Almost all aspects of plant form and development are impacted by gravity. The most basic of these is gravitropism, a change in the direction of growth in response to a physical reorientation with respect to the gravity vector. However, at 4°C, gravitropic bending is virtually undetectable in wild-type Arabidopsis thaliana (Fukaki et al., 1996), but if plants are returned to vertical at room temperature (RT), transient curvature occurs. This cold effect is known as the Gravity Persistent Signal (GPS) response and is caused by temperature effects on auxin transport (Nadella et al., 2006). To identify genes involved in the signal transduction pathway of gravitropism, a mutant screen was performed to isolate Arabidopsis lines that displayed an aberrant GPS response (referred to here as the GPS treatment) (Wyatt et al., 2002). One of these lines, gravity persistent signal 4 (gps4) showed no response after the GPS treatment (Fig. 3 & Fig. 6). However, room-temperature gravitropism in the inflorescence stem appeared to be unchanged (Fig. 6).

A T-DNA insertion was found 280 bp upstream of At1G59980 in gps4 (Fig. 1). This loci, also known as Altered Response to Gravity Like 2 (ARL2), had previously been implicated in the gravitropic response (Guan et al., 2003). To confirm that the T-DNA insertion in arl2 was responsible for eliminating the GPS response, an additional allele (arl2-4) from the SALK collection was isolated (Fig. 1) (Alonso et al., 2003). This allele has previously been characterized (Harrison and Masson, 2008b). Although not a full mRNA knockout, the insertion in the second exon causes an aberrant splicing event that leads to an early stop codon. RT-PCR results suggest that gps4 is a full knockout allele (Fig. 2).

Figure 1. Diagrammatic representation of the insertions in At1g59980. The open box represents the 5’ UTR, closed boxes represent exons, and lines represent introns. The triangles indicate the location of two T-DNA insertions.

Figure 2. RT PCR Analysis of gps4. The top row indicates bands of 750 bp produced by GPS4-specific primers that bind in the 5’UTR (forward) and span the junction between the 7th and 8th exons (reverse). Ubiquitin conjugating enzyme (UbCE: At5g25760) primers were used as a positive control.

Figure 4. GPS4/ARL2 Expression during GPS Response. Quantitative Reverse-Transcriptase PCR using SYBR Green was performed on Ws plants with primers amplifying ARL2. Time zero represents removal from the cold. Data represent the average of 5 replicates normalized to ubiquitin conjugating enzyme At5g25760. Bars represent standard error. * indicates significance of p<.001 for all samples using a pairwise students T-test. # indicates p<.005 compared to time zero.

Wild-type (Ws and Col), gps4 and arl2-4 plants were subjected to the GPS treatment, and total inflorescence stem curvature was measured. The WT plants curved an average of 55° by 60 min after return to RT (Fig. 3 & Fig. 6). In contrast, both gps4 and arl2-4 curved only 8°, a significant decrease from wild type. The gps4 mutant also showed reduced hypocotyl and root gravitropism at RT (data not shown), as previously reported for arl2 (Guan et al., 2003).
ARL2 was originally identified as part of a three-gene family that also included ARL1 and ALTERED RESPONSE TO GRAVITY 1 (ARG1) (Guan et al., 2003). While arg1 mutants, like arl2, displayed reduced gravitropism, mutants in arl1 produced no gravitropic phenotype. ARG1 and ARL2 appear to function together with PIN-FORMED 3 in a gravitropic pathway that is genetically distinct from amyloplast sedimentation in root statocytes (Harrison and Masson, 2008a; Harrison and Masson, 2008b).

Due to the knockout of the GPS response in gps4 and arl2-4, we hypothesized that the ARG1/ARL2/PIN3 pathway operating in the root may be responsible for causing the GPS response in inflorescence stems. T-DNA insertion mutants in arg1 and arl1 were obtained. The arg1 mutant was ordered directly from the ABRC. WiscDsLox264E12 (5th Exon) and SAIL_1279_E07 (last exon) were obtained for arl1. Data presented below are from WiscDsLox264E12, although SAIL_1279_E07 produced similar results.

To determine if arg1 and arl1 mutants have a GPS response phenotype, inflorescence stem curvature of vertically oriented plants was measured for 90 minutes after a 90 minute horizontal cold treatment. An average response of about 50° was seen in all lines between 45 and 60 min after cold gravistimulation (Fig. 5 & Fig. 6). There was also no significant difference in the rate at which they returned to vertical.

The robust GPS curvature in the arl1 mutant indicates that this gene is either not involved in the response, or its effects are masked by the redundant activity of another gene. In spite of its sequence similarity to two important factors in gravitropism, evidence for involvement of ARL1 in this response remains elusive. The normal GPS curvature in arg1 suggests that this gene is likewise not involved in inflorescence stem gravitropism. However, the no-response phenotype seen in gps4arl2 provides support for the GPS treatment as a viable mechanism for analyzing gravitropism and identifying physiologically relevant mutants.

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