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RESEARCH ARTICLE

Phenotypical and structural characterization of the *Arabidopsis* mutant involved in shoot apical meristem

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Abstract An *Arabidopsis* mutant induced by T-DNA insertion was studied with respect to its phenotype, microstructure of shoot apical meristem (SAM) and histochemical localization of the GUS gene in comparison with the wild type. Phenotypical observation found that the mutant exhibited a dwarf phenotype with smaller organs (such as smaller leaves, shorter petioles), and slower development and flowering time compared to the wild type. Optical microscopic analysis of the mutant showed that it had a smaller and more flattened SAM, with reduced cell layers and a shortened distance between two leaf primordia compared with the wild type. In addition, analysis of the histo-chemical localization of the GUS gene revealed that it was specifically expressed in the SAM and the vascular tissue of the mutant, which suggests that the gene trapped by T-DNA may function in the SAM, and T-DNA insertion could influence the functional activity of the related gene in the mutant, leading to alterations in the SAM and a series of phenotypes in the mutant.

Keywords *Arabidopsis*, shoot apical meristem, mutant, GUS, T-DNA

1 Introduction

Shoot apical meristem (SAM) is the source that generates all above-ground organs of higher plants after their embryonic development, and continuously produces above-ground organs including stems, leaves, flowers, and so on, through the delicate balance between proliferation and differentiation of undifferentiated stem cells (Sussex, 1989; Doerner, 1999), which is vital to the growth and development of plants. SAM in most angiosperms consists of different cellular layers as well as different zones, which in turn execute different functions.

Studies on SAM development can not only reveal its molecular mechanism, but can also provide the basis for clarifying the developmental mechanism of above-ground organs, such as stems, leaves, and flowers. At present, in our knowledge, studies on the isolation and analysis of SAM mutants, genetic control of SAM development and its interaction with the development of the whole plant have not been reported in China. Only studies on the molecular mechanism of floral meristem in wheat and rice (Chong et al., 2000; Yong et al., 2000; Duan and Guo, 2004), and the effects of plant hormones on meristem development have been documented (Chen et al., 2005; Gong and Li, 2005). Recently, more than 600 promoter trap lines from the *Arabidopsis* have been screened out in our laboratory using the T-DNA insertion technique, and a mutant with respect to the SAM function has been isolated. In the present study, we characterized this new mutant by comparing the differences between the mutant and the wild type in terms of morphology and SAM anatomy, and by localizing GUS expression precisely in the mutant, in order to lay the foundation for cloning the gene trapped by T-DNA and exploring its function. Moreover, this mutant can be used as a promising material for studing on the regulation and control of SAM developmental patterning and its association with the plant development, and provide important information for understanding the molecular mechanisms of such development.

2 Methods

2.1 Materials and growth conditions

The mutant was isolated from a population of T-DNA insertion lines from *Arabidopsis thaliana* (ecotype C24 background) using the promoter trap technique. The pMHA2 vector containing kanamycin resistant gene *npt*II and promoterless GUS (glucuronidase) gene was

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induced into *Agrobacterium* and transformed into the wild type by *Agrobacterium*-mediated vacuum infiltration method (Bechtold et al., 1993).

Matured seeds from the mutant and wild type were first vernalized at 4°C for 48 hours. Afterwards they were sowed in nutrient soil and covered with a film, and then transferred to a growth chamber with a cool-white light illumination (40001x) of 16-h light and 8-h dark photoperiod and 50% humidity to grow at 22°C. They were watered with water and Hoagland nutrient fluid alternatively every three days.

2.2 Comparative analysis of both the mutant and wild type in the phenotypes and microstructures of SAM

Differing phenotypes between the wild type and the mutant at different stages were observed, measured, photographed and statistically analyzed. SAM from 21- and 30-day-old plants were fixed in FAA (3.7% formalin, 5% acetic acid, 50% ethanol) at 4°C for 24 h, re-hydrated in an ethanol series, stained in 0.1% safranin O for 40 hours at room temperature, and dehydrated in a gradient of ethanol. The samples were embedded in paraffin and cut longitudinally with a microtome, deparaffinized in xylene, rehydrated, counterstained with 0.1% brilliant green, dehydrated again and mounted. Finally they were photographed under a Motic B5 digital microscope.

2.3 Histo-chemical localization of GUS expression

The 10-day-old whole wild type and mutant seedlings were stained in a GUS staining mixture then chlorophyll-cleared in 100% ethanol at 4°C, observed under a stereoscope and photographed. The 21-day-old shoot apices were chlorophyll-cleared in 70% ethanol, then dehydrated serially with ethanol and xylene, and finally embedded in paraffin. Longitudinal sections (8 μm) were cut and deparaffinized in xylene, dehydrated and mounted on slides. The sections were then observed and photographed.

3 Results

3.1 Phenotype of the mutant

Phenotypical observations of the mutant plants revealed that at the early stage of development, mutant seedlings were smaller and their cotyledons were more round and thicker than the wild type seedlings. In addition, cotyledons of the mutant had a dark green color, and their petioles were shorter than those of the wild type. Hypocotyls of the mutant seedlings were shorter and their roots were also shorter and fewer compared with the wild type. The mutant plants also had fewer rosette leaves, with smaller size, in contrast to the wild type. However, at the late stage of development, the rosette leaves of the mutant were still being continuously produced when the wild type had transited from vegetative to reproductive growth. The mutant transitioned 7 days later than the wild type from vegetative to reproductive growth. Additionally, the height of the matured mutant plants was about two thirds of the wild type. The development of the mutant siliques was also postponed, and the siliques were shorter and wider, being one half the length of those of the wild type. Thus, T-DNA insertion resulted in a reduction in the mutant organ volumes, delayed their development with prolonged vegetative growth, and produced a dwarf morphology (Fig. 1).

Fig. 1 Phenotypes of both wild type and mutant plants. **A**: 4-week-old wild type; **B**: 4-week-old mutant plant; **C**: 5-week-old wild type (left) and mutant plant (right).

3.2 Characteristics of the mutant SAM

The micro-sections of different stages of SAM from both the mutant and wild type plants were analyzed. By day 10, the SAM in the wild type could be compared to a plump dome, while SAM of the mutant plants appeared flattened and smaller, and no apparent appearance like vaulting was observed (data not shown). By day 21, cell layers were more than those of the mutant. Cells located in layers L1 and L2 were arranged in order. Apart from the obvious boundaries among the L1, L2 and L3 layers, the cells in the L3 layer were also arranged in order (Fig. 2A). Cell division in the outer tunica was maintained in an anticlinal orientation at the L1 and L2 positions. On day 21, the SAM in the mutant was vaulted, but still smaller than that of the wild type. Cell layers in the SAM of the mutant were also decreased, and the radian of vaulting was also reduced compared with the wild type. Additionally, the boundaries between the L1 and L2 layers in the tunica were not evident, and the cells of L2 and L3 were intermingled with each other. Further, no anticlinal cell divisions were observed in the L2 layer of the mutant tunica, and the arrangement of cells was out of order and accompanied by inhomogeneous sizes of cells as compared to the wild type (Fig. 2B).

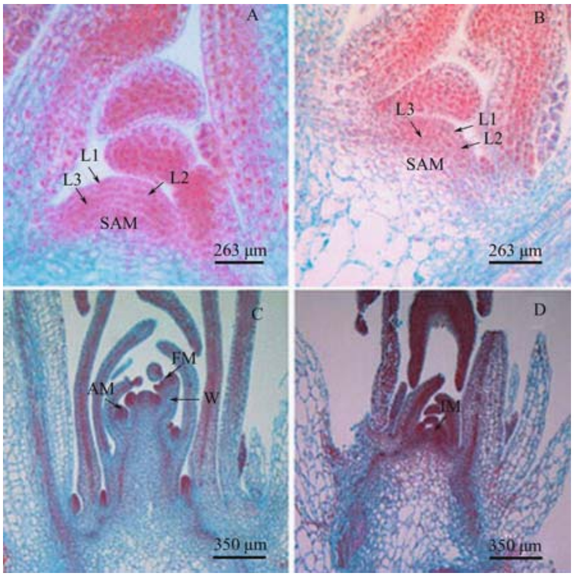

Fig. 2 Longitudinal sections of the SAM from mutant and wild type plants. **A**: 21-day-old wild type; **B**: 21-day-old mutant; **C**: 30-day-old wild type; **D**: 30-day-old mutant. AM: Axillary meristem; FM: Floral meristem; W: Whorl; IM: Inflorescence meristem.

The 30-day-old wild type and mutant SAM were also analyzed by histological methods. In the wild type, inflorescence meristem (IM) and the floral meristems (FM) came into shape. Namely, four axillary meristems (AM) and the two flower whorls (W) could be distinctly observed (Fig. 2C). FM produced compact whorls of organs, which would form four sepals, four petals, six stamens, and two carpels in the future. Meanwhile, the SAM of the mutant was converting to IM, which had an upward swelling appearance (Fig. 2D). These results were consistent with the phenotypical observations that the transition from vegetative to reproductive growth in the mutant was delayed by around 7 days (Fig. 2D).

3.3 Histo-chemical localization of GUS expression in the mutant

The promoter traps contain a promoterless reporter gene so that the GUS product β-glucuronidase can occur only when the insertion is within a transcriptional unit, leading to transcriptional fusion. The data described above indicated that T-DNA insertion resulted in alterations in phenotypes and SAM structural patterning in the mutant. Therefore, GUS expression patterning and location analysis were performed to get an insight into the expression pattern of T-DNA trap gene in the *Arabidopsis* plant, especially in the SAM.

GUS staining in different developmental stages of the mutant plants showed that at seedling stage (days 7–14), the GUS gene was specifically expressed in vascular tissues of the hypocotyls, cotyledons, rosette leaves, petioles, inflorescence stem and roots (Fig. 3A). The vascular tissues of veins in new leaves were strongly stained, whereas in the old leaves they were slightly stained. In contrast, the wild type plants did not show any blue color (Fig. 3B).

To more exactly localize the site of GUS gene expression in vascular tissues of the mutant, histo- chemical localization was performed on the longitudinal sections of SAM from 21-day-old mutant plants. The results indicated that the product of the GUS gene was specifically localized in the rib zone and procambium of the leaf primordium (Fig. 3C). Similarly, the specific blue color was not detected in the wild type.

4 Discussion

The mutant produced a series of phenotypical alterations, including delayed development, reduced size of organs such as leaves, a prolonged vegetative stage, a dwarf figure, and short siliques. These appearances were consistent with the defects of the SAM, e.g. reduced volume, decreased cell layers and cell numbers, slow development and delayed transition from SAM to FM. The reduction in SAM size and cell layers in the mutant may be correlated with reduced cell division frequency in the central zone of the SAM. Moreover, the GUS gene was also expressed specifically in the SAM and vascular tissues. All these suggested that T-DNA insertion possibly affected the functions of genes related to SAM development,

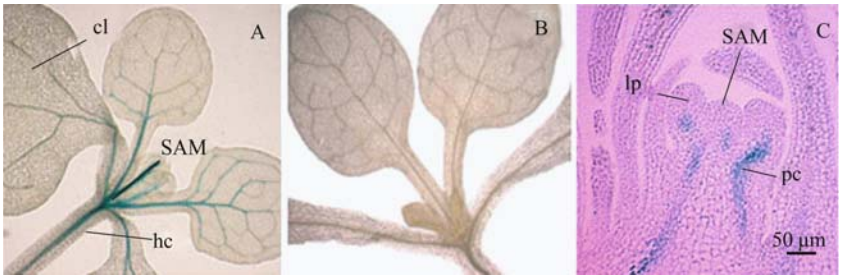

Fig. 3 Histo-chemical localization of GUS gene expression in the mutant. **A**: 10-day-old mutant plant showing GUS gene expression; **B**: 10-day-old wild plant without GUS gene expression; **C**: Longitudinal section of SAM from 21-day-old mutant plant showing GUS gene expression. SAM: Shoot apical meristem; cl: Cotyledon; hc: Hypocotyl; lp: Leaf primordium; Pc: Procambium.

and further influenced the normal development of the SAM and the plant.

The structural and functional maintenance of SAM requires a delicate signaling network in which the coordination of multiple proteins is involved (Doerner, 1999; Fletcher and Meyerowitz, 2000). To date, some mutants and genes associated with SAM activity have been isolated, and they can be divided into three kinds according to their functions: the first kind of genes is related to the initiation and maintenance of SAM, such as *shootmeristemless* (*stm*) and *wuschel* (*wus*); the second kind of genes regulates organ development and morphogenesis, such as *pinhead* (*pin*)/*zwille* (*zll*); the third kind of genes controls the proportions between differentiated and undifferentiated cells in the SAM, like *clavata* (*clv*) (Clark et al., 1996; Mayer et al., 1998; Moussian et al., 1998; Gallois et al., 2002; Lenhard et al., 2002). Mutation of those genes or changes in expression patterns usually causes abnormal SAM structure and plant morphology. For example, *clv* (*clv1*, *clv2* and *clv3*) encoded a receptor-like protein involved in the conduction of receptor kinase signals (Jeong et al., 1999; Trotochaud et al., 1999). Interaction between the CLV and WUS regulated the volume and function of SAM efficiently (Schoof et al., 2000). *clv* mutant, in which the volumes of both SAM and the floral meristem were enlarged with more cell layers and cell number, exhibited pre-mature development, flattened inflorescence stem, more floral organs, and shorter and thicker siliques compared to the wild type. The aberrance in the *clv* mutant was caused by the over-expression of CLV and an increase of the cell division frequency in the central zone. Conversely, our mutant exhibited a different phenotype compared with the *clv* mutant, such as delayed development, an extended vegetative stage and a dwarf appearance with a smaller SAM, reduced in cell layers and disordered in division direction of the L2 cells. Furthermore, the GUS gene was found specifically expressed in the SAM and vascular tissues, indicating that the gene trapped by T-DNA insertion could encode a protein required for the regulation of a complex signal network involved in SAM function. Thus, T-DNA insertion might influence the division frequency of central zone cells and affect the development of the cambium precursor cells in the rib zone of the meristem, which are related to the formation of the vascular system. The protein encoded by the gene trapped by T-DNA may act in the long-range interaction of the vascular system as a signal molecule, and then influence the development of the whole plant. Accordingly, T-DNA insertion could influence the functional activity of the related gene in the mutant, leading to a series of phenotypes in the mutant.

In summary, our mutant may be an ideal material for the study of SAM. Cloning and characterization of this gene trapped by T-DNA may provide the basis for the studies of the regulation and control between SAM patterning formation and plant development.

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