Gravity is a constant stimulus governing plant growth and development. A plant’s response to gravity is complex and involves stimulus perception, transmission of that signal to the responding tissues and the differential growth response generated by the plant growth regulator auxin. Because auxin plays such an important role in gravitropic signal transduction and the defects in the gps mutants affect auxin redistribution (Nadella et al., 2006), a primary objective of this study was to characterize members of the auxin response factor (ARF) family of proteins with respect to the gravitropic persistence signal (GPS) response. During the GPS response, plants are gravistimulated at 4°C and returned to vertical at room temperature (Wyatt et al., 2002). Because perception occurs, but auxin is not transported in the cold, this treatment allows the isolation of the events of signal transduction prior to auxin transport.

The ARF family has been well characterized, although the function of several of the ARF family members is still unknown. For example, ARF9 is expressed in all tissues during all growth stages (Zimmermann et al., 2004), but T-DNA insertional mutagenesis produced plants that showed no obvious growth phenotype (Okushima et al., 2005). Tiwari et al. (2003) reported that ARF9 was a repressive transcription factor, but its function in plant growth remains unknown.

In this study, we characterized ARF9 with respect to the GPS response in the hope of determining if ARF genes were involved early in signal transduction or in the response to auxin.

First, a transcription profile of ARF9 during the GPS treatment was performed using quantitative reverse transcriptase (RT)-PCR. Wild-type Arabidopsis (Columbia) was grown to maturity, and 56 plants with inflorescence stems 8-10cm were either gravistimulated or left upright at 4°C for 1h, then returned to room temperature upright. RNA was extracted from the inflorescence stems at seven time points: 0, 30 and 60 minutes into cold treatment and 5, 10, 15 and 30 minutes after return to room temperature. The upper 5cm of the stems was excised and flash frozen in liquid nitrogen. RNA was extracted using the TRIzol Reagent RNA isolation protocol (Gibco BRL Technologies).

The ARF9 transcript was significantly up-regulated during the cold treatment but only in the absence of gravistimulation (Fig. 1). Interestingly, when gravistimulated in the cold, the transcript did not increase until after the plants were returned to vertical at room temperature, when auxin transport was restored (Fig. 1). This may indicate a role for ARF9 in the gravitropic transduction/response mechanisms, subsequent to auxin transport.

![Figure 1. ARF9 transcript levels during the GPS treatment.](image)

A T-DNA insertion line (SALK_005473) for ARF9 was generated by the SALK Institute for Genomic Analysis and obtained from the Arabidopsis Biological Resource Center (Columbus, OH). The region flanking the insertion was sequenced, and the insertion confirmed to interrupt the transcript prior to the auxin IAA binding domain (Fig. 2). The T-DNA insertion line (SALK_005473) for ARF9 was generated by the SALK Institute for Genomic Analysis and obtained from the Arabidopsis Biological Resource Center (Columbus, OH). The region flanking the insertion was sequenced, and the insertion confirmed to interrupt the transcript prior to the auxin IAA binding domain (Fig. 2).

Figure 1. ARF9 transcript levels during the GPS treatment. Wild-type Arabidopsis were either held vertically (dashed line) or gravistimulated (solid line) for 60 min at 4°C, then returned to room temperature after 60 min. All plants were maintained vertically at room temperature. Each point represents the average gene expression in three plants as compared to the transcript for the ubiquitin conjugating enzyme as a control.

![Figure 2. Diagrammatic representation of ARF9 protein (638 amino acids) showing the B3 DNA binding domain (black oval), the auxin response domain (dark grey) and the auxin IAA binding domain (light grey). SALK_005473 T-DNA insertion is predicted to interrupt the DNA sequence upstream of the auxin IAA binding domain (white triangle). The relative location of two primers sets used for RT-PCR is indicated at the top of the figure.](image)

Plants were selected for homozygosity of the mutant allele, grown to seed, and their progeny screened for their response to the GPS treatment. Segregation analysis showed that the T-DNA insertion segregated consistently with the mutant phenotype in a classic Mendelian 3:1 ratio. Wild-type and mutant plants were grown to maturity in long day conditions (16h light, 8h dark). When inflorescence stems were 8-10cm, plants were gravistimulated at 4°C for 1h then returned to vertical at room temperature. Plants were observed every 5 min for 2h after return to room temperature. In response to the GPS treatment, the ARF9 mutant over-responded, bending almost 180° (Fig. 3). The phenotype is reminiscent of the gps3 mutant described by Wyatt et al. 2002.
To assess the ARF9 mutant for transcript, RNA was extracted from inflorescence stems using the TRIzol extraction protocol (Gibco BRL) and subjected to RT-PCR. RT-PCR using the 5' primer set indicated presence of the ARF9 transcript in SALK_005473 (Fig. 4, top). However, using the 3' primer set, no transcript was seen (Fig. 4, middle). Taken together, these data indicate that the T-DNA insertion in SALK-005473 does not fully knock out the transcription of ARF9 but does disrupt the production of a full length transcript, either by truncation or insertion of additional T-DNA sequence.

The IAA binding domain of ARF9 is responsible for dimerization of the protein. In the presence of auxin, this dimerization is released, and the protein acts as a repressive transcription factor (Tiwari et al., 2003). Clearly, the ARF9 mutant produces transcript but whether it produces a truncated protein remains to be seen. Because of the position of the insertion, a truncated protein without the IAA binding domain may be translated. If functional, this truncated protein might function constitutively as a repressive transcription factor.

If no functional protein is produced, then no repression is possible. Both are interesting prospects in light of the phenotype of the ARF9 mutant. A protein alignment of all ARF family members indicated that ARF3 encodes just such a protein (without the IAA binding domain), and ARF23 encodes an ARF family member without either the IAA binding domain or the auxin responsive element (Atschul et al. 2003). These genes may provide a unique tool for teasing apart the potential role of the ARF domains in gravitropic signal transduction. The over-achiever (gps3-like) phenotype may also provide clues to the function of GPS3.

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REFERENCES


