

SHORT COMMUNICATION



## Developmental polarity shapes thermo-induced nastic movements in plants

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### ABSTRACT

Directional and non-directional environmental cues are able to induce polar behaviors of plants, which are termed tropic and nastic movements, respectively. While molecular mechanisms underlying the directionality of tropic movements are relatively well studied, it is poorly understood how the polarity of nastic movements is determined in response to non-directional stimuli, such as ambient temperatures. It has recently been shown that thermal induction of leaf hyponasty is stimulated by developmentally programmed polar auxin transport in *Arabidopsis*. Under warm environments, the PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) transcription factor binds to the promoter of *PINOID* (*PID*) gene, whose gene product modulates the polar trafficking of the auxin transporter PIN-FORMED 3 (PIN3). Notably, PIF4 binding to the *PID* promoter occurs predominantly in the abaxial petiole cells than the adaxial petiole cells, leading to differential *PID* expression and thus asymmetric auxin accumulation in the petiole cells. In addition, ASYMMETRIC LEAVES 1 (AS1), the well-characterized leaf polarity-determining epigenetic regulator, promotes the *PID* expression by modulating the patterns of histone 4 acetylation (H4Ac) in the *PID* chromatin. These observations demonstrate that developmental programming of the thermonastic leaf movement through polar auxin distribution enables plants to bend their leaves upward in response to non-directional thermal stimuli, contributing to cooling plant body temperatures under warm temperature conditions. We propose that a developmentally predetermined polarity plays a major role in governing the directionality of various nastic movements in plants.

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### Text

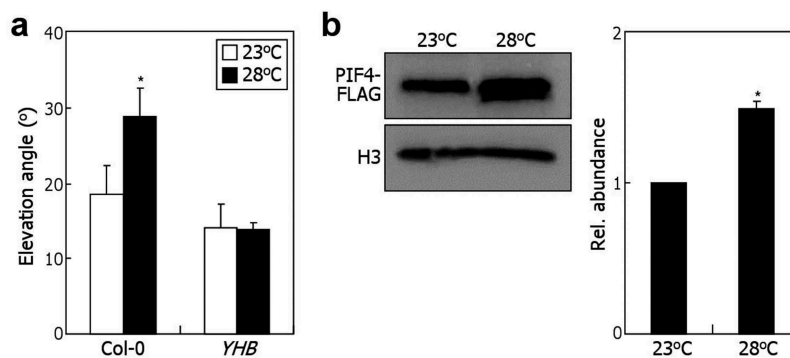
Environmental stimuli under natural conditions can be categorized into two major groups depending on whether they harbor directional information or not: directional or non-directional ones. Both two types of stimuli are capable of generating directional behaviors of specific plant organs, termed tropic and nastic movements, respectively. The tropic and nastic movements require asymmetric growth in plant tissues. The best characterized is the polar auxin transport, which directs asymmetric cell elongation during tropic movements.<sup>1,2</sup> The polar auxin flow also plays an important role in regulating the directionality of nastic movements.<sup>3</sup> A well-known example of nastic plant behaviors is the upward bending of leaves under warm environments, frequently termed leaf thermonasty.<sup>4</sup> It has been reported that thermonastic leaf movements are promoted by the asymmetric growth between the adaxial and abaxial petiole cells.<sup>3</sup> However, it is largely unknown whether and how polar auxin transport elicits the directional movements of plant organs under warm temperature conditions.

The protein kinase PINOID (*PID*) has been proven to shape the asymmetric growth of plant organs by regulating the polar localization of the auxin transporter PIN-FORMED 3 (*PIN3*).<sup>5,6</sup> It has been observed that *PIN3* is preferentially localized to the outer membranes of the abaxial petiole cells at warm temperatures. Recently, it has been observed that the thermal induction of *PID* expression is higher in the abaxial petiole cells than in the

adaxial petiole cells.<sup>3</sup> Notably, the temperature-dependent differential expression of *PID* gene is diminished in the *pif4-101* mutant that lacks the PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) transcription factor, suggesting that PIF4 is required for the polar auxin transport during leaf thermonasty. Accordingly, the *pif4-101* mutant did not exhibit leaf thermonasty. These observations indicate that PIF4 promotes leaf thermonasty by modulating the differential transcription of *PID* gene in the adaxial and abaxial petiole cells.<sup>3</sup>

A critical question is how PIF4 is associated with the *PID* function in directing polarized auxin flow during leaf thermonastic movements. Chromatin immunoprecipitation (ChIP) assays revealed that PIF4 binds directly to the *PID* promoters under warm temperature conditions. Furthermore, the DNA-binding affinity of PIF4 to the *PID* promoters was more prominent in the abaxial petiole cells, indicating that the thermo-responsive action of the PIF4 transcription factor is differentiated along the adaxial-abaxial polarity of the leaf petioles.

It is particularly interesting that the PIF4-mediated polar auxin transport occurs predominantly in the abaxial petiole cells in response to non-directional warm temperature stimuli, raising a central question as to molecular mechanisms that determine the polarity of leaf thermonasty. Light and gravity, which are two major determinants of polarized plant growth,<sup>1,2</sup> do not affect the polarity of leaf thermonasty,<sup>3</sup> suggesting that certain intrinsic signals would be involved in specifying the polarity of leaf thermonasty.



**Figure 1.** The phytochrome photoreceptors are functionally linked with leaf thermonasty.

Three measurements, each consisting of 16 individual plants grown under identical conditions, were averaged and statistically analyzed ( $t$ -test,  $*P < .01$ ). Bars indicate standard error of the mean. (a) Leaf thermonasty in *YHB* transgenic plants. The *YHB* transgenic plants express a constitutively active form of phytochrome B. Elevation angles of the 5<sup>th</sup> and 6<sup>th</sup> rosette leaves relative to the horizontal plane were measured using three-week-old plants grown either at 23°C or 28°C for 6 h. (b) Thermal induction of PIF4 protein accumulation. Transgenic plants expressing the *pPIF4:PIF4-FLAG* construct were grown at 23°C for three weeks and then exposed to either 23°C or 28°C for 6 h. Leaf petioles were harvested for total protein extraction. The PIF4-FLAG proteins were immunologically detected using an anti-FLAG antibody. Immunological detection of histone 3 (H3) was performed in parallel as loading control.

It has been well characterized that the epigenetic regulator ASYMMETRIC LEAVES 1 (AS1) modulates the expression of leaf polarity-specifying genes in plants,<sup>7–9</sup> raising a possibility that the leaf polarity specifier is functionally linked with leaf thermonasty. In support of this notion, it was found that leaf thermonasty and differential *PID* expression patterns were severely impaired in the AS1-deficient *as1-1* mutant. In addition, the thermally induced DNA binding of PIF4 to the *PID* promoters was also disrupted in the *as1-1* mutant. On the basis of the biochemical nature of AS1 function in mediating histone modifications,<sup>10</sup> the patterns of H4Ac were examined in the genomic DNA sequence harboring the *PID* locus. The results have shown that while the thermal-induction of H4Ac in the *PID* locus is prominent in the abaxial petiole cells in wild-type plants, it is compromised in the *as1-1* mutant. It is thus apparent that the AS1-mediated developmental signals determine the patterns of asymmetric growth of the adaxial and abaxial petiole cells by modulating PIF4 binding to the *PID* promoters under warm environments.

It is evident that the directional leaf hyponastic movements occur under warm environments. A next question is how temperature sensing mechanisms are functionally associated with the thermonastic leaf movement. It is known that the conversion of the PHYTOCHROME B (phyB) photoreceptor from the physiologically active Pfr form to the inactive Pr form is accelerated at warm temperatures,<sup>11</sup> supporting that the phytochrome photoreceptors act as a temperature sensor.

A challenging question is whether the phytochrome-mediated temperature sensing is related with leaf thermonastic movement. We found that overexpression of a constitutively active form of phyB, *YHB*, markedly diminished leaf hyponasty at warm temperatures (Figure 1a), suggesting that the thermal reversion of phyB is required for the leaf thermonastic leaf movement. It has been previously reported that phyB negatively regulates the protein stability of the downstream PIF4 transcription factor.<sup>12</sup> Consistent with the thermal inactivation of phyB, we observed that PIF4 protein accumulates to a higher level at warm temperatures (Figure 1b), further supporting that

the phyB-mediated temperature sensing mechanism is functionally associated with leaf thermonasty.

Plants always bend their leaves upward in response to non-directional temperature stimuli.<sup>3,4</sup> It is well known that plants exhibit distinct morphological alterations, such as hypocotyl overgrowth and increased leaf hyponasty, at warm temperatures,<sup>13–15</sup> collectively termed thermomorphogenesis. The thermomorphogenic responses are known to facilitate leaf cooling and protect the heat-labile shoot apical meristem from the warm or hot soil surface;<sup>4</sup> suggesting a possibility that leaf thermonasty contributes to cooling body temperatures. In agreement with the hypothesis, the temperature of the leaves arrested close to the soil surface was higher than the leaves exhibiting leaf hyponasty under warm environments.<sup>3</sup> Together, these results indicate that the directionality of thermonastic leaf movement enables plants to promote the cooling capacity of plant organs.

Our findings provide an excellent research system for the developmental control of environmental adaptation in plants. Polarity of thermonastic leaf movement is shaped by the developmentally programmed polar auxin transport. Why is it advantageous for plants to utilize intrinsic signals rather than environmental cues in directing thermonastic leaf movement? It is speculated that directional information given by developmental signals would be more advantageous than directional external stimuli under certain circumstances, in which plants are exposed to distorted direction of light or gravity in darkness or on a slope. It will be interesting to explore whether our leaf thermonastic model is also applicable to other nastic movements, such as flooding-induced hyponastic growth in *Arabidopsis* and nastic behaviors in *mimosa* and *Venus flytrap*:<sup>16–18</sup>

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