SUPRA-PHYSIOLOGICAL LEVELS OF GIBBERELLINS / DELLAS MODIFY THE ROOT CELL SIZE / NUMBER AND THE ROOT ARCHITECTURE IN ROOT TIPS OF *A. THALIANA* SEEDLINGS. CONNECTIONS TO THE ROOT HAIR PATTERNING AND ABUNDANCE

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ABSTRACT

A previous study (McCarthy-Suárez, 2021) showed that growing *A. thaliana* seedlings for 5 days under excessive levels of gibberellins (GAs)/DELLAs altered the arrangement, shape and frequency of root hairs in root tips. Because no changes in the distribution or number of root hairs occurred when the gai-1 (gibberellin-insensitive-1) DELLA was over-expressed at the root epidermis, it was concluded that the GAs/DELLAs might regulate the root hair patterning and abundance in *A. thaliana* seedlings by acting from the root sub-epidermal tissues. In the present study, microscopy analyses showed that excessive levels of GAs/DELLAs also modified the size and number of root tip cells in *A. thaliana* seedlings. While excessive DELLAs shortened and widened the root epidermal, cortical, endodermal and pericycle cells, excessive GAs, excepting the epidermal cells, generally narrowed them. However, no changes of root cell size occurred when gai-1 was over-expressed at the root epidermis. In addition, high levels of DELLAs often induced extra cells at the root epidermis, cortex, endodermis and pericycle, whereas high levels of GAs sometimes induced extra cells at the root cortex and pericycle. On the other hand, excessive levels of DELLAs enhanced the outgrowth of lateral roots in root tips, unlike excessive levels of GAs. Thus, the results of this study suggest that supra-physiological levels of GAs/DELLAs might modify the size/number of root tip cells by acting from the root sub-epidermal tissues. This, in turn, might impact on the patterning and abundance of root hairs and on the root architecture.

KEYWORDS

Gibberellins, DELLAs, root cell size, root cell number, root hair patterning, root architecture

1. INTRODUCTION

A previous study showed that supra-physiological levels of GAs/DELLAs altered the patterning, the morphology and the abundance of root hairs in *A. thaliana* seedlings, and that they did it by possibly acting from the sub-epidermal tissues of the root (McCarthy-Suárez, 2021). In fact, the GAs/DELLAs have a role in the production of trichomes (leaf hairs) in *A. thaliana* (Chien and Sussex, 1996; Traw and Bergelson, 2003) and participate in the organization of the cytoskeleton of microtubules (MT) (Locascio *et al.,* 2013), which is essential for trichome and root hair growth, for establishing root cell identity and shape, and for plant cell expansion and division (Bao *et al.,* 2001).

Because auxin, ethylene, abscisic acid, nitric oxide, brassinosteroids, cytokinins and strigolactones regulate the root hair patterning (Cao *et al.,* 1999; Van Hengel *et al.,* 2004; Lombardo *et al.,* 2006; Kappusamy *et al.,* 2009; Niu *et al.,* 2011), and because changes in the levels of these phytohormones are correlated to alterations in the root cell size and number and the root architecture in response to nutritional stresses, such as low availability of P, B or Fe in the soil (e.g. swelling of root cortex cells, induction of extra root cortex cells and production of lateral roots (LR)) (Schmidt *et al.,* 2000; Yang *et al.,* 2007; Martín-Rejano *et al.,* 2011), this study wanted to determine whether supra-physiological levels of GAs/DELLAs might also have an effect on the size/number of root cells, and on the production of LR, in root tips of *A. thaliana* seedlings. To this aim, the size and/or number of root tip cells were examined in Col (0) seedlings grown for 5 days under excessive levels of GAs/DELLAs, as well as in GAs (*QD*, *5X,* GID*1b*-ox) or DELLAs (*gai*-1, *HSp::gai-1*, *pGAI:gai-1:GR*, *SCR::gai-1:GR*)-overproducing mutants. Moreover, the size of root tip cells was examined in 5-day-old mutant seedlings resulting from expressing the *gai-1* (GA-insensitive) DELLA allele in different tissues of the root (UAS (GAL4-UPSTREAM ACTIVATION SEQUENCE) expression directed system lines; Dr. Jim Haselhoff's laboratory). On the other hand, the presence of LR was also analysed in root tips of *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs/DELLAs.

Results of this study suggested that the GAs/DELLAs might have a role in regulating the size, number and organization of root cells, as well as the root architecture, in root tips of *A. thaliana* seedlings.

2. MATERIALS & METHODS

2.1. Plant Material and Growth Conditions

Arabidopsis thaliana Col (0) seeds were sterilized (70 % Ethanol (v/v) and 0.01 % Triton X-100 (v/v)), sown on half-strength MS medium plates (0.8 % (w/v) agar and 1 % (w/v) sucrose), stratified for 3-4 days (4ºC, darkness), germinated, and grown vertically (22 ºC; 5-7 days) under continuous white light (Percival growth chamber E-30B) (http://www.percival-scientific.com) as described by Lee and Schiefelbein (1999).

2.2. Hormone and Chemical treatments

Stock solutions of paclobutrazol (PAC, 10 mM in acetone 100 % (v/v)), GA_4 (1 mM in 100 % ethanol (v/v)) or GA_3 (50 mM in 100% ethanol (v/v)) were conveniently diluted and added to MS agar medium or water (in the case of liquid incubation experiments) to obtain a final concentration of 0.5 µM PAC, 1 µM GA_4, and 30 µM GA_3.

2.3. Mutant Lines

In the previous study, the spatial gene expression of the root non-hair epidermal cell fate marker GL2 in root tips of *A. thaliana* seedlings was studied by using the *GL2pro::GUS* mutant line as well as those derived from crossing lines harbouring constitutively excessive levels of GAs/DELLAs with the *GL2pro::GUS* line (L*er* x *GL2pro::GUS* background). In the present study, the effect of transient increases in the levels of expression of the *gai*-1 (GA-insensitive-1) DELLA alelle on the size and number of root tip cells in *A. thaliana* seedlings was examined by using the *gai*-1 mutant lines of heat-shock inducible *HSp::gai-1* (which over-expresses gai-1 upon heat shock) and dexametasone (DEXA)-inducible *pGAI::gai-1:GR* and *SCR::gai-1:GR* (with glucocorticoid-binding domain). The *HSp::gai-1* mutant seedlings were grown at 37ºC for 4 h (heat-shock) and then at 22ºC for 2 h (recovery period), whereas the *pGAI::gai-1:GR* and *SCR::gai-1:GR* mutant seedlings were incubated in 0.2, 1.2 or 10 µM DEXA for a minimum of 6h. Root cell size and number was also studied in mutants with excessive levels of GAs/DELLAs (*gai*-1, *QD (quadruple DELLA mutant)*, *5X (quintuple DELLA mutant)* and GID1b-*ox* (which over-expresses the GA receptor GID1b (GIBBERELLIN INSENSITIVE DWARF1)), in mutants over-expressing gai-1 in different tissues of the root [*ML1::gai-1* (epidermis) and UAS expression directed system (GAL4-UPSTREAM ACTIVATION SEQUENCE) lines): *UAS::gai-1* x C24 (control, background); *UAS::gai*-1 x J0951 (epidermis of the meristematic zone (MZ)); *UAS::gai*-1 x J2812 (MZ epidermis and cortex); *UAS::gai-1* x N9142 (cortex of elongation zone (EZ)); *UAS::gai-1* x M0018 (MZ cortex and endodermis); *UAS::gai-1* x J0571 (MZ cortex and endodermis); *UAS::gai-1* x Q2393 (all tissues but the endodermis); *UAS::gai-1* x Q2500 (MZ endodermis/pericycle); *UAS::gai-1* x J0121 (EZ pericycle); *UAS::gai-1* x J0631 (all tissues of the EZ); *UAS::gai-1* x J3281 (vessels)], and in the *35S::CPC x GL2pro::GUS* and *scm* (scrambled) x *GL2pro::GUS* mutants.

2.4. GUS activity assay

GUS (β-glucuronidase) staining of the *GL2pro::GUS* reporter line was performed as described by Frigerio *et al.,* (2006), but using 8mM instead of 2 mM potassium ferro/ferricyanide and incubating the seedlings (15 min to 2h) in the reaction mixture at 4 ºC instead of 37 ºC.

2.5. Microscopy

Cell organization at the root tip was studied on ultra-thin cross sections of plastic resin-embedded roots as described by Dr. Schiefelbein Lab Protocols (http://www.mcdb.lsa.umich.edu/labs/schiefel/protocols.html). Seedlings were included in 1% agarose in 0.1M sodium phosphate buffer, pH 6.8, and stained for GUS activity. Root-containing blocks were then cut, fixed with 4% para-formaldehyde in PBS, dehydrated in ethanol series (15%, 30%, 50%, 75%, 95% and 100%, 1h each), kept in 100% ethanol overnight, incubated in Technovit ® 7100 infiltration solution for 2 days, inserted in gelatin capsules, and embedded for 9 days in Technovit ® 7100 plastic resin (Heraeus Kultzer, Germany). Ultramicrotome (Ultracut E, Reichert Jung,

Germany) cross sections of resin-embedded roots were then stained with 0.06% (w/v) toluidine blue and observed under a Nikon Eclipse E600 microscope. The GFP expression in Haselhoff crossed lines was visualized by using a Leica Confocal Microscope (Excitation: 488 nm; Detection: 500-530nm band-path filter for GFP).

3. RESULTS

3.1. Excessive levels of GAs/DELLAs modified the size and number of root tip cells in seedlings of *A. thaliana*:

Apart from altering the patterning, morphology and abundance of root hairs (McCarthy-Suárez, 2021), high levels of GAs/DELLAs also modified the size and number of root tip cells in seedlings of *A. thaliana* (Figs. 1-3; Tables 1-3). While excessive levels of GAs, excepting the epidermal cells, usually narrowed the root cells, excessive levels of DELLAs frequently widened, shortened and twisted the root epidermal, cortical, endodermal and pericycle cells, resulting in wider roots (Figs. 1-3; Tables 1-3).

Moreover, in the *HSp::gai-1* and *SCR::gai-1:GR* mutants, the widening and shortening of the root epidermal, cortical, endodermal and pericycle cells observed at 24h after heat-shock (37ºC, 4h) or after growth in DEXA (10 µM), respectively (Fig. 3), was accompanied by an alteration in the spatial expression of *GL2* and in the distribution of root hairs, as previously reported (McCarthy-Suárez, 2021). Similar changes were observed when gai-1 was over-expressed at the subepidermal tissues of the root (Figs. 1, 2 and 4; Table 1). However, when gai-1 was over-expressed at the root epidermis, no apparent changes occurred in the size of the root epidermal, cortical, endodermal or pericyclic cells (Figs. 1, 2 and 4; Table 1), in the patterning of GL2 gene expression, or the in root hair distribution (McCarthy-Suárez, 2021), what suggested that the changes in root cell size that took place when gai-1 was over-expressed at the sub-epidermal tissues of the root might have been connected to the alterations in the root hair patterning induced by excessive levels of GAs/DELLAs.

Growth of *A. thaliana* seedlings for 5 days under excessive levels of GAs, in contrast, caused the narrowing of the root cortical cells, an effect that was corroborated in the *QD, 5X* and GID1b *ox* mutants (Fig. 2; Table 1). Nevertheless, excessive levels of GAs also seemed to slightly increase the relative width of the root epidermal cells (Figs. 1, 5 and 6). Frequently, under GA_3 (30 µM) or GA_4 (1 µM), changes of cell fate at the root epidermis coincided with changes in the width of the epidermal cells and/or with changes in the size of the root cortical and endodermal cells (Fig. 3; Tables 1 and 2). In fact, estimations of tissue depth in root tips of *A. thaliana* seedlings uncovered the swelling of the root epidermal, cortical, endodermal and pericyclic cells under high levels of DELLAs (PAC) and their slight thinning under high levels of GAs (*5X* mutant) (Table 4). Moreover, growth of seedlings of the *scm* x *GL2pro::GUS* mutant in PAC (0.5 µM) for 5d caused the radial swelling of the epidermal, cortical and endodermal and pericyclic cells of the root tip (Fig. 6). On the other hand, in seedlings of the *35S::CPC* x *GL2pro::GUS* mutant, changes of cell fate at the root epidermis were accompanied by changes in the width of the underlying root epidermal and cortical cells (Fig. 3).

Interestingly, cell size changes in root tips under excessive levels of GAs/DELLAs were often accompanied by the presence of multinucleated cells at the epidermis, cortex, endodermis and pericycle of the root (Fig. 6).

Excessive levels of GAs/DELLAs also modified the radial cell organization in root tips of *A. thaliana* seedlings (Fig. 5). Treatments with PAC (5d, 7d) frequently increased the number of cells at the epidermis, cortex, endodermis and pericycle of the root (Figs. 5 and 6; Table 5) and induced anticlinal /diagonal cell divisions at the root epidermis (T-clones) (Fig. 3) as well as periclinal cell divisions at the root cortex and endodermis (middle cortex (MC)) (Fig. 6). Furthermore, growth of the *scm* x *GL2pro::GUS* mutant for 5 days in PAC induced the proliferation of the root epidermal, cortical and endodermal cells and the formation of a MC (Fig. 6). Treatments with excessive levels of GAs, in turn, sometimes increased the number of cortical and pericycle cells, but not of epidermal cells, in the radial dimension of the root, and induced epidermal T-clones and a MC (Figs. 3, 5 and 6; Table 5). Moreover, the number of root epidermal cells in the *5X* mutant decreased (Fig. 5; Table 5), what in part might have explained the lower abundance of root hairs per root radial section in this mutant (McCarthy-Suárez, 2021). In fact, in root cross sections, frequently only one epidermal cell was seen at the atrichoblast (non-hair) position under high levels of GAs,

whereas up to four cells could be seen under high levels of DELLAs (Fig. 5), in tune with the reduced number of epidermal cells in the *5X* mutant and the increased number of epidermal cells under PAC (Table 5). Furthermore, given that root non-hair cells lay over just one cortical cell, then, the observed increase in the cortex cell width under high levels of DELLAs (Figs. 2, 4 and 5; Table 1) might have accounted for the higher percentage of epidermal cells at the atrichoblast position, as well as the lower percentage of epidermal cells at the trichoblast position, detected per radial section of the root (McCarthy-Suárez, 2021). Conversely, the decrease in the width of the cortex cells seen under high levels of GAs (*5X* mutant) (Fig. 2; Table 1) might have explained the lower percentage of epidermal cells at the atrichoblast position, and the higher percentage of epidermal cells at the trichoblast position, found per radial section of the root (McCarthy-Suárez, 2021). Nevertheless, considering that the average number of epidermal cells per root radial section increased under high levels of DELLAs (PAC, *gai*-1) and decreased under high levels of GAs (*5X* mutant) (McCarthy-Suárez, 2021), then, the predicted number of epidermal cells at the trichoblast position did not change under excessive levels of GAs/DELLAs in 5 day-old *A. thaliana* seedlings (McCarthy-Suárez, 2021).

The root diameter at the MZ, in addition, increased by 40% under excessive levels of DELLAs (Table 3), in tune with the increased number of cells at the root epidermis, cortex, endodermis and pericycle (Fig. 5; Table 5), and the wider and/or deeper cells at the root cortex, endodermis and pericycle (Figs. 5 and 6; Tables 1 and 4). Conversely, under excessive levels of GAs, the root diameter decreased (Table 3), in accordance with the lower number of cells at the root epidermis (*5X* mutant) (Table 5), and the narrower and shallower cells at the root cortex and endodermis (Figs. 2, 5 and 6; Tables 1 and 4). Nevertheless, at the MZ-EZ transition zone, the root also seemed to swell under excessive levels of GAs, and there was variability of cell sizes (McCarthy-Suárez, 2021), maybe due to the swelling of the epidermal cells and to the deepening of the pericycle cells (Fig. 1; Table 4). In fact, GA_3 treatments have been shown to increase the ratio of (xilem/whole root) area (Wang *et al.,* 2015).

3.2. Excessive levels of GAs/DELLAs modified the outgrowth of lateral roots in root tips of *A. thaliana* seedlings:

Excessive levels of DELLAs also induced the outburst of LR near the root tip in *A. thaliana* seedlings, whereas excessive levels of GAs inhibited it (Fig. 7).

4. DISCUSSION

4.1. The GAs/DELLAs might regulate the size and number of root tip cells in seedlings of *A. thaliana*:

4.1.1. Root cell size. Connections to the root hair patterning and abundance:

Apart from altering the patterning, morphology and abundance of root hairs (McCarthy-Suárez, 2021), results of the present study suggest that excessive levels of GAs/DELLAs also modified the size of root tip cells in seedlings of *A. thaliana*. While excessive levels of DELLAs frequently shortened and widened the epidermal, cortical, endodermal and pericyclic cells of the root, what resulted in wider root tips, excessive levels of GAs, with the exception of the epidermal cells, often narrowed them. Thus, because root hair cells are shorter than the root non-hair cells (Salazar-Henao *et al.,* 2016), then, the inhibition of epidermal cell elongation that occurred when *gai-1* was over-expressed in tissues placed underneath the epidermis of the MZ (J2812 >> *gai-1,* J0571 >> *gai-1*, M0018 >> *gai-1*, Q2500 >> *gai-1* or Q2393 >> *gai-1* lines) or in all tissues of the EZ (J0631 >> *gai-1* line) might have contributed, in part, to the appearance of ectopic root hairs, and, therefore, to the higher density of root hairs observed near the root tip under excessive levels of DELLAs (McCarthy-Suárez, 2021). In fact, *Arabidopsis* increases root hair density by decreasing the length of root epidermal cells, as shown under P deficiency (Jiang *et al.,* 2007; Péret *et al.,* 2011; Salazar-Henao *et al.,* 2016; Janes *et al.,* 2018). Alternatively, given that GAs promote the elongation of root epidermal cells, accumulate at the endodermis of the root EZ, and affect the expansion of root EZ cells by destabilizing DELLAs and inducing expansin genes (Ubeda-Tomás *et al.,* 2008, 2009; Gou *et al.,* 2010; Bahin *et al.,* 2011; Shani *et al.,* 2013), then, the extra elongation of root epidermal cells which is known to occur under high levels of GAs (Band *et al.,* 2012) might have contributed, in turn, to the lower density of

root hairs that was observed at the root tip under this treatment (McCarthy-Suárez, 2021). In fact, in WT, cell expansion at the root EZ is strictly polar and is not accompanied by an increase in the root diameter (Bao *et al.,* 2001).

Interestingly, epidermal patterning genes instruct epidermal cell size (Löfke *et al.,* 2013). In turn, variations in the expression of HDA 19 (histidine deacetylase 19), which controls epidermal cell elongation, affect root cell elongation and, thus, root hair density (Chen *et al.,* 2015; Salazar-Henao *et al.,* 2016). In this study, however, neither the patterning or abundance of root hairs (McCarthy-Suárez, 2021), nor the epidermal cell length suffered alterations when *gai-1* was over-expressed at the root epidermis (Fig. 4), what suggests that the changes of epidermal cell size that were induced by excessive levels of GAs/DELLAs in roots of *A. thaliana* seedlings (Fig. 1) might have been orchestrated from the sub-epidermal tissues of the root. Furthermore, Wild *et al.* (2016) showed that expressing *gai-1* at the root epidermis did not affect the root length.

Results of this study also seem to point at a link between cell size and cell fate, because expressing *gai-1* at the EZ of the root (J0631 >> *gai-1*) caused the shortening and widening of the majority of the root cells (Fig. 4), along with a hairy phenotype similar to that of the *wer* mutant (McCarthy-Suárez, 2021). In fact, the DELLAs inhibit the elongation of the root EZ cells and of the primary root (Alonso-Ramírez *et al.,* 2009; Ubeda-Tomás *et al.,* 2009; Lee *et al.,* 2012) and down-regulate PIF4, a phytochrome-interacting factor which induces cell elongation genes (Achard and Genschik, 2009). Deficiencies in B, P or Fe, which increase the levels of DELLAs at the root MZ and induce ectopic root hairs, also reduce the primary root length (Martín-Rejano *et al.,* 2011; Péret *et al.*, 2011; Wild *et al.,* 2016). Furthermore, it has been suggested that production of ectopic root hairs in ectopic root hair 2 (*erh2)* occurs at late stages of root development, correlated with cell expansion. However, it has also been suggested the independence of root hair initiation from cell expansion, as *erh3* acts as soon as cell fate specification (Schneider *et al.,* 1997). Particularly, ERH3 is required for the stable fixation of positional signals at the cell wall (CW) for cell fate specification. Moreover, ERH3 codifies a MT-severing p60 katanin protein and has a role in CW biosynthesis (Webb *et al.,* 2002). On the other hand, given that the expression of cell identity markers is altered in *erh3*, it has been suggested that MT are directly active in the specification of root cell identity, and that MT disruption in *erh3* results in the development of defective identities (Webb *et al.,* 2002). In fact, in several animal systems, MT are involved in the specification of cell identity and polarity (Webb *et al.,* 2002).

Shortening and radial expansion of root cortical and endodermal cells has also been reported in the *cobra, pom-1* (both cellulose deficient), *shoebox* (GA biosynthesis-impaired), *dgl1* (GA-insensitive), TUA6/AS (α-tubulin-deficient), *erh, sabre, PLD* (phospholipase D), *wer, scm* and *jkd* mutants, in plants treated with umbelliferone (a cellulose biosynthesis inhibitor), MT-breaking drugs or 1-butanol (an inhibitor of PLD), and in plants stressed by salinity, gamma irradiation or mineral deficiency (Fe, P) (Jankay and Muller, 1976; Schiefelbein *et al.,* 1997; Schneider *et al.,* 1997; Bao *et al.,* 2001; Ma *et al.,* 2001; Scheres *et al.,* 2002; Gardiner *et al.,* 2003; Nagata *et al.,* 2004; Komorisono *et al.,* 2005; Welch *et al.,* 2007; Dinneny *et al.,* 2008; Pietra *et al.,* 2015; Janes *et al.,* 2018). Cortical cell expansion as a result of excessive DELLAs has also been described by Benfey *et al.,* (1993). Interestingly, PAC induces the expression of expansin genes at the root cortex (At4g21280 (+2,002); Arex data), being *EXPANSIN 7* a specific marker of root hair cells (Ohashi *et al.,* 2003; Gendre *et al.,* 2019). In addition, it has been suggested that the DELLA GAI might have a role in the expansion of endodermal cells in *Arabidopsis* primary roots, and that the expansion of endodermal cells determines the elongation of whole roots (Ubeda-Tomás *et al.,* 2009; Zhang *et al.,* 2014). On the other hand, the GAs control the size of the root apical meristem (RAM) in *Arabidopsis* by affecting cortical cell expansion (Nelissen *et al.,* 2012; Fonouni-Farde *et al.,* 2019). In fact, for different accessions of *Arabidopsis,* there is a correlation between cortex cell length and the length of the root MZ (Zhang *et al.,* 2014). Furthermore, when *gai-1* is over-expressed at the root endodermis (the most important tissue for GA-dependent root growth), the cessation of anisotropic cell growth expands radially the cortical cells and causes the outward protrusion of epidermal cells (Ubeda-Tomás *et al.,* 2008). With this regard, it is known that the endodermis of the EZ regulates nutrient uptake (Péret *et al.,* 2011; Shani *et al.,* 2013; Cui, 2015), whereas the cortex participates in the root response to P deficiency (Shin *et al.,* 2005).

Therefore, the shortening and widening of the root epidermal, cortical, endodermal and pericycle cells induced by excessive levels of DELLAs in seedlings of *A. thaliana* might have explained the radial expansion of the root tips observed. Interestingly, treatment with PAC also increased the root diameter in carrot (Wang *et al.,* 2015). In fact, in this study, root tips became thinner under excessive GAs (Table 3), as previously reported in carrot and *Eucalyptus grandis* (Wang *et al.,* 2015; Liu *et al.,* 2018). A wider root

diameter has also been described in the *arm, sabre, cobra, erh-1, pom-pom1* and α-tubulin under-expressing mutants, and in plants exposed to 1-butanol, umbelliferone, gamma irradiation or P deficiency (Jankay & Muller, 1976; Schneider *et al.,* 1997; Bao *et al.,* 2001; Ma *et al.,* 2001; Gardiner *et al.,* 2003; Nagata *et al.,* 2004; Hermans *et al.,* 2010; Pietra, 2014). Moreover, the reduction of the actin cytoskeleton induces the radial expansion of plant cells, making them shorter and wider, as the interphase MT determine the direction of plant cell elongation (Baluška *et al.,* 2001; Bao *et al.,* 2001). This means that the capacity of cells to elongate longitudinally depends on the orientation of the cytoskeletal MT (Dugardeyn and Van Der Straeten, 2008). Thus, a reduced expression of the α-tubulin gene in *A. thaliana* seedlings results in an abnormal expansion of the root tip (MZ and EZ), with its diameter increasing dramatically at 8 days after germination (Bao *et al.,* 2001). De-polymerization of MT by oryzalin or 1-butanol also causes the swelling of the root MZ and EZ in *A. thaliana* seedlings, whereas MT stabilization by taxol expands the root EZ and DZ (Bao *et al.,* 2001; Gardiner *et al.,* 2003).

Therefore, a connection exists between aberrant orientation of MT and reduced cell elongation, as MT regulate the oriented deposition of cellulose microfibrills that determines the direction of cell elongation (Burk and Ye, 2002). More specifically, MT are essential for anisotropic cell expansion because they direct the insertion of cellulose synthase in the CW and guide the orientation of cellulose microfibrills to a perpendicular position with respect to the growth axis, thereby restricting radial cell expansion (Jankay and Muller 1976; Lin *et al.,* 2013). Interestingly, mutations in P60 katanin protein, essential for anisotropic cell growth, cause an inappropriate feedback regulation of the *DGL1* gen for GAs biosynthesis (Komorisono *et al.,* 2005).

With this respect, a link has been proposed between aberrant orientation of MT, radial cell growth and altered root hair patterning (Baluška *et al.,* 2001; Bao *et al.,* 2001). Thus, a low expression of the α-tubulin gene (TUA6/AS transgenic lines), mutations that inhibit MT polymerization or drugs that brake the actin MT produce aberrant microtubular structures, expand radially the root tip cells, especially at the epidermis and cortex of the MZ and EZ, and induce ectopic root hairs in 5 day-old *A. thaliana* seedlings (Bao *et al.,* 2001; Collings *et al.,* 2006). On the other hand, the *erh1* and *erh3* mutants, with an altered root hair patterning, exhibit disorganized MT and radially-enlarged layers of root cortex and endodermis, what suggests a connection between radial cell expansion and root hair initiation (Schneider *et al.,* 1997; Bouquin *et al.,* 2002; Müller and Schmidt, 2004; Pietra *et al.,* 2015). Moreover, *erh2* is allelic to *pom-1*, a mutant with abnormally-expanded layers of root epidermis and cortex (Schneider *et al.,* 1997; Pietra *et al.,* 2015). In fact, not only cell length, but also cell width differs between trichoblasts and atrichoblasts (Löfke *et al.,* 2013). Deficiencies in Fe or P also induce the swelling of root cortical cells, along with ectopic root hairs (Pietra *et al.,* 2015). Another clue about the link among MT, cell expansion and cell fate is illustrated by the mutants *cobra* and *sabre*, both with an abnormal cell expansion at the root tip and ectopic root hairs (Schiefelbein *et al.,* 1997). The mutation of the SABRE protein, involved in MT organization, causes an abnormal cell expansion at the root cortex (Benfey *et al.,* 1993), whereas the mutation of the COBRA protein, which is associated to the longitudinal CW of the rapidly-growing root EZ, entails a cellulose deficiency and causes the swelling of the root epidermis and cortex (Scheres *et al.,* 2002). Interestingly, the GAs influence CW growth in mesocotyl epidermal cells (Perazza *et al.,* 1998). Therefore, under the experimental conditions of the present study, excessive levels of DELLAs might have impaired the biosynthesis, organization and/or homoeostasis of MT in root tip cells of *A. thaliana* seedlings, and this, in turn, might have caused the inhibition of cell elongation and the altered patterning of GL2 and root hairs at the MZ and EZ of the root. In fact, the DELLAS destabilize the MT, giving rise to non-polar cell growth (Locascio *et al.,* 2013).

In this respect, it is known that the levels of ploidy exert an important control over cell size, and that cell size and morphology are, in turn, linked to DNA content (Kondorosi *et al.,* 2001). Moreover, in many tissues, cell elongation is associated to the endo-reduplication of the DNA (replication without mitosis that occurs before cell elongation, resulting in a logarithmic accumulation of genome copies in each nucleus) (Sanz *et al.,* 2012). Thus, the earliest morphological signs in trichome initiation are the induction of endo-reduplication and the increase in nuclear and cellular size (Perazza *et al.,* 1998). With this regard, it is known that the GAs induce endo-reduplication in a dose-dependent manner and regulate cyclin gene expression. In fact, trichomes in the *spy5* mutant have two times more DNA than WT trichomes (Perazza *et al.,* 1998; Kondorosi *et al.,* 2001). Moreover, in GA-deficient transgenic plants, the observed root swelling, due to MT disorganization, is associated to the induction and accumulation of cyclin CYC3;1 and CYCB1;1 proteins, because the DELLAs are involved in cell cycle progression (Ubeda-Tomás *et al.,* 2009; Gou *et al.,* 2010; Sánchez-Calderón *et al.,* 2013). In addition, the halting degree of the cell cycle is related to the GA

endogenous level (Li *et al.,* 2015b). Mutations in the α-tubulin gene and drugs that inhibit MT polymerization also induce multi-nucleated cells (Bao *et al.,* 2001). Interestingly, the control of endo-reduplication in trichomes participates in the regulation of epidermal patterning (Pietra *et al.,* 2015), whereas the *RHL* genes, related to endo-reduplication, affect the fate of root epidermal cells independently from the *GL2* gene network (Guo *et al.,* 2009).

Given that stress reduces root cell length and, thus, root length (Dinneny *et al.,* 2008), then, the morphological alterations that were observed in the root cells under excessive levels of DELLAs in 5-day-old *A. thaliana* seedlings might be in tune with the known role of these proteins as mediators of the Stress-Induced Morphogenic Responses (SIMR) in plants, which are characterized by changes in MT metabolism, CW flexibility and cell cycle progression (Potters *et al.,* 2007). Moreover, it is known that stress inhibits growth by reducing GA levels and promoting the stabilization and accumulation of DELLAs (Achard and Genschik, 2009; Alonso-Ramírez *et al.,* 2009), and that the DELLAs mediate the SIMR associated to P deficiency (Jiang *et al.,* 2007). From this, it might be hypothesized that an alteration of MT homoeostasis might have been implicated in the cell size changes that were observed in roots of *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. While excessive levels of DELLAs might have disorganized the MT cytoskeleton, excessive levels of GAs might have stabilized it, giving rise to the changes of cell size and cell fate observed at the MZ and EZ of the root.

Results also suggest that cell fate decisions at the root epidermis might be synchronized with the cell size changes at the inner tissues of the root, such as the cortex. Thus, another reason for the appearance of extra root hairs in *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs/DELLAs might have been the reduction of the ratios of cortical/epidermal cell length (e.g. under excessive DELLAs, which decrease the cortex cell length) and of cortical/epidermal cell width (e.g. under excessive GAs, which decrease the cortex cell width), as they might give rise to epidermal cells laying over two cortical intersections instead of one, and, hence, to two-haired epidermal cells. This would imply that not only the length and width of epidermal cells, but also the length and width of cortical cells might contribute to the number of hairs produced by the root, although additional studies are needed to confirm this hypothesis.

On the other hand, results of this study also point at the cortex, endodermis and pericycle as root tissues from which the GAs/DELLAs might influence the root hair patterning, because trangenic lines over-expressing GAI at these root tissues produced ectopic root hairs and non-hairs (McCarthy-Suárez, 2021). In fact, it has been shown that blocking GAs signalling at the root endodermis induces morphological defects in the root epidermal cells (Löfke *et al.,* 2013; Pietra *et al.,* 2015; Janes *et al.,* 2018).

4.1.2. Root cell number. Connections to the root hair patterning and abundance:

Excessive levels of GAs/DELLAs also altered the radial cell organization in root tips of seedlings of *A. thaliana*. While excessive levels of DELLAs frequently induced additional cells at the epidermis, cortex, endodermis and pericycle of the root, excessive levels of GAs sometimes induced extra cells at the root cortex and pericycle. With this respect, whether the cell proliferation at the root cortex-endodermis-pericycle under excessive levels of DELLAs was another reason for the observed disorganization in the root hair patterning (McCarthy-Suárez, 2021), it might be worth confirming in future experiments by using inhibitors and/or mutants of cell division.

Regarding the epidermis, interestingly, the predicted number of cells at the atrichoblast position per root radial section increased under excessive DELLAs (PAC, *gai*-1), but decreased under excessive GAs (*5X* mutant) (McCarthy-Suárez, 2021). Nevertheless, as the predicted number of cells at the trichoblast position did not change, and the percentage of ectopic root hairs was higher under excessive DELLAs as compared to excessive GAs (McCarthy-Suárez, 2021), then, the higher abundance of root hairs seen under excessive DELLAs in comparison to excessive GAs was probably due to the induction of ectopic root hairs —and, thus, to the higher number of cells at the atrichoblast position— and not because of the appearance of new trichoblast positions, given that the number of cells at the trichoblast position remained unchanged (McCarthy-Suárez, 2021).

In fact, *Arabidopsis* increases root hair density in the radial dimension by increasing the number of epidermal cells that differentiate into root hair cells (Janes *et al.,* 2018). An increased number of epidermal cells in the radial domain of the root has also been described under P deficiency and in *tip1* mutants (Ma *et al.,* 2001; Grierson and Schiefelbein, 2002; Müller and Schmidt, 2004). However, under P deficiency, the

extra epidermal cells at the trichoblast position (up to 12) do not increase the abundance of root hairs in the radial axis, as ectopic non-hairs also appear (Ma *et al.,* 2001; Janes *et al.,* 2018). Interestingly, a distorted radial patterning of root cells has also been described in mutants of WRKY75, a negative regulator of root hair formation (Rishmawi *et al.,* 2014).

Radial proliferation of the root cortex cells has also been reported under stress (e.g. P deficiency) as well as in *tip1*, *erh3* and *jkd* mutants, all with an altered root hair patterning (Ma *et al.,* 2001; Müller and Schmidt, 2004; Hassan *et al.,* 2010; Cui, 2015; Janes *et al.,* 2018). Periclinal cell divisions (extra layers) of the root cortex have equally been described in mutants of JKD, which acts from the root cortex to specify the patterning of epidermal cell types (Welch *et al.,* 2007; Lyer-Pascuzzi and Benfey, 2008; Hassan *et al.,* 2010). Interestingly, the GAs restrict the production of extra cortex cell layers in *Medicago truncatula* roots, thereby generating thinner roots (Fonouni-Farde *et al.,* 2019). In contrast, PAC treatments, or mutations in components of GA signalling, increase the number of layers of root cortex cells, that is, they induce a premature middle cortex (MC) (Paquette and Benfey, 2005; Cui & Benfey, 2009). Moreover, the GAs suppress the MC formation that is proper of the root responses to stress, whereas the DELLAs promote it (Cui and Benfey, 2009; Fonouni-Farde *et al.,* 2019). Thus, the formation of a MC, due to random and periclinal cell divisions at the root endodermis, and that later on will acquire identity of root cortex, has been described under P deficiency (Cui and Benfey, 2009; Janes *et al.,* 2018). Although the production of a MC has also been reported in roots of 3-day-old WT *A. thaliana* seedlings, the presence of a premature MC in the *spy* mutant, with high levels of GAs, suggests that imbalances in GAs/DELLAs homoeostasis, which can be triggered by stress, might bring about the formation of a MC (Cui and Benfey, 2009; Cui, 2015). Maybe this was the reason, in this study, for the presence of a MC in roots of *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs (1 µM GA_4 and *QD*) (Figs. 5 and 6).

Because the PAC-inducible MC phenotype is also present in the *scr* (scarecrow) and HDA mutants, as well as in trichostatin A (TSA)-treated plants, all producing ectopic root hairs (Cui and Benfey, 2009), then, a possible link between MC formation (or ectopic cell proliferation at the cortex/endodermis) and alteration of the root hair patterning might be established. Whether this contributed to the disorganisation of the root hair patterning observed under excessive levels of DELLAs, where a cell proliferation was equally observed at the cortex/endodermis/pericycle of the root, it is not known, but might be worth studying in future experiments by using cell division inhibitors and/or mutants. In fact, the alteration of the root hair patterning in the *SCR::gai-1:GR* mutant after growth in DEXA (McCarthy-Suárez, 2021) was accompanied by random and periclinal cell divisions at the root endodermis (Fig. 3). Moreover, because the root MZ and EZ constitute cell fate-decision zones in *Arabidopsis*, then, any changes in cell division at tissues placed underneath the epidermis of the MZ/EZ might bring about changes in epidermal cell fate. In fact, the proliferation of cortex cells is known to influence the root epidermal patterning (Löfke *et al.,* 2013; Pietra *et al.,* 2015; Janes *et al.,* 2018). Interestingly, histone deacetylation, which affects the root hair patterning, has a role in the proliferation of root cortex cells (Xu *et al.,* 2005; Li *et al.,* 2015a). Increases in the number of root endodermal cells have also been reported in *erh* mutants, during P deficiency, and in *rhizobium*-infected plants (Müller and Schmidt, 2004; Ma *et al.,* 2001; Janes *et al.,* 2018). The schizorriza (*scz*) mutant, in turn, has defects in the root radial patterning, with extra periclinal cell divisions that result in multiple layers of ground tissue (cortex and endodermis) (Mylona *et al.,* 2002). Thus, the results of this study suggest that the alterations in the root hair patterning of *A. thaliana* seedlings grown under excessive levels of GAs/DELLAs might also have been related to changes in the number of the cortical/endodermal/pericycle cells of the root.

Other possible cause, in this study, for the appearance of ectopic root hairs might have been the anticlinal, diagonal or asymmetric cell divisions (T-clones) frequently observed under excessive levels of GAs/DELLAs at the root epidermis, as they gave rise to changes in the *GL2pro::GUS* patterning and the size of daughter cells (Fig. 3). These T-clones, in turn, might have been linked to alterations in the MT cytoskeleton, as MT are required for the correct positioning of cell division planes (Scheres and Benfey, 1999; Bao *et al.,* 2001; Rodriguez-Serrano *et al.,* 2014). Moreover, a reduced expression of the α-tubulin gene impairs cell division and results in defects of tissue organization at the root tip (Bao *et al.,* 2001). Also, the regulation of asymmetric cell divisions in plants is necessary for the generation of cell diversity and patterns (Pernas *et al.,* 2010). In fact, root hair cells are shorter than root non-hair cells, so that when an asymmetric cell division takes place at the epidermis of the MZ, the larger cell becomes the root non-hair cell (Salazar-Henao *et al.,* 2016). With this respect, it is known that the GAs induce cell proliferation at the root MZ and promote the division of epidermal cells (Ubeda-Tomás *et al.,* 2009; Lee *et al.,* 2012). Furthermore, the DELLAs inhibit root cell division in the longitudinal dimension when mediating the SIMR

associated to P deficiency (Jiang *et al.,* 2007; Péret *et al.,* 2011). Interestingly, in a dwarf GA-deficient mutant, the MT exhibit a oblique orientation (Bouquin *et al.,* 2002). Also, in the *erh3* mutants, which act in the same route as *cpc* and *rhd6* (root hair defective 6), but independently from WER, the CW are dis-aligned, diagonally orientated, and malformed, that is, the positioning of the cell plates and the CW is abnormal, what indicates that ERH3 participates in orienting the cell plates during cytokinesis (Webb *et al.*, 2002). The cortex-associated SABRE protein is also involved in the orientation of cell division planes (Pietra, 2014; Pietra *et al.,* 2013, 2015). In addition, GL2 is involved in the production of T-clones, whereas WER regulates cell proliferation, what suggests that genes that regulate cell specification also regulate cell division planes (Lee & Schiefelbein, 1999; Scheres *et al.,* 2002).

Thus, these results suggest that changes in cell number induced by excessive levels of GAs/DELLAs at the epidermis, cortex, endodermis and pericycle of the root tip in *A. thaliana* seedlings might influence the root hair patterning. Moreover, changes in cell number at tissues placed underneath the root epidermis might bring changes of cell fate at the root epidermal cells.

In addition, results of this study suggest that excessive levels of DELLAs in roots of *A. thaliana* seedlings might impair the biosynthesis and/or the assembly of MT, as judged by the swelling of the root tip cells (Figs. 1-4), the presence of multi-nucleated cells at the MZ (Fig. 6), as well as by the occurrence of ectopic root hairs, branched root hairs, and cells with multiple root hairs (McCarthy-Suárez, 2021). In fact, a reduced expression of the α-tubulin gene results in the disassembly and aberrant reorganization of MT (Bao *et al.,* 2001). This means that the alteration of the root hair patterning in *A. thaliana* seedlings by excessive levels of DELLAs might have been correlated to their inhibitory effect on MT organisation. In fact, MT are essential to stablish root cell identity in *Arabidopsis* (Webb *et al.,* 2002).

4.2. The GAs/DELLAs might regulate the root architecture in *A. thaliana* seedlings:

Excessive levels of DELLAs also promoted the outburst of LR near the root tip in *A. thaliana* seedlings, whereas excessive levels of GAs inhibited it. This means that any alteration in the levels of GAs/DELLAs might affect not only the root hair patterning, morphology and abundance, but also the root architecture. Thus, in seedlings of *A. thaliana,* physiologically-controlled levels of GAs/DELLAs might have a function in establishing a correct patterning and morphology of root hairs as well as in organising a proper root structure. In fact, supra-physiological levels of DELLAs mediate the root architecture changes that are associated to abiotic stress in plants (i.e., root elongation inhibition, root radial expansion, MC formation, pericycle cell proliferation and LR induction) (Yih and Clark, 1965; Jiang *et al.,* 2007; Gou *et al.,* 2010; Martín-Rejano *et al.,* 2011; Péret *et al.,* 2011; Cui, 2015; Wild *et al.,* 2016). For example, soil deficiencies of P, B, Fe or NO_3^- stimulate LR production, as nutrient concentration regulates LR production (Yih and Clark, 1965; Hermans *et al.,* 2010; Zhang *et al.,* 2014). Early production of LR has also been reported in the *erh1, jkd* and *arm* mutants, equally with a shortened primary root (Schneider *et al.,* 1997; Welch *et al.,* 2007). Interestingly, LR formation, which initiates at the pericycle, is correlated to an alteration in actin and tubulin expression (Pasternak *et al.,* 2005; Péret *et al.,* 2011; Sánchez-Calderón *et al.,* 2013). Therefore, promoting LR outburst by increasing the local levels of DELLAs in roots might constitute a mechanism used by plants to increase the specific area of the root per mass unit, in a similar way as branched root hairs do.

In conclusion, as it was previously reported for other hormones, the results of this study, and of a previous study (McCarthy-Suárez, 202?), point to a possible role for the GAs/DELLAs in mediating the changes in the distribution, shape and frecuency of root hairs, as well as in the root configuration, that take place in plants under stress situations. Moreover, the auxins, ET, ABA, BRs and SLs mediate these changes without altering the quantitative expression of *WER* and *GL2* (Schiefelbein, 2003; Yang *et al.,* 2007; Martín-Rejano *et al.,* 2011). This implies that, by regulating the elongation and/or division of root cells, as well as the production of LR, the GAs/DELLAs might be potential mediators of the changes in the root hair patterning, morphology and abundance, and of the changes in the root architecture, occurring in plants under environmental stress conditions.

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Figure legends

Fig 1. *Excessive levels of GAs/DELLAs altered the size of root epidermal cells in root tips of 5-day-old A. thaliana seedlings.* **A)** Col(0) (MS); **B)** Col(0) (0.5 µM PAC); **C)** Col(0) (30 µM GA_3); **D)** Col (0) (1 µM GA_4); **E)** L*er*; **F)** *gai-1*; **G)** *QD*; **H)** *5X*; **I)** GID1b-*ox* (MS); **J)** *HSp::gai-1* (22ºC, 4h); **K)** *HSp::gai-1* (immediately after heat shock (37ºC, 4h); **L)** *HSp::gai-1* (24h after heat-shock (37ºC, 4h); **M)** *HSp::gai-1* (48h after heat-shock (37ºC, 4h); **N)** *pGAI::gai-1:GR* (MS); **O)** *pGAI::gai-1:GR* (10 µM DEXA); **P)** *SCR::gai-1:GR* (MS, leaky line); **Q)** *SCR::gai-1:GR* (10 µM DEXA); **R)** *UAS::gai-1* x C24; **S)** *UAS::gai-1* x J0951; **T)** *UAS::gai-1* x J2812; **U)** *UAS::gai-1* x J0571; **V)** *UAS::gai-1* x Q2500; **W)** *UAS::gai-1* x J0121; **X)** *UAS::gai-1* x J0631; **Y)** *UAS::gai-1* x J3281. Magnification: 20X. Propidium iodide staining.

Fig 2. *Excessive levels of GAs/DELLAs altered the size of root cortical cells in root tips of 5-day-old A. thaliana seedlings.* **A)** Col(0) (MS); **B)** Col(0) (0.5 µM PAC); **C)** Col(0) (1 µM GA_4); **D)** L*er*; **E)** *gai-1*; **F)** *QD*; **G)** *5X*; **H)** GID*1b-ox;* **I)** *pGAI::gai-1:GR* (3d in MS); **J)** *pGAI::gai-1:GR* (3d in 10 µM DEXA); **K)** *SCR::gai-1:GR* (3d in MS); **L)** *SCR::gai-1:GR* (3d in 10 µM DEXA); **M)** *HSp::gai-1* x *GL2pro::GUS* (24h after 22ºC for 4h); **N)** *HSp::gai-1* x *GL2pro::GUS* (24h after heat-shock (37ºC for 4h)); **O)** *GL2pro::GUS* (heat-shock control) (24h after 22ºC for 4h); **P)** *GL2pro::GUS* (heat-shock control) (24h after heat shock (37ºC for 4h)); **Q)** *UAS::gai-1* x C24; **R)** *ML1::gai-1*; **S)** *UAS::gai-1* x J2812; **T)** *UAS::gai-1* x M0018; **U)** *UAS::gai-1* x Q2500; **V)** *UAS::gai-1* x N9142; **W)** *UAS::gai-1* x J0121; **X)** *UAS::gai-1* x 0631; **Y)** *UAS::gai-1* x J3281. Magnification: 20X. Propidium iodide staining.

Fig. 3. *Excessive levels of GAs/DELLAs induced cell size changes and T-clones at the epidermis, cortex and endodermis of root tips of 5-day-old A. thaliana seedlings.* **A)** *GL2pro::GUS* (30 µM GA_3) (all layers): expansion of an epidermal cell and narrowing of a cortical cell coincide with a change of epidermal cell fate; **B)** *35S::CPC* x *GL2pro::GUS* (epidermis): A change in epidermal cell size coincides with a change in epidermal cell fate; **C)** *35S::CPC* x *GL2pro::GUS* (cortex): A change in cortical cell width coincides with a

change in epidermal cell fate; **D)** *35S::CPC* x *GL2pro::GUS* (all layers): epidermis and cortex vary in cell size; **E)** *HSp::gai-1* x *GL2pro::GUS* (48h after 22ºC for 4h) (all layers), 20X; **F)** *HSp::gai-1* x *GL2pro::GUS* (48h after heat-shock (37ºC, 4h) (all layers): swelling of the root epidermal, cortical, endodermal and pericyclic cells, 20X; **G)** *SCR::gai-1:GR* (MS) (all layers), 20X; **H)** *SCR::gai-1:GR* x *GL2pro::GUS* (24h in 10 µM DEXA) (all layers): swelling of the root cortex, endodermis and pericycle, 20X; **I)** Epidermal T-clones in PAC (0.5 µM), 40X; **J)** Epidermal T-clones in GA_3 (30 µM), 40X; **K)** *SCR::gai-1:GR* (0.2 µM DEXA): epidermal T-clones, 20X; **L)** *SCR::gai-1:GR* (1.2 µM DEXA): periclinal cell division at the cortex, 20X; **M)** *SCR::gai-1:GR* (MS) (all layers), 40X: arrow on endodermis; **N)** *SCR::gai-1:GR* (10 µM DEXA) (all layers): periclinal cell divisions at the endodermis, 40X; **O)** *SCR::gai-1:GR* (10 µM DEXA) (all layers): periclinal cell divisions at the endodermis, 40X; Propidium iodide or GUS staining.

Fig. 4. *Over-expression of* gai-1 *in different tissues of the root modified the cell size in root tips of 5-day-old A. thaliana seedlings.* **A)** *UAS::gai-1* x C24 (Background); **B)** *UAS::gai-1* x J0951 (epidermis of MZ); **C)** *UAS::gai-1* x J2812 (epidermis and cortex of the MZ); **D)** *UAS::gai-1* x N9142 (cortex of EZ); **E)** *UAS::gai-1* x J0571 (cortex and endodermis); **F)** *UAS::gai-1* x Q2500 (endodermis/pericycle of MZ); **G)** *UAS::gai-1* x J0121 (pericycle of EZ); **H)** *UAS::gai-1* x Q2393 (all tissues but the endodermis); **I)** *UAS::gai-1* x J0631 (elongating tissues); **J)** *UAS::gai-1* x J3281 (vessels). Magnification: 40X. Propidium iodide staining.

Fig. 5. *Radial cell organization in root tips of 5 or 7 day-old A. thaliana seedlings grown under (or harbouring) excessive levels of GAs/DELLAs.* **A)** Col(0) (MS, 5d); **B)** Col(0) (0.5 µM PAC, 5d); **C)** Col(0) (1 µM GA_4, 5d); **D)** Col(0) (MS, 7d); **E)** Col(0) (0.5 µM PAC, 7d); **F)** Col(0) (1 µM GA_4, 7d); **G)** L*er* (5d); **H)** *gai*-1 (5d); **I)** *QD* (5d); **J)** *5X* (5d). Magnification: 40X. Toluidine blue staining.

Fig. 6. *Excessive levels of GAs/DELLAs induced multinucleated cells, a middle cortex (MC) and extra cortical cells in root tips of A. thaliana seedlings.* **A)** PAC (0.5 µM, 5d): Epidermal multinucleated cell; **B)** PAC (0.5 µM, 5d): Cortical and endodermal multinucleated cells; **C)** PAC (0.5 µM, 5d): Pericyclic multinucleated cell; **D)** GA_4 (1 µM, 5d): Epidermal and cortical multinucleated cells; **E)** PAC (0.5 µM, 5d): MC; **F)** PAC (0.5 µM, 7d): MC (arrow) and 10 cortical cells; **G)** GA_4 (1 µM, 5d): MC (arrow) and 9 cortical cells; **H)** *scm* x *GL2pro::GUS* (MS, 5d); **I)** *scm* x *GL2pro::GUS* (0.5 µM PAC, 5d): MC (arrow) and 9 cortical cells. Magnification: 40X. Toluidine blue staining.

Fig. 7. *Over-expression of gai-1 induced an early outburst of lateral roots in root tips of A. thaliana seedlings.* **A)** *SCR::gai-1:GR* (MS, 5d) (Leaky line), 4X; **B)** *SCR::gai-1:GR* (10 µM DEXA, 5d), 4X; **C)** *UAS::gai-1* x C24 (control, 5d), 4X; **D)** *UAS::gai-1* x J2812 (5d), 20X; **E)** *UAS::gai-1* x M0018 (5d), 4X; **F)** *UAS::gai-1* x J0571 (5d), 4X; **G)** *UAS::gai-1* x Q2500 (5d), 4X; **H)** *UAS::gai-1* x N9142 (5d), 20X; **I)** *UAS::gai-1* x J3281 (8d) (aborted primary root), 4X.

Table 1. *Average length and width of root cortical cells in root tips of 5-day-old A. thaliana seedlings grown under (or harbouring) excessive levels of GAs/DELLAs.* Analyses performed on electron micrographs of root cortex cells, 20X. (*) At 48h after a 4h-heat-shock experiment.

	Root cortical cell			
	Nº Cells analysed	Length (µm)	Nº Cells analysed	Width (µm)
Col (0) (MS)	25	171 ± 28 *(100 %)*	66	31 ± 3 *(100 %)*
PAC (0.5 µM)	23	153 ± 34 (*89 %*)	60	43 ± 3 (*142 %*)
GA_4 (1 µM)	26	209 ± 52 (*122 %*)	26	27 ± 2 *(87 %)*
PAC (0.5 µM) + GA_4 (1 µM)	24	173 ± 49 *(101 %)*	28	19 ± 2 *(61 %)*
L*er*	28	188 ± 29 *(100 %)*	18	30 ± 4 *(100 %)*
ML1::gai-1	12	171 ± 33 *(91 %)*	28	30 ± 3 *(97 %)*
gai-1	71	138 ± 41 *(73 %)*	72	37 ± 7 (*123 %*)
QD	33	180 ± 34 *(96 %)*	53	27 ± 3 *(90 %)*
5X	15	187 ± 34 *(99 %)*	30	27 ± 4 *(90 %)*
GID1b-ox	23	210 ± 54 *(112%)*	34	24 ± 4 *(80 %)*
Hsp::gai-1x GL2pro::GUS (22ºC)	48	184 ± 39 *(100 %)*	47	27 ± 4 *(100 %)*
Hsp::gai-1 x GL2pro::GUS (37ºC)	38	158 ± 52 *(86 %)*	33	31 ± 4 *(115 %)*
*Hsp::gai-1 x GL2pro::GUS (22ºC) 48h**	23	195 ± 43 *(100 %)*	34	29 ± 3 *(100%)*
*Hsp::gai-1 x GL2pro::GUS (37ºC) 48h**	36	61 ± 19 *(31 %)*	44	46 ± 9 *(159 %)*
pGAI::gai-1:GR (MS)	20	180 ± 56 *(100 %)*	20	34 ± 4 *(100 %)*
pGAI::gai-1:GR (10 µM DEXA)	29	129 ± 31 *(72 %)*	29	46 ± 5 (*135 %*)
SCR::gai-1:GR (MS)	77	140 ± 51 *(100 %)*	77	34 ± 5 *(100%)*
SCR::gai-1:GR (10 µM DEXA)	64	70 ± 32 *(50 %)*	63	53 ± 10 *(156 %)*
UAS::gai-1 x C24 (control)	46	154 ± 49 *(100 %)*	52	28 ± 4 *(100 %)*
UAS::gai-1 x J0951 (epidermis)	54	155 ± 42 *(101%)*	72	31 ± 4 *(111 %)*
UAS::gai-1 x J2812 (epi + cortex)	79	116 ± 48 *(75 %)*	91	35 ± 6 *(125 %)*
UAS::gai-1 x J0571 (cortex + endo)	74	71 ±18 *(46 %)*	74	48 ± 8 *(171 %)*
UAS::gai-1 x M0018 (cortex + endo)	49	89 ± 31 *(58 %)*	46	46 ± 9 *(164 %)*
UAS::gai-1 x Q2500 (MZ endo/pericycle)	79	51 ± 13 *(33 %)*	85	41 ± 8 *(146 %)*
UAS::gai-1 x Q2393 (all but endo)	23	174 ± 59 *(113 %)*	32	32 ± 5 *(114 %)*
UAS::gai-1 x J0631 (elong. tissues)	73	46 ± 8 *(30 %)*	67	37 ± 6 *(132 %)*
UAS::gai-1 x J0121 (EZ pericycle)	16	177 ± 45 *(115 %)*	19	27 ± 3 *(96 %)*

Table 2. *Cortical and endodermal cell area in cross sections of root tips of 5-day-old A. thaliana seedlings grown under (or harbouring) excessive levels of GAs/DELLAs.* Measurements of transversal cell length and width were performed on electron micrographs of cross sections of resin-embedded roots (40X). Area was calculated by multiplying cell length (µm) by cell width (µm).

	Nº cross sections analysed	Cortical cell area (μm^2)	Nº cross sections analysed	Endodermal cell area (μm^2)
Col (0) (MS)	84	507 ± 7 *(100 %)*	82	169 ± 3 *(100 %)*
PAC (0.5 µM)	105	737 ± 17 *(145 %)*	82	215 ± 7 *(127 %)*
GA_4 (1 µM)	98	375 ± 13 *(74 %)*	81	157 ± 5 *(93 %)*
L*er*	8	509 ± 9 *(100 %)*	24	172 ± 4 *(100 %)*
gai-1	100	464 ± 5 *(91 %)*	89	186 ± 4 *(108 %)*
QD	16	264 ± 6 *(52 %)*	23	138 ± 4 *(80 %)*
5X	7	270 ± 2 *(53 %)*	51	143 ± 3 *(83 %)*

Table 3. *Average width of the root MZ in root tips of 5-day-old A. thaliana seedlings grown under (or harbouring) excessive levels of GAs/DELLAs.* Measurements of root width were performed on electron micrographs of whole root tips (10X, longitudinal view) or of cross sections of resin-embedded roots (40X).

	Root width (µm)			
	Nº roots analysed	Whole root tips	Nº cross sections analysed	Root cross sections
Col (0) (MS)	12	115 ± 4 *(100 %)*	84	144 ± 10 *(100 %)*
PAC (0.5 µM)	19	161 ± 12 (*140* %)	107	164 ± 14 (*114* %)
GA_4 (1 µM)	19	99 ± 12 (*86* %)	96	128 ± 18 *(89 %)*
L*er*	10	122 ± 5 *(100 %)*	8	145 ± 4 *(100 %)*
gai-1	18	125 ± 18 *(102 %)*	100	148 ± 13 (*102* %)
QD	16	107 ± 18 (*88* %)	41	129 ± 7 (*89* %)
5X	5	111 ± 0 (*91* %)	50	132 ± 8 *(91 %)*

Table 4. *Estimated tissue depth in roots tips of 5-day-old A. thaliana seedlings grown under (or harbouring) excessive levels of GAs/DELLAs.* Measurements of tissue diameter (µm) were performed on electron micrographs of cross sections of resin-embedded roots (40X; MZ to early EZ). Estimated epidermal depth = [Root diameter (data from table 3) – (Cortex-Endodermis-Pericycle-Vessels diameter)]/2. Estimated cortical depth = [(Cortex-Endodermis-Pericycle-Vessels diameter) – (Endodermis-Pericycle-Vessels diameter)]/2. Estimated endodermal depth = [(Endodermis-Pericycle-Vessels diameter) – (Pericycle-Vessels diameter)]/2. Estimated pericycle depth = [(Pericycle-Vessels diameter) – (Vessels diameter)]/2. Endo: Endodermis; Peri: Pericycle. Number of cross sections analysed: Control (27-29), PAC (46-49), GA_4 (74-78), L*er* (21-22), *gai-1* (36), *QD* (34-36), *5X* (47-48).

	Col (0) (MS)	PAC (0.5 µM)	GA_4 (1 µM)	L*er*	*gai-1*	*QD*	*5X*
Cortex-Endo-Peri-Vessels diameter	95 ± 6 *(100 %)*	110 ± 11 *(116 %)*	83 ± 12 *(87 %)*	90 ± 7 *(100 %)*	91 ± 5 *(101 %)*	81 ± 5 *(90 %)*	82 ± 4 *(91 %)*
Endo-Peri-Vessels diameter	59 ± 6 *(100 %)*	70 ± 7 *(119 %)*	58 ± 7 *(98 %)*	60 ± 4 *(100 %)*	59 ± 3 *(98 %)*	59 ± 6 *(98 %)*	55 ± 2 *(92 %)*
Peri-Vessels diameter	45 ± 5 *(100 %)*	54 ± 5 *(120 %)*	45 ± 6 *(100 %)*	45 ± 3 *(100 %)*	44 ± 2 *(98 %)*	45 ± 5 *(100 %)*	40 ± 2 *(89 %)*
Vessels diameter	35 ± 3 *(100 %)*	40 ± 3 *(114 %)*	32 ± 5 *(91 %)*	33 ± 3 *(100 %)*	34 ± 2 *(103 %)*	33 ± 4 *(100 %)*	30 ± 2 *(91 %)*
Estimated Epidermal depth	25 *(100 %)*	27 *(108 %)*	22 *(88 %)*	27 *(100 %)*	28 *(104 %)*	24 *(89 %)*	25 *(93 %)*
Estimated Cortex depth	18 *(100 %)*	20 *(111 %)*	13 *(72 %)*	15 *(100 %)*	16 *(107 %)*	11 *(73 %)*	13 *(87 %)*
Estimated Endodermis depth	7 *(100 %)*	8 *(114 %)*	6 *(86 %)*	8 *(100 %)*	7 *(88 %)*	7 *(88 %)*	7 *(88 %)*
Estimated Pericycle depth	5 *(100 %)*	7 *(140 %)*	6 *(120 %)*	6 *(100 %)*	5 *(83 %)*	6 *(100 %)*	5 *(83 %)*

Table 5. *Radial cell organization in roots of 5 or 7-day-old A. thaliana seedlings grown under (or harbouring) excessive levels of GAs/DELLA.* Analyses performed on electron micrographs of cross sections of resin embedded-roots (40X).

	Nº root cross sections analysed	Epidermis	Cortex	Endodermis	Pericycle
Control (5d)	75	20–25	8	8–10	13–15
PAC (5d)	56	25–34	8–9	9–13	14–18
GA_4 (5d)	56	17–25	8–9	8–10	12–16
Control (7d)	14	24–27	8	8–10	14–15
PAC (7 d)	18	26–33	8–10	11–13	14–18
GA_4 (7d)	45	23–25	8–9	8–10	14–16
L*er* (5d)	13	18–24	8	8	13–14
gai-1 (5d)	57	19–25	8	8–9	12–14
QD (5d)	24	22–24	8–11	8–11	14–18
5X (5d)	54	18–21	8–9	7–8	12–16

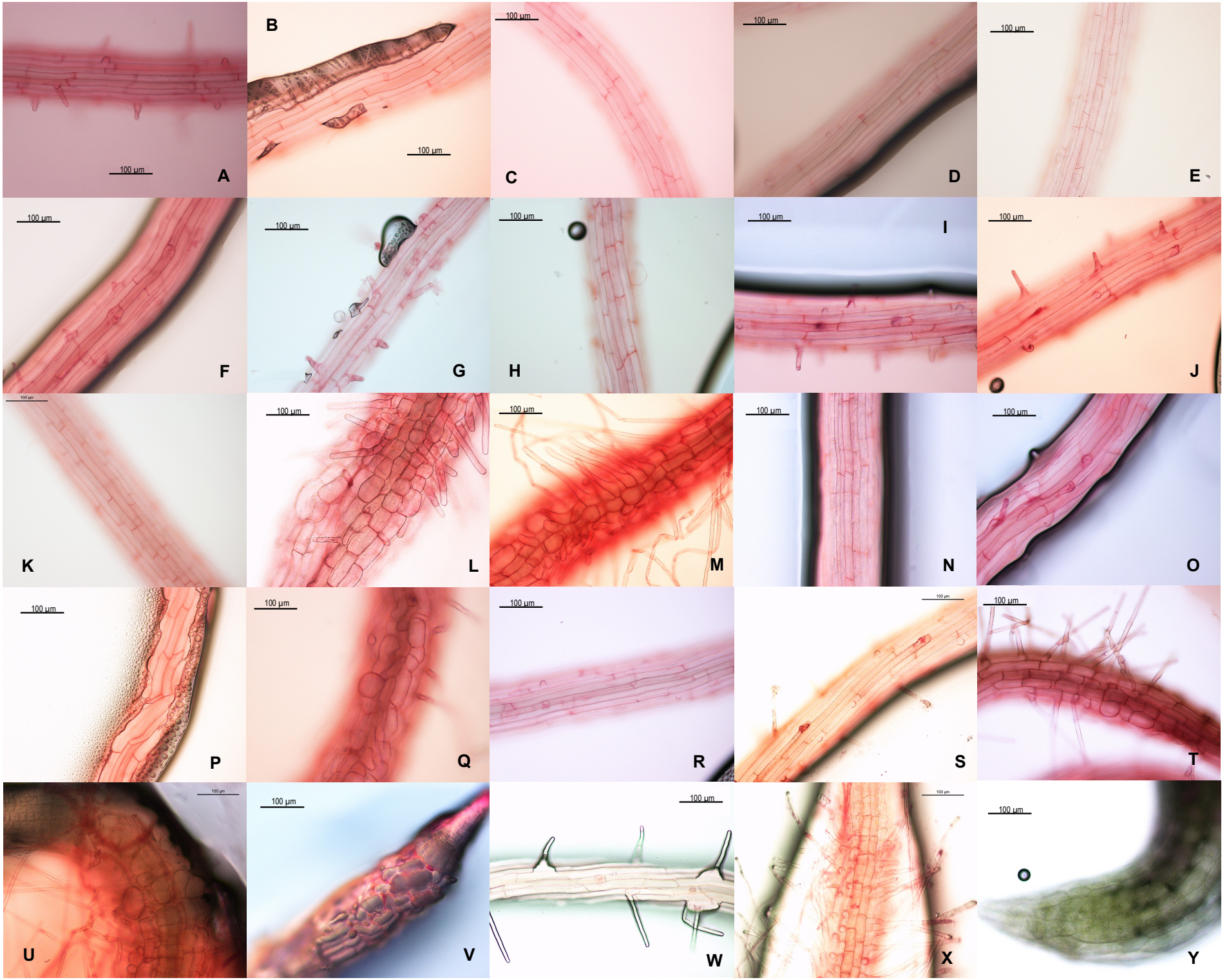
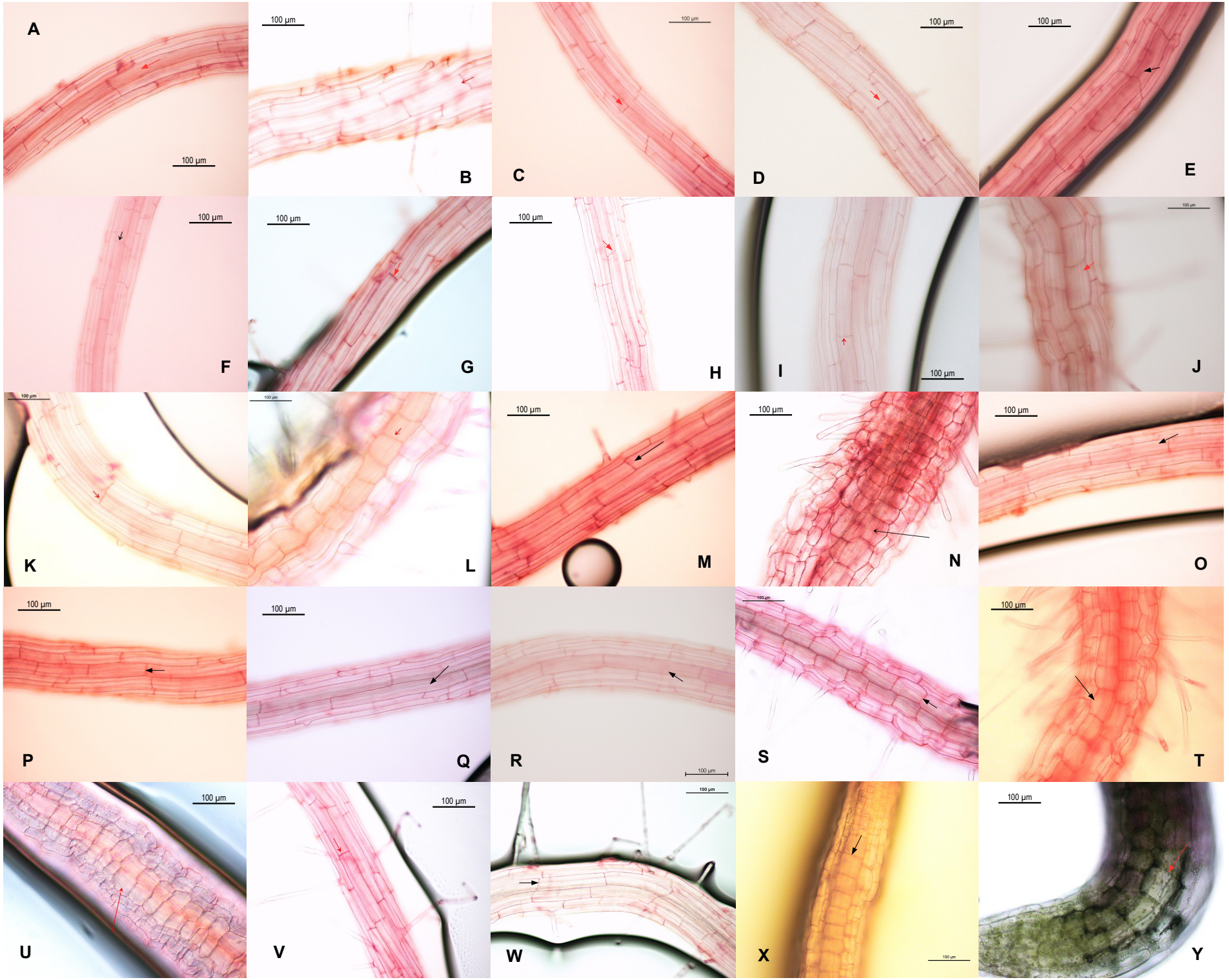


Figure 1



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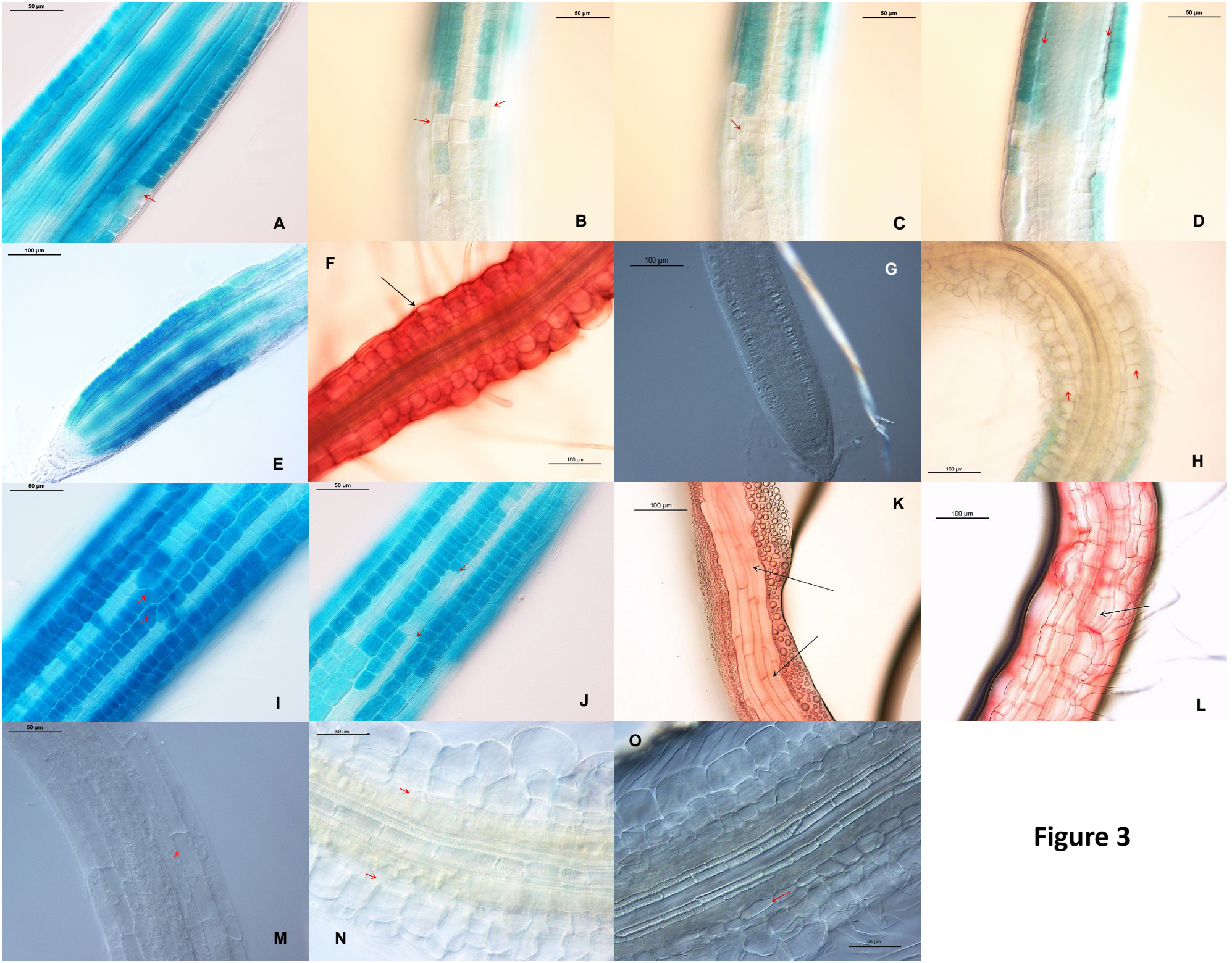
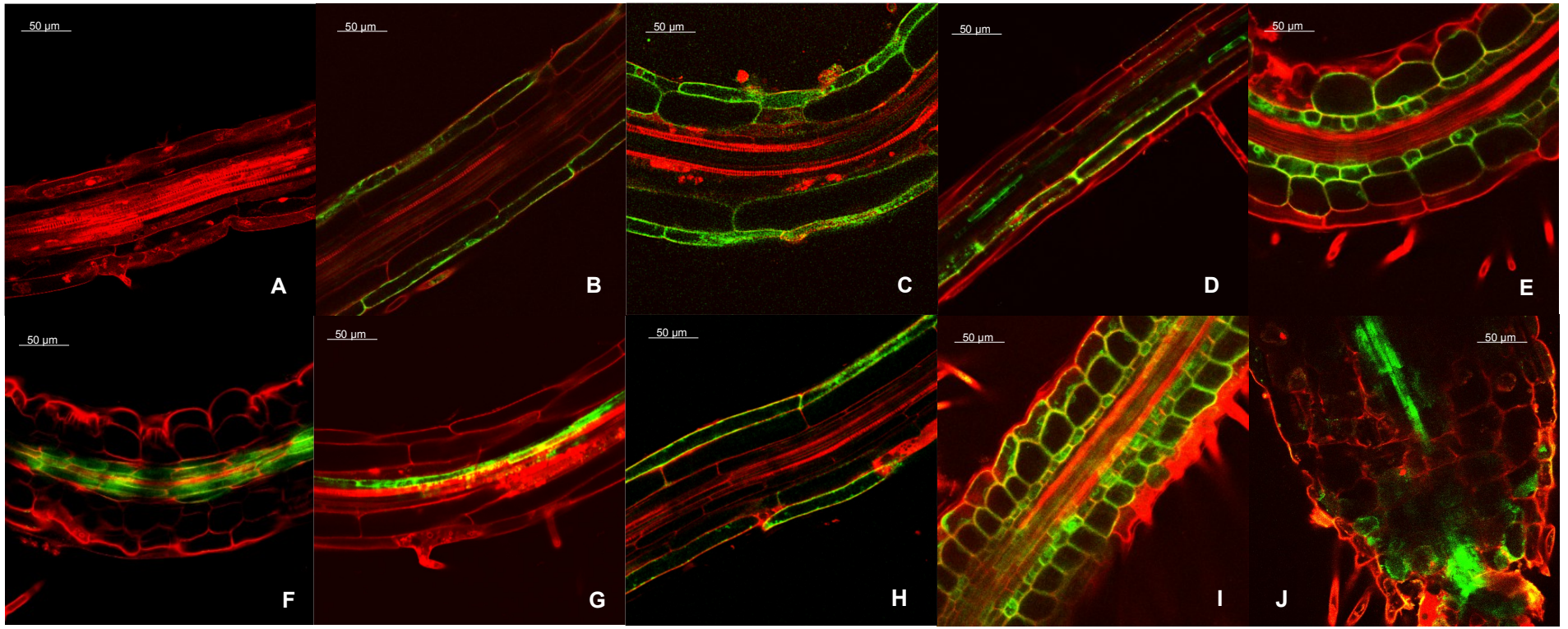


Figure 3

Figure 4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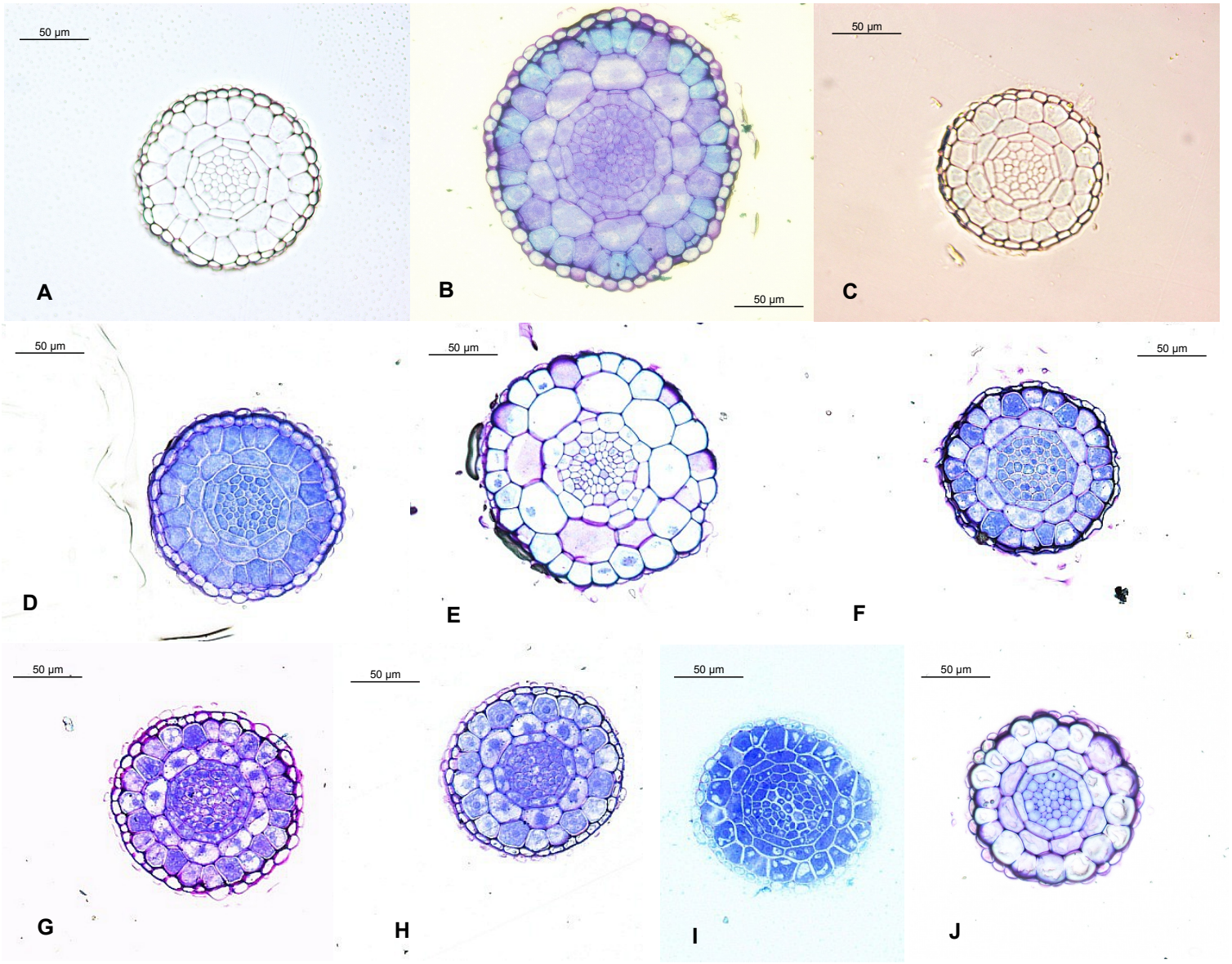
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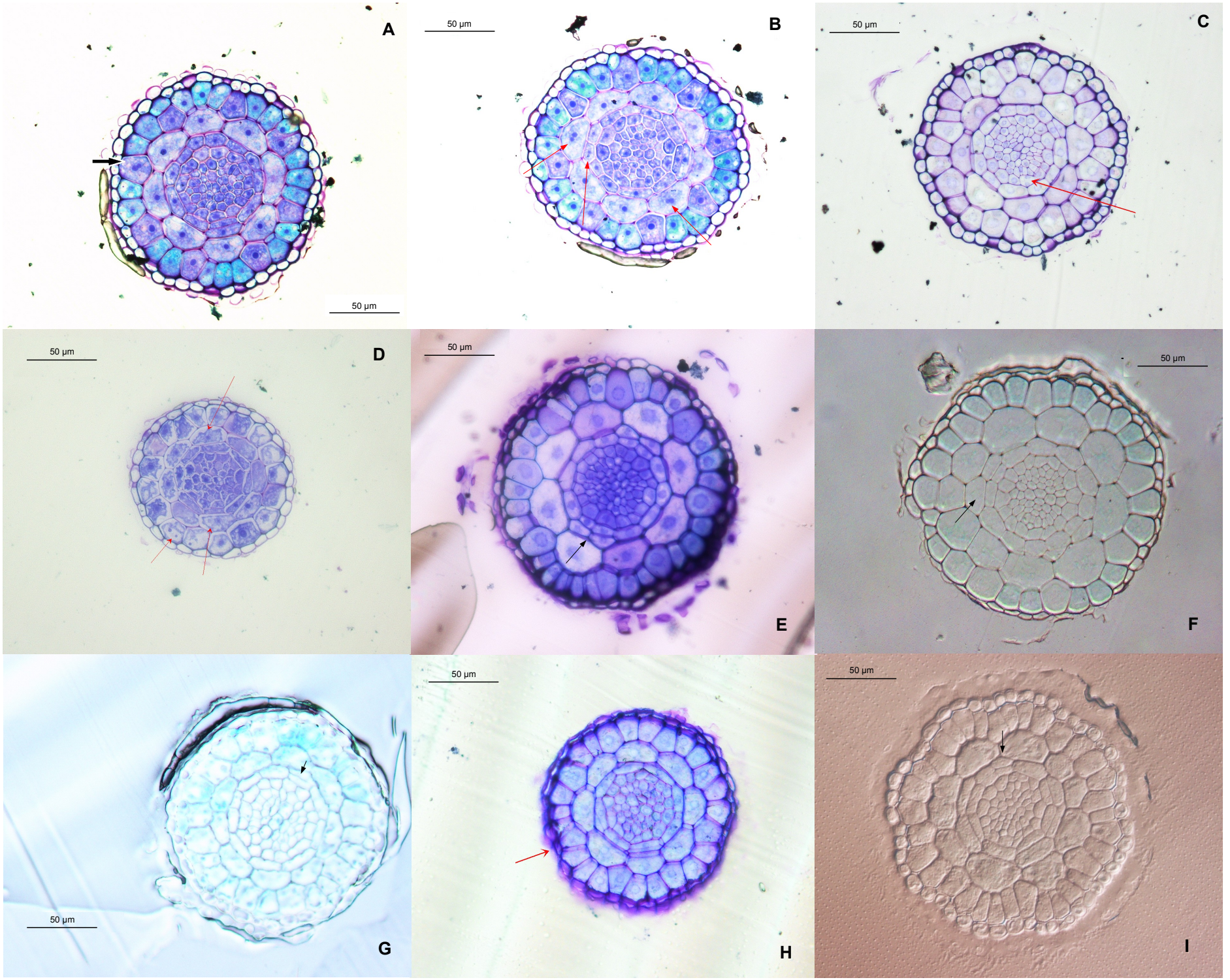

Figure 6

Figure 7


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