The Inflorescence Stem Fibers of Arabidopsis thaliana Revoluta (ifl1) Mutant

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ABSTRACT

Arabidopsis thaliana is gradually gaining significance as a model for wood and fiber formation. revolute/ifl1 is an important mutant in this respect. To better characterize the fiber system of the revolute/ifl1 mutant, we grew plants of two alleles (rev-9 in Israel and rev-1 in the USA) and examined the fiber system of the inflorescence stems using both brightfield and polarized light. Microscopic examination of sections of plants belonging to the two different alleles clearly revealed that, contrary to previous views, in 18 (13 in Israel and 5 in Ohio) out of 30 stems (20 in Israel and 10 in Ohio) the mutant produced the primary wavy fiber system of the inflorescence stems. Our findings are further supported by the fact that fibers are seen in the figures published in other studies of the mutant even when it was stated that there were no fibers. The impression of a total lack of the wavy band of fibers is in many cases just a result of poorly lignified secondary walls. This specific gene that reduces lignification in fibers is of great significance for biotechnological developments for the paper industry and thus for the global economy and ecology. We propose that revoluta, the first name given to this mutant (Talbert and others 1995), is more appropriate than ifl1.

Key words: Arabidopsis thaliana; fibers; INTERFASCICULAR FIBERLESS1 (IFL1); Inflorescence stems; Lignification; REVOLUTA

INTRODUCTION

The developing ability and increasing tendency to use Arabidopsis thaliana as a model for wood and fiber formation (Dolan and others 1993; Lev-Yadun 1994, 1997; Dolan and Roberts 1995; Zhong and others 1997, 2001; Zhong and Ye 1999, 2001; Zhao and others 2000; Beers and Zhao 2001; Burk and others 2001; Lev-Yadun and Flaishman 2001; Altamura and others 2001; Chaffey 2002; Chaffey and others 2002; Funk and others 2002; Ye 2002; Ye and others 2002; Little and others 2002; Flaishman and others 2003; Kirst and others 2003; Oh and others 2003; Ko and others 2004; Nieminen and others 2003) offer prospects for great advances in understanding the biology of the formation of these tissues, which are not easy to study in trees. Genes that reduce lignification in fibers are of great sig-
nificance for biotechnological developments in the paper industry and thus for the global economy and ecology. One of the promising mutants in this respect, inducing alterations in fiber differentiation, is the *A. thaliana* mutant of the gene *REVOLUTA* (*REV*) (Talbert and others 1995), which was studied and cloned in parallel as a different gene *INTERFASCICULAR FIBERLESS 1* (*IFL1*) (Zhong and others 1997, 2001; Ratcliffe and others 2000; Zhong and Ye 1999, 2001, 2004). However, these genes were found to be identical (Ratcliffe and others 2000). The gene encodes a class III homeodomain-leucine zipper protein (HD-ZIP) and is involved in the regulation of interfascicular fiber differentiation in inflorescence stems of *A. thaliana* (Zhong and Ye 1999; Otsuga and others 2001), as well as in regulating other apical meristem functions (Meyerowitz 1997; McConnell and others 2001; Emery and others 2003; Greb and others 2003; Mattsson and others 2003). The *REVIFL1* gene was found to be one of the putative target sequences of microRNA 165 that cleave the wild-type *REVIFL1* mRNA (Floyd and Bowman 2004; Zhong and Ye 2004). The name *INTERFASCICULAR FIBERLESS 1* implies that interfascicular fibers do not form. This is not merely a semantic issue but a substantial developmental matter. Ye (2002) also stated that the *ifl1* mutation leads to a block of vascular cambium activity at the basal parts of inflorescence stems of *A. thaliana*, a description based on the assumption that the interfascicular fibers are part of the secondary xylem formed by the cambium. This blockage was shown to be associated with the reduced expression of the auxin efflux carriers PIN3 and PIN4 (Zhong and Ye 2001; Ye 2002). In Figure 5J, Talbert and others (1995) show, however, a cambium formed in both interfascicular and fascicular zones, probably a regular case of variability concerning expression of cambial development in the *revoluta* mutant. Such variability seems to be common in various genotypes of *A. thaliana* and is probably influenced by growth conditions (M.A. Flaishman and S. Lev-Yadun unpublished).

The examination of the published histological figures of the mutant (Talbert and others 1995; Zhong and others 1997, 2001; Zhong and Ye 1999, 2001) raised the possibility that fibers are formed in the inflorescence stems and that the mutation mainly alters the secondary cell-wall composition rather than causing its absence. To clear this issue, we grew plants of the mutant and characterized the interfascicular fiber band of their inflorescence stems.

**Materials and Methods**

**Plant Materials and Growth Conditions**

Seeds of the mutant *revoluta* of *Arabidopsis thaliana* were obtained from the *Arabidopsis* Biological Resource Center (Ohio State University). In Israel, the *rev-9* allele was used and they were germinated in a growth chamber under short day conditions (9 h light/15 h dark, 22 ± 1 °C, 45 μS−1m−2). Two sets of single, four-week-old rosettes were transferred to 0.5 liter pots filled with a mixture of peat/tuff/perlite (40%/40%/20% v/v) fertilized once a week with Osmocot (NPK), irrigated twice a week and grown in a greenhouse. In Ohio, the *rev-1* allele was used and they were germinated in a growth room under short day conditions (9 h light/15 h dark, 21-22 °C, 75 μS−1m−2). After two weeks, 10 rosettes were moved to long day conditions (9 h light/15 h dark with the same illumination) and were grown until they flowered. Control wild-type plants were grown under the same conditions.

**Sampling and Histological Examination**

In the plants grown in Israel, in one group of eight plants, the inflorescence stems were sampled when they were flowering. In two other groups of plants, one of seven and one of five, the plants flowered and had green siliques when sampled. In five of the plants we also sampled the main root to determine whether it performs secondary growth. A segment about 1 cm long was cut off the lower part of 20 inflorescence stems or the upper part of the main root and fixed in a mixture of 3:1 ethanol and glacial acetic acid overnight at room temperature. Control wild-type plants were also sampled in the lower part of 10 inflorescence stems. After fixation, samples were washed three times for 15 min each in PBS (pH 7.2), dehydrated in a series of ethanol solutions (25, 50, 75, 96, and 100%), and embedded in paraffin. Serial cross-sections, 10 μm thick, were prepared from each stem segment with a rotary microtome (American Optical model 820, Spencer) from the whole width of each stem segment, stained with Safranin and Fast Green and mounted with Permount (Fisher Scientific, Cat. No. SP15-100). Slides were examined under brightfield and polarized light with a Leitz Dialux 20 microscope equipped with a Nikon F3 camera, at magnifications of X40 to X160.

In the plants grown in Ohio, ten inflorescence stems were sampled 1 cm above the soil line. The stem segments were embedded in 5% agar, and sections 100 μm thick were made using a vibratome 1500