNA gel blot	Mena et al. (1995)

Supplemental Table 3. *AGL6* homologs included in this study

<i>Species</i>	Family/Subfamily	Gene	Accession no
<i>Crocus sativus</i>	Iridaceae/—	Cs <i>AGL6a</i>	EF041505
		Cs <i>AGL6b</i>	EF041506
<i>Lilium lancifolium</i>	Liliaceae/—	Ll <i>AGL6</i>	<u>GQ496626</u>
<i>Hyacinthus orientalis</i>	Hyacinthaceae/—	Ho <i>AGL6</i>	AY591333
<i>Asparagus officinalis</i>	Asparagaceae/—	Ao <i>M3</i>	AY383559.1
<i>Agapanthus africanus</i>	Agapanthaceae/—	Aaf <i>AGL6</i>	<u>GQ496627</u>
<i>Elaeis guineensis</i>	Arecaceae/—	Eg <i>AGL6-1</i>	AY739701
<i>Tradescantia virginiana</i>	Commelinaceae/—	Tv <i>AGL6</i>	<u>GQ496625</u>
<i>Elegia sp</i>	Restionaceae/—	Es <i>AGL6</i>	Malcomber and Christensen (unpublished)
<i>Joinvillea ascendens</i>	Joinvilleaceae/—	Ja <i>AGL6</i>	<u>GQ496631</u>
<i>Streptochaeta angustifolia</i>	Poaceae/Anomochlooideae	Sa <i>AGL6</i>	<u>GQ496633</u>
<i>Pharus sp.</i>	Poaceae/Pharoideae	Ps <i>AGL6</i>	<u>GQ496632</u>
<i>Dendrocalamus latiflorus</i>	Poaceae/Bambusoideae	Dl <i>MADS17</i>	AY599754.1
		Dl <i>MADS18</i>	AY599755.1
<i>Lithachne humilis</i>	Poaceae/Bambusoideae	Lh <i>AGL6</i>	<u>GQ496639</u>
<i>Oryza sativa</i>	Poaceae/Ehrhartoideae	Os <i>MADS6</i>	U78782.1
		Os <i>MADS17</i>	AF109153.1
<i>Oryza barthii</i>	Poaceae/Ehrhartoideae	Ob <i>MADS6</i>	<u>GQ496637</u>
		Ob <i>MADS17</i>	<u>GQ496630</u>
<i>Oryza glaberrima</i>	Poaceae/Ehrhartoideae	Og <i>MADS6</i>	<u>GQ496635</u>
		Og <i>MADS17</i>	<u>GQ496629</u>
<i>Oryza meridionalis</i>	Poaceae/Ehrhartoideae	Om <i>MADS6</i>	<u>GQ496636</u>
		Om <i>MADS17</i>	<u>GQ496628</u>
<i>Leersia sp.</i>	Poaceae/Ehrhartoideae	Ls <i>AGL6</i>	<u>GQ496638</u>
<i>Zizania aquatica</i>	Poaceae/Ehrhartoideae	Za <i>AGL6</i>	<u>GQ496634</u>
<i>Brachypodium distachyon</i>	Poaceae/Pooideae	Bd <i>AGL6</i>	<u>GQ496641</u>
<i>Triticum aestivum</i>	Poaceae/Pooideae	Ta <i>MADS37</i>	DQ512353.1
		Ta <i>MADS12</i>	AB007505.1

<i>Triticum monococcum</i>	Poaceae/Pooideae	Tm <i>AGL6</i>	<u>GQ496643</u>
<i>Hordeum vulgare</i>	Poaceae/Pooideae	Hv <i>AGL6</i>	<u>GQ496642</u>
<i>Lolium temulentum</i>	Poaceae/Pooideae	Lt <i>AGL6</i>	<u>GQ496646</u>
<i>Lolium perenne</i>	Poaceae/Pooideae	Lp <i>MADS4</i>	AY198329.1
<i>Phalaris canariensis</i>	Poaceae/Pooideae	Pc <i>AGL6</i>	<u>GQ496640</u>
<i>Avena strigosa</i>	Poaceae/Pooideae	Ast <i>AGL6</i>	<u>GQ496644</u>
<i>Avena sativa</i>	Poaceae/Pooideae	As <i>AGL6</i>	<u>GQ496645</u>
<i>Poa annua</i>	Poaceae/Pooideae	Pa <i>MADS1</i>	AF372840.1
<i>Eleusine indica</i>	Poaceae/Chloridoideae	Ei <i>AGL6</i>	<u>GQ496648</u>
<i>Eragrostis pilosa</i>	Poaceae/Chloridoideae	Ep <i>AGL6</i>	<u>GQ496650</u>
<i>Eragrostis tef</i>	Poaceae/Chloridoideae	Et <i>AGL6</i>	<u>GQ496649</u>
<i>Chasmanthium latifolium</i>	Poaceae/Centothecoideae	Cl <i>AGL6</i>	<u>GQ496647</u>
<i>Setaria viridis</i>	Poaceae/Panicoideae	Sv <i>AGL6</i>	<u>GQ496659</u>
<i>Setaria italica</i>	Poaceae/Panicoideae	Si <i>AGL6</i>	<u>GQ496658</u>
<i>Pennisetum glaucum</i>	Poaceae/Panicoideae	Pg <i>AGL6</i>	<u>GQ496657</u>
<i>Megathyrsus maximus</i>	Poaceae/Panicoideae	Mm <i>AGL6</i>	<u>GQ496655</u>
<i>Panicum miliaceum</i>	Poaceae/Panicoideae	Pm <i>AGL6</i>	<u>GQ496656</u>
<i>Sorghum bicolor</i>	Poaceae/Panicoideae	Sb <i>AGL6</i>	<u>GQ496651</u>
<i>Zea mays</i>	Poaceae/Panicoideae	ZAG3	NM_001111862.1
		ZAG5	NM_001112222.1
<i>Tripsacum dactyloides</i>	Poaceae/Panicoideae	Td <i>AGL6.1</i>	<u>GQ496652</u>
		Td <i>AGL6.2</i>	<u>GQ496653</u>
<i>Coix sp.</i>	Poaceae/Panicoideae	Cos <i>AGL6</i>	<u>GQ496654</u>

Voucher information for each sequence is included in GenBank. Newly generated sequences are underlined.

Supplemental Table 4. Primers used for isolation of Os *MADS6*-like sequences and Os *MADS17*-like sequences, and expression analysis in Poaceae and other monocots.

Method	Species	Primer	Primer sequence
Isolation of Os <i>MADS6</i>-like genes			
	<i>Lilium lancifolium</i>	147F	SAG CCG HGG CAA RCT CTA
	<i>Agapanthus africanus</i>	147F	SAG CCG HGG CAA RCT CTA
	<i>Tradescantia virginiana</i>	147F	SAG CCG HGG CAA RCT CTA
	<i>Xyris sp.</i>	147F	SAG CCG HGG CAA RCT CTA
	<i>Joinvillea ascendens</i>	163F	TAC GAR TTC GGY AGC GCC
	<i>Streptochaeta angustifolia</i>	163F	TAC GAR TTC GGY AGC GCC
	<i>Pharus sp.</i>	163F	TAC GAR TTC GGY AGC GCC
	<i>Oryza barthii</i>	157F	AAG CTS TAC GAR TTC GGY AG
	<i>Oryza glaberrima</i>	215F	GYT GCT AYA ATG CTC AAG ATT CC
	<i>Oryza meridionalis</i>	215F	GYT GCT AYA ATG CTC AAG ATT CC
	<i>Lithachne humilis</i>	157F	AAG CTS TAC GAR TTC GGY AG
	<i>Leersia sp.</i>	157F	AAG CTS TAC GAR TTC GGY AG
	<i>Zizania aquatica</i>	157F	AAG CTS TAC GAR TTC GGY AG
	<i>Brachypodium distachyon</i>	157F	AAG CTS TAC GAR TTC GGY AG
	<i>Triticum monococcum</i>	270F	GAG YTG GTA CCA KGA AAT GTC AAA
	<i>Lolium temulentum</i>	157F	AAG CTS TAC GAR TTC GGY AG
	<i>Phalaris canariensis</i>	270F	GAG YTG GTA CCA KGA AAT GTC AAA
	<i>Avena strigosa</i>	215F	GYT GCT AYA ATG CTC AAG ATT CC
	<i>Avena sativa</i>	270F	GAG YTG GTA CCA KGA AAT GTC AAA
	<i>Eleusine indica</i>	215F	GYT GCT AYA ATG CTC AAG ATT CC
	<i>Eragrostis pilosa</i>	215F	GYT GCT AYA ATG CTC AAG ATT CC
	<i>Eragrostis tef</i>	215F	GYT GCT AYA ATG CTC AAG ATT CC
	<i>Chasmanthium latifolium</i>	215F	GYT GCT AYA ATG CTC AAG ATT CC
	<i>Setaria viridis</i>	157F	AAG CTS TAC GAR TTC GGY AG
	<i>Setaria italica</i>	157F	AAG CTS TAC GAR TTC GGY AG
	<i>Pennisetum glaucum</i>	163F	TAC GAR TTC GGY AGC GCC
	<i>Megathyrsus maximus</i>	270F	GAG YTG GTA CCA KGA AAT GTC AAA
	<i>Panicum miliaceum</i>	270F	GAG YTG GTA CCA KGA AAT GTC AAA
	<i>Sorghum bicolor</i>	270F	GAG YTG GTA CCA KGA AAT GTC AAA
	<i>Tripsacum dactyloides</i>	157F	AAG CTS TAC GAR TTC GGY AG
	<i>Coix sp.</i>	163F	TAC GAR TTC GGY AGC GCC
Isolation of Os <i>MADS17</i>-like sequences			
		150F	CCG TGG CAA ACT GTA TGA ATT
		177F	CGC GGG CAT TAA CAA AAC
		192F	AAC CCT GGA AAA ATA TAA CAG CTG
		257F	GCG AAC ATC AGA GCT GGT ATC
		157F	AAG CTS TAC GAR TTC GGY AG
		163F	TAC GAR TTC GGY AGC GCC
		650R	CGC ACA AAC TGA TAA TAG CCA A
		711R	ATC ACA AAG TTG TTG TTC TGA TCG
		714R	TCA GAA CAA CAA CTT TGT GAT GG
		725R	ACT TTG TGA TGG GCT GGC
			CCG GAT CCT CTA GAG CGG CCG CTT TTT
		polyT	TTT TTT TTT TTT
Q-PCR			
		ACTIN591F	GAG RGG TTA CTC CTT CAC VAC
		764R	GGA CCT CGG GGC ACC TGA ACC TCT
	<i>Streptochaeta angustifolia</i>	StrepAGL6_1F	TGC TAT TCT ATA CCT AAG TCG TTG GA
		Strep_3UTR	TTA TAC CCA CCT TGG ATG TCG
	<i>Oryza sativa</i>	OsMADS6_2F	TCT GTA GTT GCT GGT GGA ACC
		OSMADS6_5CT113	ATT ATT AGC CCC AAC TTC TGA CAG
		OsMADS17_1F	CGT GCT ATA ATA TAT AGT TCG GCA AA
		OSMADS17_3UTR451	CAA ACA TAC ACT CAT AGG TAC GTG C
	<i>Eleusine indica</i>	EiAGL6_2F	TTT GAC CTA CAG ATC CAT CCG
		Ei3UTR	ACG ACC ACA CAA TCA CGG T

RT-PCR and in situ hybridization

<i>Lolium temulentum</i>	LtAGL6_1F Lt3UTR	CTA ATA AAA TGC GTG TTC TGC TTC T TTC AAA TAA TCA AAA CGT AGA AGT GG
<i>Setaria italica</i>	SiAGL6_1F Seta3UTR	TTC TAT GTG ATT GTA TTG TGG CAC TT ATA CGT ACC GGT AGA GAT AAT TTT GG
<i>Sorghum bicolor</i>	SbAGL6_2F Sb3UTR	GTG GTG TGG CAC TTC TAT GTT TT ATA AGT ACC GTT GCA GAT AAT TTT GG
<i>Triticum monoccocum</i>	TmAGL6_1F Tri_3UTR	TCG TTA TTT GTG CGT GTA AGA AGTA GCA TGC ATG GAT AGC TTG G
<i>Agapanthus africanus</i>	147F 559r	SAG CCG HGG CAA RCT CTA CCC AGC CCA GCA TAA AGT T
<i>Joinvillea ascendens</i>	JO_5CT158 JO23_3UTR Jo5CT JO32_3UTR	CTC TGC AAA TTG GGC AGT ATC AGA GCT TAA ATT TTA CAA GGT CC GGA GAA ATT AAC AAA CAA CTC AAA AAC TTT TTG GCA GTC AAG GGA ATA
<i>Streptochaeta angustifolia</i>	Strep_5CT270 Strep_5CT Strep_3UTR	CGT AGA CTG AGT TTA TCT GCC TAG C TAA CAG GCA GCT CAA AAA CAA G TTA TAC CCA CCT TGG ATG CCG
<i>Lithachne humilis</i>	LITHA_5CT316 Litha_181_3UTR	GTG GGT TCT TTA AGC TTG CTT G TTT GAG TTG CTA GGG AAT TTG AA
<i>Oryza sativa</i>	OsMADS6_5CT OSMADS6_5CT113 OsMADS6_3UTR OSMADS17_5CT100 OSMADS17_3UTR451 OsMADS17_5CT OsMADS17_3UTR	CTG GGT GAA ATT AAT AGG CAA CTC ATT ATT AGC CCC AAC TTC TGA CAG CAG CTA GCA GTA GCT TAC ACA CAA A AAG CTA GAA GCT GAA GCC GAT A CAA ACA TAC ACT CAT AGG TAC GTG C CAG GCT CGA CAA CGA AAG A ACC GGA TAG GGG AGA AAT ATA CA
<i>Leersia sp.</i>	LE_5CT202 Le_3UTR	CGG CCA TGG ACT CTG AAC AAA CAC AGC CAC GTA CGT AGA AC
<i>Hordeum vulgare</i>	Hv5CT Hv3UTR	GCG TCA GCT GGG AGA CAT AAT TGT CCA GAA ATT ATG GTT GTA CT
<i>Triticum monococum</i>	Tri_5CT Tri_3UTR	GGC AGC AAC AGC AAC AAC TA GCA TGC ATG GAT AGC TTG G
<i>Lolium temulentum</i>	Lt5CT Lt3UTR	CAG CAG CAG CCA AAT CAC T TTC AAA TAA TCA AAA CGT AGA AGT GG
<i>Phalaris canariensis</i>	270F 805R	GAG YTG GTA CCA KGA AAT GTC AAA TTC ATG CTG GGR TGG GTT
<i>Eleusine indica</i>	Ei5CT Ei3UTR	TGG GAG AAA TGA ACA GGC A ACG ACC ACA CAA TCA CGG T
<i>Eragrostis tef</i>	270F 805R	GAG YTG GTA CCA KGA AAT GTC AAA TTC ATG CTG GGR TGG GTT
<i>Setaria viridis</i>	Seta5CT Seta3UTR	GAG AAA TGA ACA GGC AAC TCA AG ATA CGT ACC GGT AGA GAT AAT TTT GG
<i>Setaria italica</i>	Seta5CT Seta3UTR	GAG AAA TGA ACA GGC AAC TCA AG ATA CGT ACC GGT AGA GAT AAT TTT GG
<i>Pennisetum glaucum</i>	Pg5CT Pg3UTR	GTC ACC TGG GAG AAA TGA ACA CGG CAG CAT AGA AGT ATT TTT ACA
<i>Megathyrsus maximus</i>	270F 805R	GAG YTG GTA CCA KGA AAT GTC AAA TTC ATG CTG GGR TGG GTT
<i>Sorghum bicolor</i>	Sb5CT Sb3UTR	GAA CAG GCA ACT CAA ACA CAA G ATA AGT ACC GTT GCA GAT AAT TTT GG

Statistical tests

Statistical tests of nucleotide sequences (tabulated results at Supplemental Table 5 bellow)

We performed a Shimodaira–Hasegawa (SH) test (Goldman et al. 2000) to test whether we could statistically reject alternative hypotheses for the timing of the *AGL6/Os MADS6/Os MADS17* duplication and the *zag3/zag5* duplication.

We tested whether the duplication could have taken place at the base of the grasses after the divergence of Joinvilleaceae. Also, we explored alternative hypothesis for the *Os MADS6/Os MADS17* duplications based on previous studies that suggested that the two copies are the result of rice genome duplication event (Arora et al., 2007). Each topology differed in the placement of *Oryza MADS17* sequences as follows: (1) *Oryza MADS17-like* sequences sister to the *Oryza MADS6* clade (testing whether the duplication could have occurred just prior to the origin of the genus *Oryza*), (2) *Oryza MADS17-like* sequences sister to the Oryzeae clade sequences (testing whether the duplication could have occurred just prior to the origin of the tribe Oryzeae), (3) *Oryza MADS17-like* sequences sister to the Ehrhartoideae (testing whether the duplication could have occurred just prior to the origin of the subfamily Ehrhartoideae), (4) *Oryza MADS17-like* sequences sister to the grass family (testing whether the duplication could have occurred just prior to the origin of the grasses), (5) *Oryza MADS17-like* sequences sister to the BEP clade (testing whether the duplication could have occurred just prior to the common ancestor of Bambusoideae, Ehrhartoideae, and Pooideae).

In addition, phylogenetic analysis could not determine precisely when the duplication of *zag3/Td AGL6.2* and *zag5/Td AGL6.1* happened. We tested whether *zag3/Td AGL6.1* and *zag5/Td AGL6.2* genes are sister taxa, meaning that the duplication occurred prior the divergence of maize and *Tripsacum*.

A priori constraint trees were created in MacClade (Maddison and Maddison 2003). Each constraint tree was tested against the unconstrained ML phylogeny using PAUP* version 4.0b10 (Swofford 2001). The SH test was performed using 1000 bootstrap replicates and the REL (resampling estimated log likelihoods) option.

Tests for positive selection (tabulated results at Supplemental Tables 6-9 bellow)

The tests are based on estimates of the ratio of non-synonymous to synonymous substitutions (dN/dS, or ω). If this ratio is appreciably less than 1, then we infer that the

protein has been subject to purifying selection, such that mutations causing amino acid changes have been selectively eliminated. If the ratio is near 1, we infer that selection pressures are relaxed, and if the ratio is greater than 1, we infer that there has been selection for diversification (positive selection).

The best-supported tree obtained from Bayesian inference analysis was pruned to include only full-length sequences, and likelihood scores were calculated and statistically compared using the program *codeml* from the PAML package, version 4 (Yang, 2007). PAML calculates the likelihood of the data given the tree under a set of increasingly complex models of evolution. We began with the simplest model (M_0), which calculates a single ω ratio averaged over the entire tree (Yang et al., 1998). We compared this to a set of models that allow the ω ratio to vary among sites (among codons or amino acids in the protein) (Nielsen and Yang 1998; Yang et al. 2000). Model 1a assumes two site classes with $0 < \omega_0 < 1$ and $\omega_1 = 1$, Model 2a is like 1a but also assumes positive selection, Model 3 uses three site classes, Model 7 assumes variable selection among sites, and Model 8 is like Model 7, but with the addition of positive selection. We repeated this set of tests on an alignment including all sequences but excluding the MADS box, and on another alignment including only the C terminus of all sequences.

We also wanted to test for positive selection on particular branches of the *AGL6-like* gene tree. In particular, we were interested in testing for positive selection along all branches subsequent the *AGL6/Os MADS6* and *Os MADS17*. For this test we used the following models that incorporate heterogeneity over sites and branches: Model A (assumes $0 < \omega_0 < 1$ estimated from the data and $\omega_1 = 1$) and Model B (determines ω_0 and ω_1 as free parameters estimated from the data). We tested the data set that included only the IKC regions and excluded the MADS-box, which we had shown to be largely invariant.

Models were compared using the likelihood ratio test statistic ($2\Delta \ln L$).

Tajima's relative rate test (tabulated results at Supplemental Tables 10 bellow)

To test whether *Os MADS17/Os MADS6*, as well as *zag5/zag3* duplicates show differences in evolutionary rates, we used the test proposed by Tajima (1993) using MEGA4 (Tamura et al., 2007). This method compares three sequences, where one of them is an outgroup sequence. The test compares the identical sites in all three

sequences (m_{iii}), the divergent sites in all three sequences (m_{ijk}), the unique differences in sequence A (m_{ijj}), the unique differences in sequence B and the unique differences in the outgroup sequence. Statistical significance among these is assessed under the χ^2 distribution. We compared the evolutionary rate between Os *MADS17* vs. Os *MADS6* and *zag5* vs. *zag3* using Es *AGL6* and Os *MADS6* as outgroups, respectively.

Supplemental Table 5. Results of the Shimodaira-Hasegawa test performed to evaluate whether we could statistically reject alternative hypotheses for the timing of the *Os MADS6*/*Os MADS17* duplication and *ZAG3*/*ZAG5* duplication. Statistically worse trees as compared to the best tree are marked with an asterisk (*), and *P* values < 0.05.

Tree	-ln Likelihood	Difference from best tree (-ln <i>L</i>)	<i>P</i> value
Likelihood tree (GTR+I+G)	8550.63754	(best)	
<i>Oryza MADS17</i> -like sequences sister to <i>Oryza MADS6</i> clade	8586.81569	36.17815	0.000*
<i>Oryza MADS17</i> -like sequences sister to the Oryzeae clade sequences	8579.31828	28.68074	0.004*
<i>Oryza MADS17</i> -like sequences sister to the Ehrhartoideae subfamily	8572.32382	21.68628	0.020*
<i>Oryza MADS17</i> -like sequences sister to the grass family	8551.18172	0.54418	0.691
<i>Oryza MADS17</i> -like sequences sister to the BEP clade	8572.40916	21.77162	0.020*
<i>zag3</i> /Td <i>AGL6.1</i> and <i>zag5</i> /Td <i>AGL6.2</i> are sister taxa	8551.78152	1.14398	0.327

Supplemental Table 6. Likelihoods and estimated parameters under different site models of codon substitution using the pruned data set with sequences that expand the MIKC regions. Abbreviations: $-\ln L$, log likelihood, p , parameter; Kappa (ts/tv), transition/transversion ratio; NA, non applicable; ω , dN/dS value calculated under each model and applied to all branches in the phylogeny. Parameters in parenthesis are not free parameters.

Model	p	$-\ln L$	Kappa (ts/tv)	ω	Additional Parameters Calculated
0	1	-4129.210037	2.72999	0.0954	NA
1a (neutral)	2	-4245.967313	2.69268	0.4632	$p_0 = 0.53680, (p_1 = 0.46320)$
2a (positive)	4	-4245.967313	2.69267	0.4632	$p_0 = 0.53682, p_1 = 0.46318, (p_2 = 0.00000)$ $\omega = \infty$
3 (discrete)	3	-4015.407265	1.81912	0.1158	$p_0 = 0.54898, (p_1 = 0.35404), p_2 = 0.09698$ $\omega_0 = 0.01390, \omega_1 = 0.13578, \omega_2 = 0.61991$
7 (β)	2	-4054.198096	1.80986	0.1162	$p = 0.33480, q = 2.45264$
8 (B + ω)	4	-4054.328221	1.79949	0.5511	$p_0 = 0.99940, p = 0.33622, q = 2.45648,$ $(p_1 = 0.00060), \omega = 724.93092$

Supplemental Table 7. Likelihoods and estimated parameters under different site models of codon substitution using the data set excluding the MADS-box. Abbreviations: $-\ln L$, log likelihood, p , parameter; Kappa (ts/tv), transition/transversion ratio; NA, non applicable; ω , dN/dS value calculated under each model and applied to all branches in the phylogeny. Parameters in parenthesis are not free parameters.

Site Model	p	$-\ln L$	Kappa (ts/tv)	ω	Additional Parameters Calculated
0	1	-3842.679439	2.09356	0.1317	NA
1a (neutral)	2	-3784.514745	2.15909	0.1977	$p_0 = 0.88116, (p_1 = 0.11884)$
2a (positive)	4	-3784.514745	2.15909	0.1977	$p_0 = 0.88116, p_1 = 0.11884, (p_2 = 0.00000)$ $\omega = \infty$
3 (discrete)	3	-3770.470138	2.01294	0.1590	$p_0 = 0.61960, (p_1 = 0.27508), p_2 = 0.10532$ $\omega_0 = 0.04262, \omega_1 = 0.20315, \omega_2 = 0.72797$
7 (β)	2	-3777.500304	1.99916	0.1636	$p = 0.54167, q = 2.70239$
8 (B + ω)	4	-3777.500402	1.99916	0.1636	$p_0 = 1.00000, p = 0.54167, q = 2.70239$ $(p_1 = 0.00000), \omega = 6.48257$

Supplemental Table 8. Likelihoods and estimated parameters under different site models of codon substitution using only the C-terminus. Abbreviations: $-\ln L$, log likelihood, p , parameter; Kappa (ts/tv), transition/transversion ratio; NA, non applicable; ω , dN/dS value calculated under each model and applied to all branches in the phylogeny. Parameters in parenthesis are not free parameters.

Site Model	p	$-\ln L$	Kappa (ts/tv)	ω	Additional Parameters Calculated
0	1	-1640.637362	2.15397	0.1994	NA
1a (neutral)	2	-1690.315486	3.46592	0.7867	$p_0 = 0.21335$, ($p_1 = 0.78665$)
2a (positive)	4	-1690.318441	3.46590	0.7866	$p_0 = 0.21337$, $p_1 = 0.78663$, ($p_2 = 0.00000$) $\omega = \infty$
3 (discrete)	3	-1618.117196	2.03426	0.2106	$p_0 = 0.70167$, ($p_1 = 0.29833$), $p_2 = 0.000000$ $\omega_0 = 0.10034$, $\omega_1 = 0.46658$, $\omega_2 = 999.000$
7 (β)	2	-1614.406920	2.06628	0.2117	$p = 0.76756$, $q = 2.81030$
8 (B + ω)	4	-1614.497092	2.06632	2.1553	$p_0 = 0.99758$, $p = 0.76772$, $q = 2.81123$, ($p_l = 0.00242$), $\omega = 804.09372$

Supplemental Table 9. Hypothesis tested using likelihood ratio tests. Abbreviations: $2\Delta \ln L$, the likelihood ratio statistic; df, degrees of freedom (is the difference in the number of parameters calculated under the models being compared).

Model	Data set	H ₀ (null)	H ₁ (alternate)	$2\Delta \ln L$	df	<i>P</i> value (χ^2)
Site Models						
	MIKC					
		0	3	227.60	4	0.000
		1a	2a	0.00	2	1.000
		7	8	-0.26	2	1.000
	IKC					
		0	3	144.41	4	0.000
		1a	2a	0.00	2	1.000
		7	8	0.00	2	1.000
	C-terminus					
		0	3	45.04	4	0.000
		1a	2a	0.00	2	1
		7	8	-0.18	2	1
Branch site Models						
	IKC	Os <i>MADS17</i>				
		Model A null	A	-12.46	2	1.000
		Model B null	B	-14.42	1	1.000
		<i>AGL6/Os MADS6</i>				
		Model A null	A	-11.42	2	1.000
		Model B null	B	-14.42	1	1.000
		Gain of palea expression				
		Model A null	A	-10.19	2	1.000
		Model B null	B	-8.87	1	1.000

Supplemental Table 10. Results from Tajima's relative rates test. The asterisk indicates *P* values less than 0.05 is often used to reject the null hypothesis of equal rates between lineages. dN/dS ratio was estimated using codeml as implemented in PAML 3.14 package (Yang 1997).

Test	Gene	dN/dS = ω	Unique sites	<i>P</i> value
<i>ZAG5</i> vs <i>ZAG3</i>	<i>ZAG5</i>	0.09049	7	0.36571
	<i>ZAG3</i>	0.08171	4	
<i>Os MADS17</i> vs <i>Os MADS6</i>	<i>Os MADS17</i>	0.57442	25	0.01963*
	<i>Os MADS6</i>	0.21785	11	

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