Molecular Plant • Volume 3 • Number 5 • Pages 807–817 • September 2010 RESEARCH ARTICLE

Overexpression of *ACL1* (*abaxially curled leaf 1*) Increased Bulliform Cells and Induced Abaxial Curling of Leaf Blades in Rice

Ling Li[a,b], Zhen-Ying Shi[a], Lin Li[a], Ge-Zhi Shen[c], Xin-Qi Wang[c], Lin-Sheng An[a] and **Jing-Liu Zhang[a,1]**

a National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, China
b Graduate School of the Chinese Academy of Sciences, 19 Yuquan Road, Beijing 100039, China
c The Plant Breeding and Cultivation Research Institute, Shanghai Academy of Agriculture Sciences, 2900 Beidi Road, Shanghai 201106, China

---

ABSTRACT **Understanding the genetic mechanism underlying rice leaf-shape development is crucial for optimizing rice configuration and achieving high yields; however, little is known about leaf abaxial curling. We isolated a rice transferred DNA (T-DNA) insertion mutant, *BY240*, which exhibited an abaxial leaf curling phenotype that co-segregated with the inserted T-DNA. The T-DNA was inserted in the promoter of a novel gene, *ACL1* (*Abaxially Curled Leaf 1*), and led to overexpression of this gene in *BY240*. Overexpression of *ACL1* in wild-type rice also resulted in abaxial leaf curling. *ACL1* encodes a protein of 116 amino acids with no known conserved functional domains. Overexpression of *ACL2*, the only homolog of *ACL1* in rice, also induced abaxial leaf curling. RT–PCR analysis revealed high expressions of *ACLs* in leaf sheaths and leaf blades, suggesting a role for these genes in leaf development. *In situ* hybridization revealed non-tissue-specific expression of the *ACLs* in the shoot apical meristem, leaf primordium, and young leaf. Histological analysis showed increased number and exaggeration of bulliform cells and expansion of epidermal cells in the leaves of *BY240*, which caused developmental discoordination of the abaxial and adaxial sides, resulting in abaxially curled leaves. These results revealed an important mechanism in rice leaf development and provided the genetic basis for agricultural improvement.**

Key words: **Cell expansion; leaf/vegetative development; rice; *ACL1*; *ACL2*; LRI; T-DNA; bulliform cells.**

INTRODUCTION

The morphological traits of leaves, such as size and shape, are important components of plant architecture that strongly affect yield performance (Chen et al., 2001; Yuan, 1997). By affecting light interception, leaf temperature, and water loss, leaf shape plays a crucial role in photosynthesis and plant development (O'Toole and Cruz, 1980). Leaf morphology, among other yield-related components, is a highly complex trait that is not yet fully understood. As a result of recent developments in genetic methodology, several mutants with the curling-leaf traits have been identified (Kessler and Sinha, 2004; Luo et al., 2007; Tsukaya, 2006; Kee et al., 2009). Studies on these mutants have revealed several pathways that contribute to leaf curling, such as miRNA and *HD-ZIPIIIs*-mediated leaf polarity establishment and hormone biosynthesis or signaling activation (Byrne, 2005; Hake et al., 2004). It is reported that adaxial leaf curling could increase stomatal resistance, reduce water loss, and cause erection of the leaf blade (King and Vincent, 1996; Moulia, 2000; Sakamoto et al., 2006). To date, several genes related to adaxial leaf curling in rice and maize have been identified (Juarez et al., 2004; Shi et al., 2007; Yan et al., 2008; Zhang et al., 2009). Among these genes, mutation of *NARROW LEAF 7* results in smaller bulliform cells and thereby induces adaxial leaf curling (Fujino et al., 2008). *Rld1* mutant in maize is characterized by polarity character conversion, in which patches of ligule, macrohairs, and bulliform cells are found on the abaxial side of the leaf (Candela et al., 2008; Nelson et al., 2002). In the loss-of-function mutant of *SHALLOT-LIKE1* (*SLL1*), a member of the *KANADI* family, sclerenchymatous cells and bulliform cells are formed on the abaxial side

---

[1] To whom correspondence should be addressed. E-mail jlzhang@sippe.ac.cn, fax +86-021-54924015, tel. +86-021-54924079.

doi: 10.1093/mp/ssq022, Advance Access publication 21 May 2010
Received 13 November 2009; accepted 19 April 2010

of the leaf, which results in adaxial leaf curling (Zhang et al., 2009).

In monocotyledons such as rice, single leaf development from the founder cells requires the integration of several developmental events, including differentiation of basic leaf structures, development of various affiliated structures such as leaf ligules, and elongation of leaf blade and leaf sheath (Itoh et al., 2005). Very young rice leaf curls inherently when it is still enwrapped in the sheath, via a largely unknown mechanism. In contrast, mature leaves usually curl adaxially under conditions of water deficiency—a phenomenon that might be related to the bulliform cells, which are a special kind of bulky cells on the adaxial epidermis of the leaf (Botwright et al., 2005; Fujino et al., 2008). It is proposed that under water stress, bulliform cells lose turgor, which results in leaf rolling; when water stress is relieved, bulliform cells absorb water and swell up again (Price et al., 1997). However, the actual role of bulliform cells in leaf curling under conditions of water deficiency is controversial (Price et al., 1997). Some studies suggest that they participate in the expansion of very young leaves, which still remain rolled up in the leaf sheath (Alvarez et al., 2008).

Until now, few genes controlling abaxial leaf curling have been reported. In this study, we isolated a rice mutant with abaxially curled leaves, *BY240*, from our T-DNA insertion population (Wang et al., 2004) and showed that the abaxial curling phenotype co-segregated with the inserted T-DNA. A novel gene, *ACL1* (A̲baxially C̲urling L̲eaf 1), was identified to be overexpressed in *BY240*. Artificial overexpression of *ACL1* also caused abaxial leaf curling. Furthermore, we cloned *ACL2*, the only homolog of *ACL1* in rice, and found that *ACL2* could also induce abaxial leaf curling when overexpressed. Histological analysis revealed that the number of bulliform cells in the *ACL1* or *ACL2* overexpressing lines was increased. Furthermore, *ACL1* and *ACL2* were highly expressed in the leaf sheath and leaf blade, and their expression levels increased as the leaves developed. These data suggested that *ACL1* and *ACL2* played important roles in leaf shape configuration in rice.

RESULTS

BY240 Developed Abaxially Curled Leaf Blades

To identify genes essential for leaf development in rice, we screened our T-DNA insertion population (Wang et al., 2004) and isolated a mutant, *BY240*, with abaxially curled leaves. In wild-type ZH11, the mature leaves were usually upright at the vegetative and early reproductive stage, and gradually flattened at later reproductive stages. *BY240* seedlings exhibited abaxially curled leaves at three-leaf-stage; the leaves showed more obvious abaxial curling along with the growth process. At later reproductive stages, the leaves curled so seriously (Figure 1A) that LRIs (L̲eaf-r̲olling I̲ndex) of all the first three leaves from the top reached 100 (Figure 1B). The leaf length and width of the top three leaves in *BY240* were not different from that of the wild-type. No difference in heading date and tiller number was found, either, although the total plant height of *BY240* was slightly lower than that of the wild-type (Table 1).

The Inserted T-DNA Co-Segregated with the Abaxial Curling Leaf Trait in *BY240*

Genetic analysis was carried out to investigate whether the abaxial leaf curling character was caused by the inserted T-DNA in *BY240*. Hybrids between homozygous *BY240* and the wild-type ZH11 were generated, including an original cross and a reciprocal cross. Leaves of the F1 hybrids showed abaxial curling.

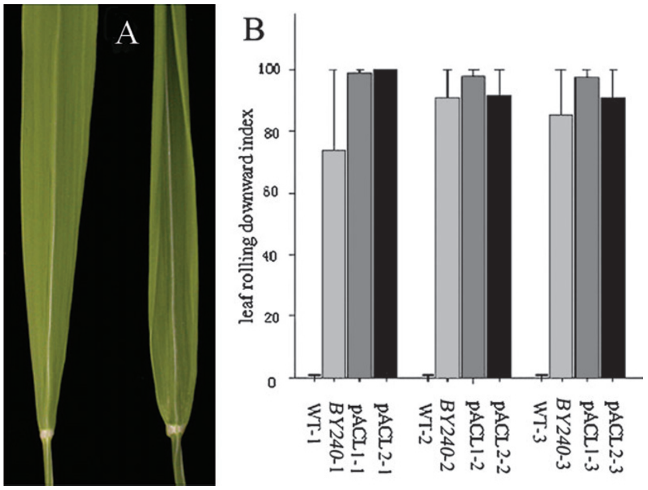

Figure 1. Characteristics of Wild-Type ZH11 and Mutant *BY240*.

(A) Flag leaf from *BY240* (right) and ZH11 (left). The photo was taken from the abaxial side of the leaf.

(B) LRIs of ZH11 ('WT' in the Figure), mutant *BY240*, and pACL1 and pACL2 transgenic plants. LRIs for each of the top three leaves (1, flag leaf; 2, the second leaf from top; and 3, the third leaf from top) at 20 DAFs were shown.

Table 1. Morphological Characteristics of Wild-Type ZH11 and Mutant *BY240*.

Traits	ZH11	*BY240*
Plant height (cm)	86.7 ± 6.0	73.7 ± 8.0
Heading date*	71.8 ± 3	72.1 ± 2
Panicle length (cm)	19.2 ± 1.5	20.2 ± 1.2
Tillering number	8.8 ± 2	9.2 ± 3
Flag leaf length (cm)	33.3 ± 2.4	33.0 ± 2.9
Second leaf from top length (cm)	45.8 ± 3.1	45.0 ± 2.7
Third leaf from top length (cm)	50.4 ± 2.6	51.1 ± 2.4
Flag leaf width (cm)	1.5 ± 0.1	1.5 ± 0.1
Second leaf from top width (cm)	1.2 ± 0.1	1.2 ± 0.1
Third leaf from top width (cm)	1.0 ± 0.1	1.0 ± 0.1

* Heading date was measured in the greenhouse (LD, light:dark = 14:10) and other traits in the field (Hainan, China, 2006, winter). The values were shown as mean ± SE (*n* = 20).