ARTICLE ADDENDUM

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Pleiotropic effects of ZmLAZY1 on the auxin-mediated responses to gravity and light in maize shoot and inflorescences

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Abbreviations: AUX1, auxin permease 1; LAX, like-AUX1; PKC, protein kinase catalytic; Aux/IAA, auxin/indole-3-acetic acid; PIN, PIN-FORMED; ABCB/PGP, ATP-binding cassette/P-Glycoproteins; *bif1*, *barren inflorescence1*; *bif2*, *barren inflorescence2; ba1*, *barren stalk1*; NDL1, N-MYC Downregulated-Like1; BR2, Dwarf Brachytic2; PHOT, Phototropin; WAG, Wavy Root Growth; D6PK, D6 Protein Kinase

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Auxin has been found to control both gravitropism and inflorescence development in plant. Auxin transport has also been demonstrated to be responsible for tropism. Maize, a key agricultural crop, has distinct male (tassel) and female (ear) inflorescence, and this morphogenesis depends on auxin maximum and gradient. The classic maize mutant *lazy plant1 (la1)* has defective gravitropic response. The mechanism underlining maize gravitropism remains unclear. Recently, we isolated the *ZmLA1* gene by map-based cloning, and our findings suggest that ZmLA1 might mediate the crosstalk between shoot gravitropism and inflorescence development by regulating auxin transport, auxin signaling, and auxin-mediated light response in maize. Here, we propose a model describing the ZmLA1-mediated complex interactions among auxin, gravity, light, and inflorescent development.

Auxin, as the first phytohormone recognized in plant, plays important roles in multiple physiological and developmental processes, particularly in regulating gravitropism and inflorescence development.[1,2] These biological functions of auxin depend on the establishment of localized auxin gradients through the coordination between local auxin biosynthesis and cell-to-cell auxin transport. The directional transport of auxin, also named as Polar Auxin Transport (PAT), is a unique characteristic of auxin. Several auxin transporter proteins have been identified in *Arabidopsis*, including AUX1 and LAXs as influx carriers, PINs and ABCB/PGPs as efflux transporters.[3] PAT contributes to the establishment of proper auxin gradients more significantly compared with local auxin synthesis. Therefore, disruption of the auxin transporters, through either changes in accumulation or ectopic plasma membrane localization, interferes with multiple auxin-related developmental processes.[4] Given this, plant scientists have been intrigued by auxin-related mutants for over a century. For instance, auxin transporter mutants *aux1*, *pin2*, *pin3*, abcb4, and *abcb1 abcb19* double mutant either lose or have significantly reduced positive root gravitropism.[5] Among all the transporters mutants, *pin3* mutant shows abnormal shoot gravitropic response,[6] while *abcb1 abcb19* double mutant displays enhanced hypocotyl gravitropic curvature,[7] suggesting that negative shoot gravitropism might be regulated by an unique mechanism. Another common phenotype associated with PAT-related mutants is aberrant inflorescence. For example, maize *bif1*, *bif2* and *ba1* mutants show similar defective morphogenesis of tassel and ear though to different extend.[2] In conclusion, those studies on the mutants demonstrate that accurate PAT is indispensible for proper gravitropism and inflorescence development. However, whether there are PAT-related genes regulating both processes simultaneously still need to be further investigated.

We recently reported the identification of a gene involved in shoot gravitropsim in maize. The gene also mediates inflorescence development through regulating PAT.[8] We screened our *MuDR* mutant library by phenotype and selected a *prostrate1* (*ps1*) mutant. The horizontally placed *ps1* mesocotyl-coleoptiles show a reduced gravitropic response compared with the recovery rate of the wild type (WT) samples. At seedling stage, the *ps1* mutant completely loses gravitropic response. Interestingly, the *ps1* mutant exhibits normal gravitropic root growth, suggesting that the *ps1* mutant is a shoot specific agravitropism mutant. However, when growing in field, the *ps1* mutant seedlings gradually bend until reaching complete prostrate growth in random directions at seven-leaves stage. We speculate that the *ps1* mutation disrupts the asymmetric elongation of the responsive cells in shoot gravitropism and the mutant adopts the prostrate growth pattern passively. Environmental factors such as wind or rain randomly exert influences over plants. The *ps1* mutant is insensitive to gravity; thus, it might maintain the prostrate growth in the direction where the influencing power is the most significant.

We also identified the *PS1* gene by map-based cloning. We found that the *ps1* mutant is allelic to the classic maize *lazy1* (*la1*) mutant, which has been demonstrated to have defective gravitropic response.[9] Consistent with the maize mutant in the *LAZY1* gene (*ZmLA1*), studies on the orthologs in rice and *Arabidopsis* have shown that shoot agravitropism also occurs in the respective mutants.[10-12] ZmLA1 represses basipetal PAT but promotes lateral PAT in maize, which is consistent with LA1 function in rice.[11] However, unlike its counterparts in rice and *Arabidopsis*, *ZmLA1* in maize plays a unique role in regulating inflorescence development. *ZmLA1* is highly expressed in reproductive organs in maize. Basipetal PAT is accelerated in both tassel and ear in the *la1* mutant, suggesting that a ZmLA1-depended mechanism is involved in the regulation of basipetal PAT. We speculate that LAZY1 might represent a conserved and diverse PAT model in different species, which is similar to previously identified PAT transporter. BIF2 in maize and rice displays similar expression pattern and inflorescence function as its ortholog PINOID in *Arabidopsis*,[13] indicating the partially conserved roles of auxin transporters between monocots and dicots. However, remarkably diverse PAT mechanisms exist in mono- and dicots, as suggested in the comparison of orthologs from *Arabidopsis* and grass. For example, the *Arabidopsis* mutants of *abcb1* show mild or even indiscernible phenotype,[14] in contrast, single gene mutants of *brachytic2* (*br2*) and *dwarf3* (*d3*), the *ABCB1* ortholog in maize and sorghum respectively, have compact stalk resulting from reduced PAT and cell elongation.[15] Further analysis on PAT-related orthologs in grass and *Arabidopsis* at larger scale is required to clarify the evolution of auxin transport in different species.

RNA-SEQ analyses on the *la1* mutant reveal putative genes that are subject to ZmLA1-related network, including several auxin transport related genes. For instance, *ZmPIN1c* and *ZmNDL1* are greatly downregulated; while maize *BR2* (*ZmABCB1*) is significantly upregulated in the *la1* mutant. This bidirectional regulation on transcription might produce a ZmLA1 with the dual function of basipetal and lateral PAT. Therefore, we propose that basipetal and lateral PATs may be mediated by different regulators of auxin transport in maize, and ZmLA1 may act as a link or switch between the regulators. The RNA-SEQ results also reveal that abundant auxin-responsive genes and genes involved in light signaling are upregulated in the *la1* mutant, implying that ZmLA1 may act as a negative regulator for both light signaling and auxin signaling. In addition, because *ZmLA1* promoter contains multiple auxin responsive elements and light responsive elements, treatment with exogenous auxin leads to a repression-then-induction pattern for *ZmLA1* expression. Dark dramatically induces the accumulation of *ZmLA1* transcript but light inhibits it, indicating a mystical crosstalk between ZmLA1 and light at transcriptional level.

Yeast two-hybrid screening with ZmLA1 as the bait further results in the identification of two proteins interacting with ZmLA1: an AGC kinase family member containing a PKC-like domain and an Aux/IAA family member, named as PKC and IAA17 respectively. Based on the bimolecular fluorescence complementation (BiFC) assays, we also found that ZmLA1 physically contact PKC near the plasma membrane and contact IAA17 in the nucleus, which is in accordance with the subcellular locations of ZmLA1. Although the nucleus localization of AtLAZY1 is found to be nonessential for controlling gravitropic branch orientation in *Arabidopsis*,[10] we believe that both nucleus and membrane localization are important for proper ZmLA1 function in maize. The ZmLA1-IAA17-mediated auxin signaling might be associated with ear and tassel development, while ZmLA1-PKC-mediated auxin transport might contribute to gravitropic response in maize. Recently, several plant-specific members of the AGC family have been characterized for their phosphorylation on PINs, including PHOT1, PHOT2,[16] WAG1,WAG2,[17] and D6PK.[18,19] The PKC domain is believed to endow their phosphorylation activity to control PINs polarity. Further investigation on ZmLA1-PKC interaction is needed to elucidate the mechanisms underlying the functions of ZmLA1 and PKC in PAT.

Compared with the defects of axillary meristem initiation observed in the auxin transport mutants such as *bif1* and *bif2,* inflorescence defects at early stage in the *la1* mutant are relatively mild. The initiation of axillary meristems appears quite normal, and only the organization of AMs shows certain degree of abnormality. ZmLA1 physically interacts with a putative auxin transport regulator and a putative auxin signaling protein. A large number of genes associated with auxin transport and auxin response are differentially expressed in the *la1* mutant compared with the wild type. Thus, the roles of ZmLA1 in maize inflorescence might be indirect. ZmLA1 might function through regulating PAT and/or auxin signaling. In addition, there are probably paralogs or other genes that act redundantly to regulate maize tassel and ear development. Genetic analysis on the interactions between ZmLA1 and genes regulating PAT or auxin signaling (including the newly identified interacting proteins) will shed new light on the roles of ZmLA1 and auxin in inflorescence development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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