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ARTICLE ADDENDUM

Xyloglucans fucosylation defects do not alter plant boundary domain definition

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ABSTRACT

The CUP-SHAPED COTYLEDON (CUC) transcription factors play a fundamental role in plant morphogenesis by defining boundary domains throughout plant development. Despite their central roles in plant development, little is known about the CUC molecular network. In a recent work, we identified a role for MUR1, a protein involved in the production of GDP-L-Fucose, in this network and showed that fucose *per se* is required for proper boundary definition in various developmental contexts. Which pathway involving fucose is required to determine boundary is not yet known. Here, we use a previously described mutant and transgenic line with reduced fucosylated xyloglucans (XyG) to explore one such pathway. By quantitatively comparing leaf shape, we show that defects in XyG fucosylation do not impact leaf serrations development suggesting that fucose absence in XyG does not impact boundary development in *mur1-1* mutant. Thus another – not yet identified – pathway or fucosylated compound contribute to boundary domain definition.

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Boundaries act both as frontiers defining functional units and as organizing centres providing positional clues to control the fate of neighbouring cells.[1,2] Both functions are important to correctly pattern developing organs. Failure to establish and maintain boundaries can result in organ fusion, meristem loss and developmental arrest.[3] Boundaries often display reduced cell proliferation,[4,5] while adjacent tissues or organs actively grow out *via* an auxin-dependent mechanism.[6,7] As boundary definition regulators, CUP-SHAPED COTYLEDON transcription factors are involved in both shoot meristem formation[8] and correct organ separation in various developmental contexts.[3,9,10] In addition, *CUC* genes are key regulators of leaf shape through their roles on leaf margin development.[11–13] They are expressed at the sinus of leaf margin serrations where they are thought to repress growth while allowing it in adjacent serration tips in a manner that is similar to other boundaries. While wild-type Arabidopsis leaves are serrated, *cuc2* loss-of-function mutants have smooth leaves with no serrations.[11,12] Our recent work uses this system and joins the decades long effort to characterize the molecular network centred around the *CUC* genes.[14] In this work we identified a mutation that simplifies leaf dissection and affects the protein encoded by *MURUS1* (*MUR1*),[14] which participates in GDP-L-fucose production.[15] Expression analyses revealed that CUC2 levels are reduced in *mur1* mutant backgrounds. Our study shows that GDP-L-fucose has an important role in different developmental contexts where it contributes to organ separation in the same pathway as CUC2. Key questions remain on how fucose modifies CUC2 expression and how it contributes to boundary definition. Here we expand on our understanding of this role by showing complementary results on the role of fucosylated xyloglucans in leaf margin development.

Fucose is naturally incorporated into various cell wall glycoconjugates such as xyloglucans (XyG), rhamnogalacturonan II (RGII), and arabinogalactans.[16–18] In addition, fucose participates in post-translational protein glycosylation.[19] GDP-L-fucose is synthesised in three steps catalysed by two different enzymes the first of which GDP-D-mannose 4,6-dehydratase is encoded by *MUR1*. Fucose content of cell wall components in the *mur1* mutant is strongly reduced in aerial parts but only partially in root tissues, which may be due to the presence of its homologue *GMD1*.[15,20,21] It has been hypothesized that activity of this homolog together with the residual fucose and/or its replacement by α-L-galactosyl residues in aerial parts is responsible for the weak phenotype of *mur1* mutants.[20,22] Nevertheless incorporation of fucose into the different cell wall components is important for plant development as illustrated by the phenotypes of loss of GDP-L-fucose import into the Golgi lumen,[23] and inhibition of fucosyltransferase activity.[24,25]

Among the diverse roles of GDP-L-Fucose, its impact on XyG structure and its implications in terms of growth are of especial interest. XyG is the most abundant hemicellulose in dicot cell walls and is thought to play a crucial role in cell elongation and cell wall rigidity.[26] XyG molecules can be hydrolysed *in muro* and the resulting oligosaccharides can act as signalling molecules (termed oligosaccharins[27]). Fucosylated XXFG oligosaccharins in particular have been shown to antagonize at low concentration the synthetic auxin 2,4-D-stimulated elongation

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of pea stem segments.[28] Furthermore, a role for fucosylated XyG in an auxin dependent *in muro* remodeling of XyG has been suggested. The overexpression of the xyloglucan fucosyl hydrolase AXY8/FUC95A[29] is able to complement the short hypocotyl phenotype of dark-grown seedlings of transgenic lines impaired in auxin responses while removing XyG fucosyltransferase activity with *mur2*-1 mutation[30] is not.[31] CUC2 and auxin act together to regulate boundary domain formation, lateral organ development and leaf margin development in an intricate feed-back loop mechanism.[6,7,32] Therefore we hypothesized that the decrease in fucosylated XyG and consequent reduced levels of XXFG residues in the *mur1* mutant may result in modified auxin responses and subsequent developmental defects in boundary and leaf development.

To test whether XyG fucosylation impacts leaf margin patterning, we studied two previously reported lines with reduced XyG fucosylation levels: the *35S:AXY8* line overexpressing the fucosidase AXY8/FUC95A[29] and the *mur2-1* mutant line defective for a XyG specific fucosyltransferase.[30] While the near total absence of fucose in the *mur1-1* mutant results in smooth leaf margins compared to the wild-type control in short-day (Fig. 1A), as previously reported in long-day conditions (Gonçalves et al. 2017), neither the *mur2-1* mutation nor the overexpression of AXY8 altered mature leaf shape in short day conditions (Fig. 1A). To compare leaf serration levels between genotypes we calculated the dissection index (DI)[33,34] for leaves 11, 12 and 13 of plants grown in short-day conditions. Our analysis shows that leaf serration as measured by its DI is not significantly reduced in the *mur2-1* mutant or *35S::AXY8* plants when compared to the wild-type, while DI for *mur1-1* is significantly reduced compared to the wild-type (ANOVA, $p < 0.0001$, Fig. 1B).

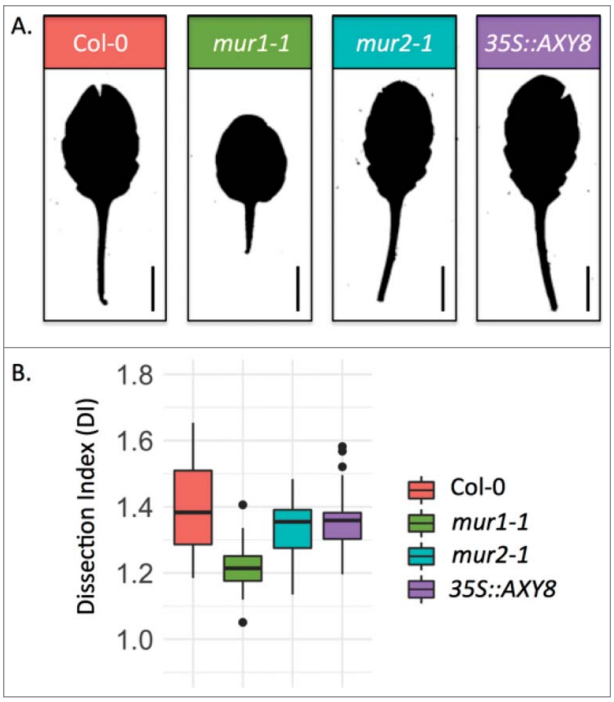

Figure 1. (A) Representative silhouettes of rank 13 leaves from 8 weeks-old plants grown in short-day conditions from Col-0, *mur1-1*, *mur2-1* and *35S::AXY8* plants. (B) Quantification of leaf shape (pooled leaves of rank 11, 12 and 13) from 8 weeks-old plants grown in short-day conditions from Col-0 (n = 27), *mur1-1* (n = 36), *mur2-1* (n = 35) and *35S::AXY8* (n = 33) plants using the Dissection Index as a shape descriptor (DI = leaf perimeter^2/(4π.leaf area).

Together these results show that correct leaf margin patterning can still occur in the absence of XyG fucosylation, suggesting that our previously reported boundary definition defects in the *mur1* mutants are independent of the lack of fucose in XyG. Because GDP-L-fucose deficiency in *mur1* mutants leads to defects in several glycosylation processes, it is probable that another fucosylated compound is responsible for the defects in boundary domain definition in *mur1* mutants. The pectic polymer RGII is a key component of the cell wall and it has been suggested that its cross-linked dimerization has a role in cell wall expansion.[17,35] Interestingly, fucosylation state of RGII has been shown to impact the stability of the borate di-ester facilitated cross-linked dimers.[17] Therefore we can hypothesize that absence of fucose in *mur1* mutants impacts cell wall expansion properties affecting leaf margin development through a reduction of cross-linked RGII. Alternatively it is possible that the fucosylation of a protein within the CUC2 molecular network is required for its function.

Competing interests

The authors declare no competing or financial interests.

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