

Short Communication

Development of universal primers for isolating fragments of the *LEAFY* gene

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ABSTRACT. *LEAFY* orthologs are found throughout land plants. Degenerate primers were designed for amplifying exon 3 region fragments of the *LEAFY* gene. Sixteen taxa from different plants were sampled, all of which were successfully isolated, which indicated that this set of primers is universally applicable for all land plants. With the use of this method, there may be potential for applying *LEAFY* exon 3 as a single-copy nuclear marker to resolve reticulate evolution at high taxonomic levels and to clarify the origin and evolutionary history of the *LEAFY* gene.

Key words: Land plants; LEAFY gene; Degenerate primers

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INTRODUCTION

The genetic regulation of the transition to reproduction in plants is controlled by a group of genes termed floral meristem identity genes, of which *LEAFY* is arguably the most important. *LEAFY* orthologs are found throughout the diversity of land plants and are essential for angiosperm reproduction. These genes have also been implicated in reproductive development in gymnosperms (Siriwardana and Lamb, 2012a). In the model annual plant *Arabidopsis thaliana*, the floral meristem identity gene, *LEAFY*, is essential for the formation of fertile flowers and plays a role in the control of several aspects of floral development (Siriwardana and Lamb, 2012b). Analyses of loss of *LEAFY* function revealed that this gene is essential for reducing cortical cell elongation at the adaxial side of the pedicel base (Yamaguchi et al., 2012).

Since Weigel et al. (1992) isolated the *LEAFY* gene in *A. thaliana*, many homologs of the gene have been isolated from other species (Maizel et al., 2005; Ordidge et al., 2005; Yu et al., 2005; Kanrar et al., 2008). In these studies, *LEAFY* gene homologs were mostly isolated from a cDNA library (An et al., 2011), which was both a time-consuming and an expensive process. Furthermore, a set of degenerate primers was designed for all plants, except algae, and the procedure for polymerase chain reactions (PCRs) of sequences with especially high GC contents was optimized.

METHODS AND RESULTS

To test the new primer set, we sampled different plants from 16 taxa. Total genomic DNA was extracted from fresh leaves using the DNAquick Plant System (TIANGEN Biotech Ltd., Beijing, China). Based on available *LEAFY* sequences of plants in GenBank, a universal primer set - LEAFYF+LEAFYR - was designed and focused on conserved regions of exon 3 for amplification of the partial sequences of *LEAFY* (LEAFYF: 5'-TAYATIAAYAARCCIAARATG-3', LEAFYR: 5'-ARIYKIGTIGGIACRTACCA-3') (Yu et al., 2010). Alignment and primer design were performed using DNAMAN (Figure 1). The PCR amplifications were performed in 25-µL reaction volumes containing 20-200 ng template DNA, 15.3 µL ddH₂O, 2 µL DMSO, 2.5 µL 10X buffer, 2.0 µL 10 µM dNTPs, 1.0 µL each of 10 µM primers, and 0.5 U Taq polymerase (Yu et al., 2010). Thermal cycles were completed with a 2-min denaturation step at 94°C, followed by 35 cycles of 94°C for 30 s, 42°C for 30 s, 72°C for 30 s, followed by a 10-min final extension at 72°C. The total volumes of PCR products were run on 2% agarose gels containing ethidium bromide, and bands were excised and purified with TIANgel Midi Purification Kit (TIANGEN Biotech Ltd.). The sequencing products were analyzed using the ABI 3730xl DNA analyzer (Applied Biosystems).

Using the universal primer set, the sequences of interest from all 16 taxa sampled (*Nephrolepis auriculata*, *Shibataea nanpingensis*, *Rhododendron pulchrum*, *Fagus longipetiolata*, *Davidia involucrata*, *Ilex cornuta*, *Osmanthus fragrans*, *Scindapsus aureus*, *Podocarpus macrophyllus*, *Canna generalis*, *Nandina domestica*, *Pharbitis nil*, *Prunus majestica*, *Porella pinnata*, *Bougainvillaea glabra*, and *Trachycarpus fortunei*) were amplified successfully. We obtained 16 *LEAFY* fragments, 236 bp in length. A BLAST search against National Center for Biotechnology Information sequences (http://www.ncbi.nlm.nih.gov/blast/Blast. *cgi*) revealed that all of our sequences showed the highest identities to *LEAFY*, suggesting that they belonged to the *LEAFY* gene family. The homology of our amplicons was further confirmed by alignment analysis (Figure 2).

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Primers for LEAFY gene fragments

XP 002511083	E <mark>KC</mark> PTKVTNQVFRYA <mark>KKA</mark> GA <mark>S</mark> YINKPKMRHYVHCYA <mark>L</mark> HCLD <mark>EEA</mark> SN <mark>A</mark> LRRA <mark>F</mark> K <mark>ER</mark> GENVG <mark>A</mark> WRQACY <mark>K</mark> PLV <mark>AIAARQG.W</mark>	337
AAC96098	HK <mark>S</mark> PTKVTNQVFRYA <mark>KKV</mark> GA <mark>S</mark> YINKPKMRHYVHCYA <mark>L</mark> HCLD <mark>EEA</mark> SDALRRA <mark>YKAR</mark> GENVG <mark>A</mark> WRQACYAPLV <mark>DISARHG.F</mark>	345
AAF66099	EKCPTKVTNQVFRYA <mark>KKA</mark> GA <mark>S</mark> YINKPKMRHYVHCYA <mark>L</mark> HCLDEDASNALRRAEKERGENVG <mark>A</mark> WRQACYKPLV <mark>AIAARQG.W</mark>	361
AAF77118	EKCPTKVTNOVFRYAKKAGASYINKPKMRHYVHCYA <mark>L</mark> HCLDEEVSNTLRRAFKERGENVGTWROACY <mark>K</mark> PLV <mark>AIAALOG.W</mark>	328
AAF77609	EKCPTKVTNQVFRYAKSMGASYINKPKMRHYVHCYA <mark>L</mark> HCLDEASSDRLRRAFKERGENVGAWRQACYOPLV <mark>VMAAHHS.W</mark>	343
AAG41992	HKSPTKVTNOVFRYASKVGASYINKPKMRHYVHCYALHCLDOEASDALRRAYKARGENVGAWROACYAPLVDIAAGHG.F	346
AA043174	H <mark>KS</mark> PTKVTNQVFRYA <mark>NKC</mark> GA <mark>S</mark> YINKPKMRHYVHCYA <mark>L</mark> HCLD <mark>EEASNA</mark> LRRA <mark>YKSR</mark> GENVG <mark>A</mark> WRQACYAPLV <mark>EIAARHG.</mark> F	346
ABC69153	HK <mark>S</mark> PTKVTNOVFRYA <mark>NKC</mark> GA <mark>S</mark> YINKPKMRHYVHCYALHCLD <mark>EEASD</mark> ALRRA <mark>MKSR</mark> GENVG <mark>A</mark> WROACY <mark>A</mark> PLVEIAARHG.F	344
ABR13035	DKCPTKVTNOVFRYAKKMGACYINKPKMRHYVHCYA <mark>F</mark> HCLD <mark>EEGSNA</mark> LRRAYK <mark>ER</mark> GENVG <mark>A</mark> WRQACY <mark>K</mark> PLV <mark>AIAARHGTW</mark>	349
BAE78663	oksptkvtnovfryaskvgasyinkpkmrhyvhcya <mark>l</mark> hcld <mark>edasda</mark> lrra <mark>ykar</mark> genvg <mark>awr</mark> oacy <mark>aplvdiaarhg.</mark> f	345
NP 200993	EKCPTKVTNOVFRYAKKSGASYINKPKMRHYVHCYALHCLDEEASNALRRAFKERGENVGSWROACYKPLVNIACRHG.W	357
NP_001053742	HKSPTKVTNQVFRYAKKVGASYINKPKMRHYVHCYALHCLDEEASDALRRAYKAFGENVGAWRQACYAPLVDISARHG.F	345
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XP 002511083	DIDAIFNAHPRUAIWYVPTKLROLCHAERNSATASSSVSGGGGDHLRF	385
AAC96098	DIDAVFAAHPRUAIWYVPTRLROLCHOARSSHAAAAAAL	388
AAF66099	DIDAIFNAHPRUAIWYVPTKLROLCHSBRSNAAAAASSSVSGGVADHLPHF	412
AAF77118	DIDAIFNAHPRUSIWYVPTKLROLCHAERSNNAASSSSAAAAAAAAGPIAGPEF	382
AAF77609	DIDAIFNANEKICIWYVPTKLROLCHOORTAAAASMGTAHLROM	387
AAG41992	DVDAVFAAHPRUAIWYVPTRLFOLCHOARSAHEAAAANANANGAMPPFPPPPM	399
AA043174	DIDAVEAAHPRUAVWYVPTRUROLCHOARGSHAHAAAGUP	390
ABC69153	DIDAVFAAHPRLAVWYVPTRLROLCHOARGSHAHAAAGLP	388
ABR13035	DIDAVENAHERLAIWYVPTKLROLCHLARSNATATGELOPP	396
BAE78663	DIDAVEAAHPRLAIWYVPTRLRQLCHQARSAHDTAAAHA	391
NP 200993	DIDAVENAHRUIVIALAIVIALAINA DIDAVAAAAALVGGISCTGSSTSGR <mark>G</mark> GCGGDDLRF	420
NP 001053742	DIDAVFAAHPREAIWYVPTREROLCHOARSSHAAAAAALPPPL	388

Figure 1. Relevant sequence alignment for primer design.

в.	glabra	YMNKPKMR <mark>H</mark> YVHCYALHCLDEETS <mark>NALRRAF</mark> KERGENVGAWRQACYKPLVATAARQGWDIDAIFNSHPRISIWYVPTR	78
т.	fortunei	YMNKPKMR <mark>H</mark> YVHCYALHCLD <mark>EEA</mark> S <mark>NALRRAYKER</mark> GENVGAWRQACYKPLVAISARHGWDIDAVFNAHPRISIWYVPTS	78
с.	generalis	YMNKPKMR <mark>H</mark> YVHCYALHCLD <mark>EET</mark> SNVLR <mark>RAF</mark> KERGENVGAWRQACYKPLVSMAARQGWDIDAIFNAHPRLTIWYVPTN	78
D.	involucrata	YMNKPKMR <mark>H</mark> YVHCYALHCLD <mark>EEA</mark> STALR <mark>RAFKER</mark> GENVGAWROSCYKPLVATAAROGWDIDAIFNAHPRLAIWYVPTS	78
F.	longipetiolata	YMNKPKMR <mark>H</mark> YVHCYALHCLD <mark>EEASNALRRGF</mark> KERGENVGAWRQACYKPLVA IAARQGWDIDAIFNAHPRLSVWYVPTN	78
I.	cornuta	YMNKPKMR <mark>H</mark> YVHCYALHCLD <mark>EAAS</mark> NVLR <mark>RAF</mark> KERGENVGAWRQACYKPLVAIAARQGWDIDTIFNTHPRLSIWYVPTH	78
Ν.	auriculata	YMNKPKMR <mark>H</mark> YVHCYALHCLDK <mark>E</mark> KS <mark>NLLRKIFKER</mark> GENVGAW <mark>ROACYY</mark> PLV <mark>DMA</mark> RDN <mark>GW</mark> DIEGVFVRNEKLRIWYVPTR	78
Ν.	domestica	YMNKPKMR <mark>H</mark> YVHCYALHCLD <mark>EEA</mark> S <mark>NALR<mark>RAFK</mark>EKGENVGAWRQACYKPLVSMASHHGWDIDGIFNAHPRLSIWYVPTR</mark>	78
ο.	fragrans	YMNKPKMR <mark>H</mark> YVHCYALHCLD <mark>EEA</mark> S <mark>NALRRAYKER</mark> GENVGAWROACYKPLV <mark>AIASROGW</mark> DIDVIFNTHPRLSIWYVPTN	78
P.	macrophyllus	YMNKPKMR <mark>H</mark> YVHCYALHCLDS <mark>E</mark> QS <mark>N</mark> HLR <mark>RIYKER</mark> GENVGAW <mark>RQA</mark> CYYPLVD IA RENNWDIEGVENKNEK <mark>I</mark> KIWYVPTH	78
P.	majestica	YMNKPKMR <mark>H</mark> YVHCYALHCLD <mark>EEA</mark> S <mark>NALRRVFK</mark> ERGENVGAWRQACYKPLVAIAAGQGWDIDAIFTSHPRLSIWYVPTN	78
P.	nil	YMNKPKMR <mark>H</mark> YVHCYALHCLD <mark>EEA</mark> S <mark>NALRRAF</mark> KERGENVGAWRQACYKPLVAIAALQAWDIDAIFNAHPRLSIWYVPTN	78
P.	pinnata	YMNKPKMR <mark>G</mark> YVHCYALHCLDVETS <mark>NTLRKEYKER</mark> GENVGAWCQACYFPLVTLARQNEWDIDELE <mark>NRNDKLRI</mark> WYVPTH	78
R.	pulchrum	YMNKPKMR <mark>H</mark> YVHCYALHCLD <mark>EE</mark> ES <mark>DALRRAFK</mark> ERGENVGAWROACYKPLV <mark>AIAAQQGW</mark> DIDAIFNAHPRLSVWYVPTN	78
s.	aureus	YMNKPKMR <mark>H</mark> YVHCYALHCLD <mark>EEA</mark> S <mark>NALRRAF</mark> KERGENVGAWRQACYKPLVAISARHGWDIDAVFNAHPRLSIWYVPTH	78
s.	nanpingensis	YMNKPKMR <mark>H</mark> YVHCYALHCLD <mark>EEAS</mark> NTLR <mark>RVYKAR</mark> GENVGAWR <mark>QACYA</mark> PLVD <mark>ISARHG</mark> FDIDAV <mark>BAAHP</mark> LLA <mark>I</mark> WYVPTR	78

Figure 2. Sequence alignments of *LEAFY* fragments from different plants.

CONCLUSIONS

This study showed that the newly designed primers successfully amplified and sequenced the *LEAFY* fragment of the exon 3 region across all of the sampled species from each of the plants selected. *LEAFY* exon 3 sequences were variable, which suggests that this region will be particularly useful for high taxonomic-level phylogenetic analyses. Thus, there may be potential for applying *LEAFY* exon 3 as a single-copy nuclear marker to resolve reticulate evolution at high taxonomic levels, and to clarify the origins and subsequent evolution of the *LEAFY* gene.

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