Modification and co-option of leaf developmental programs for the acquisition of flat structures in monocots: unifacial leaves in Juncus and cladodes in Asparagus

Hokuto Nakayama1, Takahiro Yamaguchi2 and Hirotaka Tsukaya2*

1 Department of Bioresource and Environmental Sciences, Faculty of Life Sciences, Kyoto Sangyo University, Kyoto, Japan
2 Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan

INTRODUCTION

Plants display remarkable morphological diversities (Bell, 2008), which are largely a result of morphological variations of the shoot consisting of leaves, internodes, and axillary buds (flowers are considered as reproductive shoots consisting of modified leaves; von Goethe, 1790; Tsukaya, 1995; Honma and Goto, 2001). Recent studies have revealed the mechanisms underlying the morphological diversification of shoots (Guo et al., 2008). This repression is partially mediated by a protein complex containing ASYMMETRIC LEAVES1/RIGHT(HAND)SHEATH2/PHANTASTICA-KNOX (ARP-KNOX) module. After the initiation of leaf primordia marked by the repression of KNOX1, an ad–ab polarity is established within leaf primordia. Cell fates in the root meristem are determined mainly by a positional cue rather than cell lineage (van den Berg et al., 1999; Scheres, 2001), and the positional cue plays an important role in the establishment of ad–ab polarity in leaf primordia (Warldaw, June 2013| Volume 4| Article 248 | www.frontiersin.org

Based on these results, we discuss the importance of modification and co-option of GRN for the acquisition of flat structures in monocots.

BASIC GRNs OF LEAF ORGANOGENESIS REGARDING LEAF INITIATION AND ESTABLISHMENT OF DORSIVENTRALITY

The mechanisms underlying the morphological diversity of leaves remain poorly understood, whereas the basic mechanisms of leaf development are beginning to be characterized (Tsukaya, 2006; Koenig and Sinha, 2010; Moon and Hake, 2010; Horiguchi and Tsukaya, 2011; Tsukaya, 2013). Since leaf primordia develop from a group of undifferentiated cells on the flank of the SAM, initiation of leaf primordia requires a transition of cell fate from pluripotent to determinate. In Arabidopsis, KNOX1 genes are known to be the transcription factors that maintain meristem activity in shoots (Long et al., 1996). Therefore, the expression of KNOX1 genes is down-regulated at initiation sites of leaf primordia within the SAM (Long et al., 1996; Guo et al., 2008). This repression is partially mediated by a protein complex containing ASYMMETRIC LEAVES 1 (AS1), a MYB domain transcription factor, and ASYMMETRIC LEAVES 2 (AS2), a LRR domain protein (Su et al., 2003; Ueno et al., 2007), and this interaction is known as the ASYMMETRIC LEAVES 1/ROUGHSHOOT2/PHANTASTICA-KNOX (ARP-KNOX) module.

Keywords: Asparagus, cladode, evolution, gene regulatory networks, Juncus, leaf, leaf-like organ, unifacial leaf
known that the establishment of the middle domain marked
mordia (W aites and Hudson, 1995). During outgrowth, it is
juxtaposition between the adaxial and abaxial sides of leaf pri-
and adaxial cell fate, respectively. According to the established
Therefore, miR165/166 and tasiR-ARF also specify the abaxial
cursor transcripts, represses the expression of
hand, tasiR-ARF, which is derived from non-coding TAS3 pre-
leaf primordia (McConnell and Barton, 1998; McConnell et al.,
ial fate ( Eshed et al., 2001; Kerstetter et al., 2001; Pekker et al.,
mutants of
phb-1d
, which are negatively regulated by
KAN
, as a complex with AS2 (Iwakawa et al.,
Further, ARF4
), as a complex with AS2 (Iwakawa et al.,
HOMEODOMAIN-LEUCINE ZIPPER
(HD-ZIP III) transcription factors, PHAULOSA (PHB), REV-
OLUTA (REV), and PHAULOSA (PHV), specify adaxial fate in
leaf primordia (McConnell and Barton, 1998; McConnell et al.,
Similarly, ARF3 is expressed in the abaxial domain of leaf prim-
PD
, a member
HD-ZIP III
 photographed, whereas an
expression analysis was performed to confirm ad–ab polarity of
leaf blades of
J. prismatocarpus
). As a result, a HD-ZIP III homolog
It was weakly expressed in cylindrical unifacial leaves of
KAN
), which are negatively regulated by
KAN
, as a complex with AS2 (Iwakawa et al.,
2003). On the other hand, tasiR-ARF, which is derived from non-coding TAS3 pre-
cleaves mRNA of
ARF3
, ARF4
, and
KANADI
 (KAN) specify abaxi-
imal fate (Eshed et al., 2001; Kerstetter et al., 2001; Pekker et al.,
Additionally, two small RNAs play an important role in establishment of ad–ab polarity. miR166
strands, whereas an ARF3 homolog was expressed throughout the
entire outer region of the leaf blade (Figure 1D). These results
demonstrated that the leaf blade of unifacial leaves was abaxialized,
as suggested by morphological observations. Additionally, the
results suggested that modification of the GRN involved in estab-
lishment of ad–ab polarity in leaf may have resulted in abaxialized
leaves in the genus
Juncus
. Thus, unifacial leaves can be used as a model
to investigate the evolution of leaf morphology, focusing on mod-
ification of ad–ab polarity (Kaplan, 1975; Rudall and Bugallo, 2002;
Yamaguchi and Tsukaya, 2010).

Recently, Yamaguchi et al. (2010) explored the molecular mech-
anism underlying the development and evolution of unifacial
leaves in the genus
Juncus
. Juncus primaticarpus has flat unifac-
leaves (Figures 1B,C). In addition to morphological analysis,
expression analysis was performed to confirm ad–ab polarity of
leaf blades of
J. prismatocarpus
. As a result, a HD-ZIP III homolog
was expressed in the presumptive region of xylem in procambial
strands, whereas an ARF3 homolog was expressed throughout the
entire outer region of the leaf blade (Figure 1D). These results
demonstrated that the leaf blade of unifacial leaves was abaxialized,
as suggested by morphological observations. Additionally, the
results suggested that modification of the GRN involved in estab-
lishment of ad–ab polarity in leaf may have resulted in abaxialized
leaves in the genus
Juncus
. To our knowledge, this was the first case
in which abaxialized leaves were confirmed by gene expressions
of both adaxial and abaxial marker genes, excluding mutants of
different species.

Moreover, the study using
Juncus
 identified an alternative
mechanism of leaf blade flattening without establishing ad–ab
polarity by analyzing hybrids of a pair of closely related species;
namely,
J. primaticarpus
 (with flattened unifacial leaves) and
J. wallichianus
 (with cylindrical unifacial leaves). The results demon-
strated that an ortholog of
DROOPING LEAF
 (DL), a member of
CRABS CLAW/DF subfamily of
KAN
 genes, promotes
flattening of blades of unifacial leaves in the genus
Juncus
. (Yam-
aguchi et al., 2010; Figure 1E). The DL ortholog was strongly
expressed in flattened unifacial leaves of
J. primaticarpus
, whereas
it was weakly expressed in cylindrical unifacial leaves of
J. wal-
lichianus
. Genetic analysis using interspecific hybrids between
the two species revealed that the DL locus from
J. primaticarpus

flattens the unifacial leaf blade (Yamaguchi et al., 2010). An
important function of
KAN
 genes is thought to be the promo-
tion of directional cell proliferation in the lamina (Nakayama et al.,
2010; Yamaguchi et al., 2012), in addition to the induction of leaf
lamina-specific genetic programs and shut-down SAM programs
Saromam et al., 2010). DL in
Oryza sativa
 thickens the midrib by
promoting cell proliferation toward the shoot apex (Yamaguchi et al.,
2004). Similarly, the DL ortholog in unifacial leaves flattens
FIGURE 1 | Unifacial leaves in the genus *Juncus*. (A) Schematic diagrams of leaf polarities in bifacial and unifacial leaves. (B) Seedling. (C) Transverse section of the leaf blade. (D,E) Expression patterns of orthologs in leaf primordia: HD-ZIP III and ARF3 (D) and DL ortholog (E). (F) Model of acquisition and blade flattening of unifacial leaves in the genus *Juncus* (B–F). *J. prismatocarpus* in (C–F), top of the image is the adaxial side. Bars = 1 cm in (B) and 200 μm in (C).

CO-OPTION OF GRN FOR LEAF DEVELOPMENT: CLADOSES

Foliage leaves in the genus *Asparagus* are reduced in size. Instead, *Asparagus* has unusual organs called cladodes in the axils of scale leaves (Figure 2A). Interestingly, the morphology of cladodes is leaf-like, although it develops in an axil where a lateral shoot generally arises (Figures 2A,B). Therefore, cladodes have received much attention from the morphological and evolutionary standpoints. However, the uniqueness of cladodes makes it difficult to understand their origin. Arber (1924) concluded that cladodes

the abaxialized leaf blades by promoting cell proliferation toward the shoot apex. This flattening by the DL ortholog may allow the leaf blades to stand straight to make the efficiency of light capture to be better.

These results suggested that modification of the GRN involved in the establishment of ad–ab polarity may have resulted in the evolution of unifacial leaves, and co-option of the GRN involved in directional cell proliferation may have resulted in the evolution of flattened unifacial leaves (Yamaguchi et al., 2010; Figure 1F).
are prophylls of abortive lateral shoots in the axis based on their leaf-like morphology and other anatomical features. Meanwhile, Cooney-Sovetts and Sattler (1986) and Kubitzki and Rudall (1998) concluded that cladodes are modified lateral branches based on their axillary position. Additionally, it is known that the morphology of cladodes in the genus is diverse (Kubitzki and Rudall, 1998; Fukuda et al., 2005). Recent molecular phylogenetic studies indicated that cladodes have evolved from a leaf-like (flattened) to a rod-like (cylindrical) form (Fukuda et al., 2005; Kubota et al., 2012). Currently, the positions of the xylem and the phloem were inverted in cladodes compared to foliage leaves, suggesting that the cladodes differ from leaves (Nakayama et al., 2012a).

To further characterize the developmental processes of cladodes, expression analyses of orthologs of gene involved in leaf and/or shoot development were performed. The results showed that an ortholog of the KNOX1 gene was expressed in the cladode primordia. However, its expression was limited to the peripheral region of primordia and ultimately decreased. Indeed, an ortholog of AS1 was also expressed in the cladode primordia. Moreover, orthologous genes involved in the establishment of ad-ab polarity, such as PHB, REV, and miR166, were expressed in a leaf-like manner (Nakayama et al., 2012a). Thus, cladodes are modified axillary shoots that have evolved by co-option of the GRN and establish ad-ab polarity in the axillary shoots, as well as confer the leaf-like planar form (Nakayama et al., 2012a,b).
Asparagus will increase our understanding of acquisition processes. Molecular phylogenetic studies have suggested that the genus Asparagus has undergone rapid radiation in arid regions (Fukuda et al., 2005). The reduced surface area is thought to limit evaportranspiration and reduce water loss. Therefore, the morphological alteration from leaf-like to cylindrical forms likely contributed to the adaptation to arid and semiarid regions.

Overall, it was proposed that cladodes are modified axillary shoots, which have evolved by co-opting the GRN of leaf development. Subsequent alteration of the co-opted GRN has led to the cylindrical form of cladodes in the genus Asparagus (Nakayama et al., 2012a,b, Figure 2D). Therefore, the GRN of leaf development is likely required for the acquisition of joint-like organs in the case of cladodes in the genus Asparagus.

**Modification and Co-option of the Leaf GRN in Plant Morphological Diversity**

In unifacial leaves and cladodes, modifications and co-option of existing GRNs have led to the flat form of cylindrical leaves and lateral shoots. However, the flattening processes differ between the two cases. In Asparagus cladodes, the GRN involved in leaf development is thought to be co-opted. Therefore, it seems likely that the regulation of flattening and subsequent lamina outgrowth in cladodes is similar to that in leaves: ad–ab polarity dependent lamina outgrowth. This was supported by analysis of cylindrical cladodes in A. officinalis (Nakayama et al., 2012a).

On the other hand, in unifacial leaves of Juncus, flattening and subsequent lamina outgrowth are regulated by a distinct mechanism. For flattening, the DL ortholog promotes cell proliferation of abaxialized leaf primordia toward the SAM. Therefore, unlike bifacial leaves, flattening of primordia of unifacial leaves is independent of leaf ad–ab polarity. Additionally, the DL and DES expression patterns demonstrated that subsequent marginal outgrowth is also independent of ad–ab polarity and occurs along newly established central–marginal axes. Analysis of radial leaf mutants in J. prismatocarpus indicated that the rearrangement is induced by initial leaf flattening and is important to marginal outgrowth of unifacial leaves in Juncus (Yamaguchi et al., 2010). These suggested the existence of multiple mechanisms of flattening originally cylindrical structures.

Recently, Katayama et al. (2010) reported that flattened roots in Podostemaceae, which is a family of aquatic angiosperms, have evolved by co-option of genes involved in shoot development. Therefore, the mechanism of root flattening also should be explored for further understanding of acquisition processes of planar forms. In nature, many plants show flattened structures such as phylloclades, winged stems, and pericentral stems. Additionally, many of these structures, unifacial leaves, and cladodes have evolved repeatedly during plant evolution. Therefore, studies of Juncus and Asparagus will increase our understanding of acquisition processes of these flattened structures.

Knowledge gained from evo-devo studies of Juncus unifacial leaves and Asparagus cladodes has an interesting similarity: modifications of GRNs have led to abaxialization of each organ. Such abaxialization has been observed in diversified plant forms (e.g., Kim et al., 2003, Toriba et al., 2010). Therefore, abaxialization may be a common way in which novel structures and morphology arise, regardless of organ type. As opposed to abaxialization, adaxialization has not been reported to result in morphological diversification, although it leads to cylindrical structures (McConnell et al., 2001). One possible reason is the original identity of the leaf primordia. It is known that establishment of ad–ab polarity in leaf primordia requires an adaxializing signal from the SAM, which may be mediated by GABA shunt metabolites (Sussex, 1981; Toyokura et al., 2011). Microsurgical experiments that separate a primordium from a meristem tip generate an abaxialized leaf, indicating that the original identity of leaf primordia is abaxial. Thus, it may be difficult to convert the identity of the whole leaf primordia into adaxial identity. Alternatively, some sort of morphological or physiological differences between adaxial and abaxial sides may be subjected to evolutionary constraints. In fact, it is known that the abaxialization was easily caused by a simple mutation in miR165/166 target sites of HD-ZIP genes (McConnell et al., 2001; Bhoades et al., 2002). Nevertheless, the adaxialized organs or forms have not been seen in nature. Therefore, the latter hypothesis may be worth considering. In any case, further analyses will reveal whether abaxialization is a general trend during evolution of plants and constraints of adaxialization and abaxialization.

In this review, we focused on the relation between morphological diversification and alteration of GRNs for leaf development taking Juncus and Asparagus as examples. These instances indicate that modification and co-option of existing GRNs are an effective way to give rise to novel forms and organs. Additionally, these instances shed light on the importance of abaxialization in morphological diversity. However, many questions in alteration processes of GRNs remain unanswered. One is how specific part of newly co-opted GRN is modified without any perturbation of other developmental processes. The other is why specific GRNs are co-opted repeatedly in various contexts of development during evolution. In addition to revealing function of individual genes, elucidation of these questions about evolution of GRN will allow us to better understand how novel forms or organs arise in nature.

**Acknowledgments**

The authors thank all members of Tsukaya laboratory for discussion. This work was partially supported by the following sources: Grants-in-Aid for Scientific Research (A) to Hirokazu Tsukaya and for Creative Scientific Research to Hirokazu Tsukaya (18680031) from the Japan Society for the Promotion of Science, and for Scientific Research on Priority Areas to Hirokazu Tsukaya (19060002), an grant for Young Scientists (B) to Takahiro Yamaguchi (19770041) from the Ministry of Education, Culture, Sports, Science and Technology, Japan; a grant from the Mitsubishi Foundation to Hirokazu Tsukaya; the Sumitomo Foundation to Takahiro Yamaguchi; and a Research Fellowship from the Japan Society for the Promotion of Science to Hukuo Nakayama.
REFERENCES

Arber, A. (1924). Myriophyllum and Arabidopsis. Jens Bry 38, 635–678. doi: 10.1111/j.1359-0916.1924.tb00153.x

Bell, A. D. (2008). Plant Form: An Illustrated Guide to Flowering Plant Morphology. Portland: Timber Press. doi: 10.4161/psb.20913

Chenwood, D. H., Guo, M., Nogueira, F. T., and Timmermans, M. C. (2007). Establishing leaf polarity: the role of small RNA signals in the shoot apex. Development 134, 813–25. doi: 10.1242/dev.01053.x

Cosma, R. S., and Salter, R. (1988). Phyllode development in the Asparagaceae: an example of homostasis. Bot. J. Linn. Soc. 94, 327–371. doi: 10.1111/j.1095-8339.1988.tb01053.x

Nakayama, H., Yamaguchi, T., and Tsukaya, H. (2012a). Clades. leaf-like organs in the genus Arabidopsis. Plant Cell 24, 929–941. doi: 10.4159/tpc.2011.3478

Nakayama, H., Yamaguchi, T., and Tsukaya, H. (2012b). Clades. leaf-like organs in Arabidopsis. show the significance of co-option of pre-existing genetic regulatory circuit for morphological diversity of plants. Plant Signal Behav. 7, 965–966. doi: 10.4161/psb.20913

Nakayama, H., Yamaguchi, T., and Tsukaya, H. (2012c). Clades. leaf-like organs in Arabidopsis. show the significance of co-option of pre-existing genetic regulatory circuit for morphological diversity of plants. Plant Signal Behav. 7, 965–966. doi: 10.4161/psb.20913
Nakayama et al. Modification of leaf developmental programs

T o yokura, K., W atanabe, K., Oiwaka, A., Kusano, M., T ameshige, T ., T atematsu, K., et al. (2011). Succinic semi-aldehyde dehydrogenase is involved in the robust patterning of Arabidopsis leaves along the adaxial–abaxial axis. Plant Cell Physiol. 52, 1340–1353. doi: 10.1093/pcp/pcr079

T ukaya, H. (1995). “The genetic control of morphogenesis in Arabidopsis and its relevance to the development of biodiversity,” in Biodiversity and Evolution, eds R. Arai, M. Kato, and Y . Doi (Tokyo: The National Science Museum Foundation), 253–265. doi: 10.1093/pcp/pcf051

T ukaya, H. (2006). Mechanism of leaf-shape determination. Annu. Rev. Plant Biol. 57, 477–496. doi: 10.1146/annurev.arplant.57.032905.105320

T ukaya, H. (2013). “Leaf development,” in The Arabidopsis Book, ed. K. Torii (Rockville: American Society of Plant Biologists). doi: 10.1199/tab.0163 (published online e0163).

Ueno, Y ., Ishikawa, T ., W atanabe, K., T erakura, S., Iwakawa, H., Okada, K., et al. (2007). Histone deacetylases and ASYMMETRIC LEAVES2 are involved in the establishment of polarity in leaves of Arabidopsis. Plant Cell 19, 440–457. doi: 10.1105/tpc.019044

X u, L., Xu, Y ., Dong, A., Sun, Y ., Pi, L., Xu, Y ., et al. (2005). Novel and auxin defects in leaf adaxial–abaxial polarity reveal the requirement for ASYM1/2 and ERECTA functions in specifying leaf adaxial identity. Development 130, 4097–4107. doi: 10.1105/tpc.019044

Y amaguchi, T ., Nukazuka, A., and T ukaya, H. (2010). Evolutionary and developmental studies of unifacial leaves in monocots: Juncus as a model system. J. Plant Res. 123, 35–41. doi: 10.1105/tpc.110.076927

Y amaguchi, T ., Y ano, S., and T ukaya, H. (2010). Genetic framework for flattened leaf blade formation in unifacial leaves of Juncus prismatocarpus. Plant Cell 22, 2141–2155. doi: 10.1105/tpc.110.076927

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 April 2013; accepted: 19 June 2013; published online: 09 July 2013.

Citation: Nakayama, H., Y amaguchi, T ., and T ukaya, H. (2013). Modification and co-option of leaf developmental programs for the acquisition of flat structures in monocots: artificial leaves in Juncus and cladodes in Asparagus. Front. Plant Sci. 4:248. doi: 10.3389/fpls.2013.00248

This article was submitted to Frontiers in Plant Evolution and Development, a specialty of Frontiers in Plant Science. Copyright © 2013 Nakayama, Yamaguchi and T ukaya. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and any copyright notices concerning any third-party graphics etc.