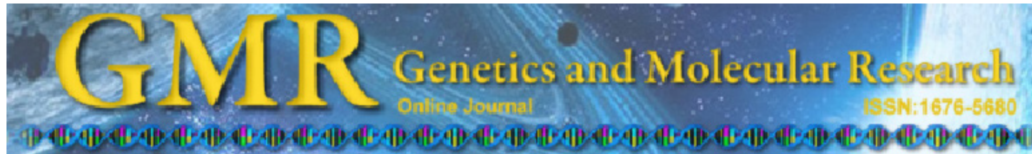

Short Communication

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

L.X. Yu and B. Yan

Faculty of Landscape Architecture, Southwest Forestry University, Kunming, China

Corresponding author: B. Yan
E-mail: yanbodr@yahoo.com.cn

Genet. Mol. Res. 12 (2): 1777-1780 (2013)
Received August 28, 2012
Accepted February 15, 2013
Published June 6, 2013
DOI http://dx.doi.org/10.4238/2013.June.6.1

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

 ©FUNPEC-RP www.funpecrp.com.br

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 ddH$_2$O, 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).

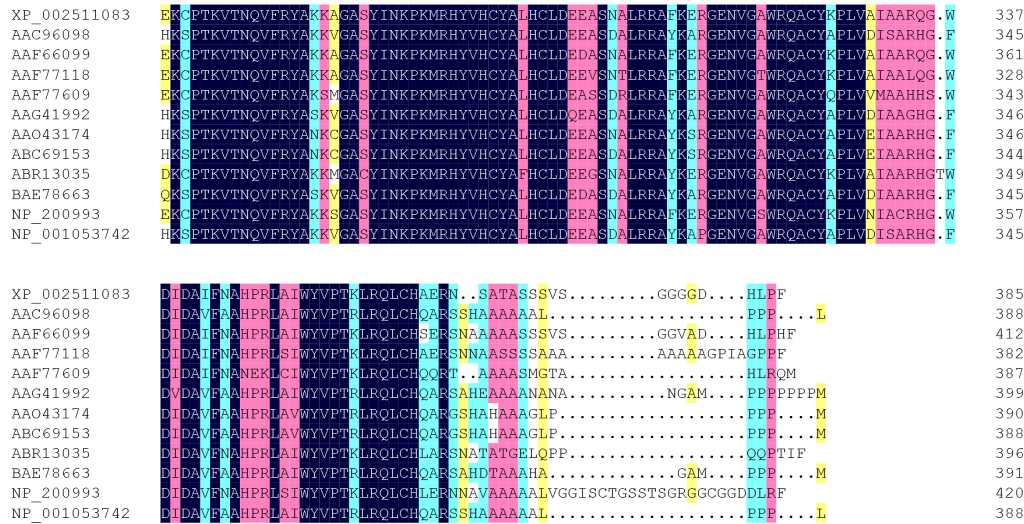

Figure 1. Relevant sequence alignment for primer design.

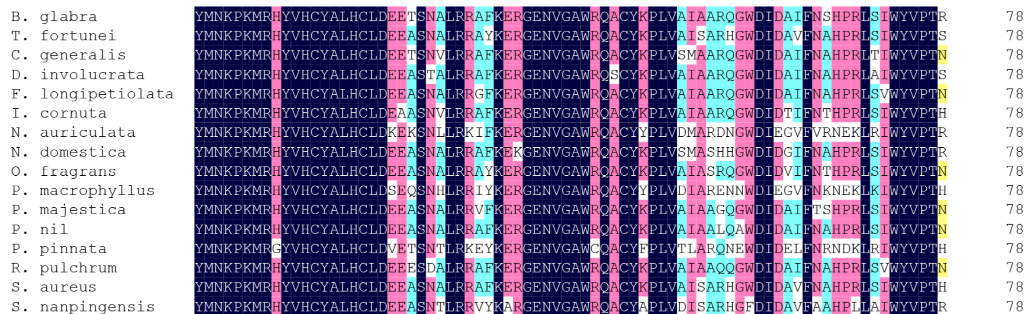

Figure 2. Sequence alignments of *LEAFY* fragments from different plants.

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.

ACKNOWLEDGMENTS

Research supported by the Natural Science Foundation of China (#31060042 and #31160177).

REFERENCES

An XM, Wang DM, Wang ZL, Li B, et al. (2011). Isolation of a *LEAFY* homolog from *Populus tomentosa*: expression of PtLFY in *P. tomentosa* floral buds and PtLFY-IR-mediated gene silencing in tobacco (*Nicotiana tabacum*). *Plant Cell Rep.* 30: 89-100.

Kanrar S, Bhattacharya M, Arthur B, Courtier J, et al. (2008). Regulatory networks that function to specify flower meristems require the function of homeobox genes *PENNYWISE* and *POUND-FOOLISH* in *Arabidopsis*. *Plant J.* 54: 924-937.

Maizel A, Busch MA, Tanahashi T, Perkovic J, et al. (2005). The floral regulator *LEAFY* evolves by substitutions in the DNA binding domain. *Science* 308: 260-263.

Ordidge M, Chiurugwi T, Tooke F and Battey NH (2005). *LEAFY*, *TERMINAL FLOWER1* and *AGAMOUS* are functionally conserved but do not regulate terminal flowering and floral determinacy in *Impatiens balsamina*. *Plant J.* 44: 985-1000.

Siriwardana NS and Lamb RS (2012a). The poetry of reproduction: the role of *LEAFY* in *Arabidopsis thaliana* flower formation. *Int. J. Dev. Biol.* 56: 207-221.

Siriwardana NS and Lamb RS (2012b). A conserved domain in the N-terminus is important for *LEAFY* dimerization and function in *Arabidopsis thaliana*. *Plant J.* 71: 736-749.

Weigel D, Alvarez J, Smyth DR, Yanofsky MF, et al. (1992). *LEAFY* controls floral meristem identity in *Arabidopsis*. *Cell* 69: 843-859.

Yamaguchi N, Yamaguchi A, Abe M, Wagner D, et al. (2012). *LEAFY* controls *Arabidopsis* pedicel length and orientation by affecting adaxial-abaxial cell fate. *Plant J.* 69: 844-856.

Yu L, Wu X, Tang X and Yan B (2010). Simple and efficient method for isolating cDNA fragments of *lea3* genes with potential for wide application in the grasses (Poaceae). *Genet. Mol. Res.* 9: 1321-1325.

Yu Q, Moore PH, Albert HH, Roader AH, et al. (2005). Cloning and characterization of a *FLORICAULA/LEAFY* ortholog, *PFL*, in polygamous papaya. *Cell Res.* 15: 576-584.

 ©FUNPEC-RP www.funpecrp.com.br