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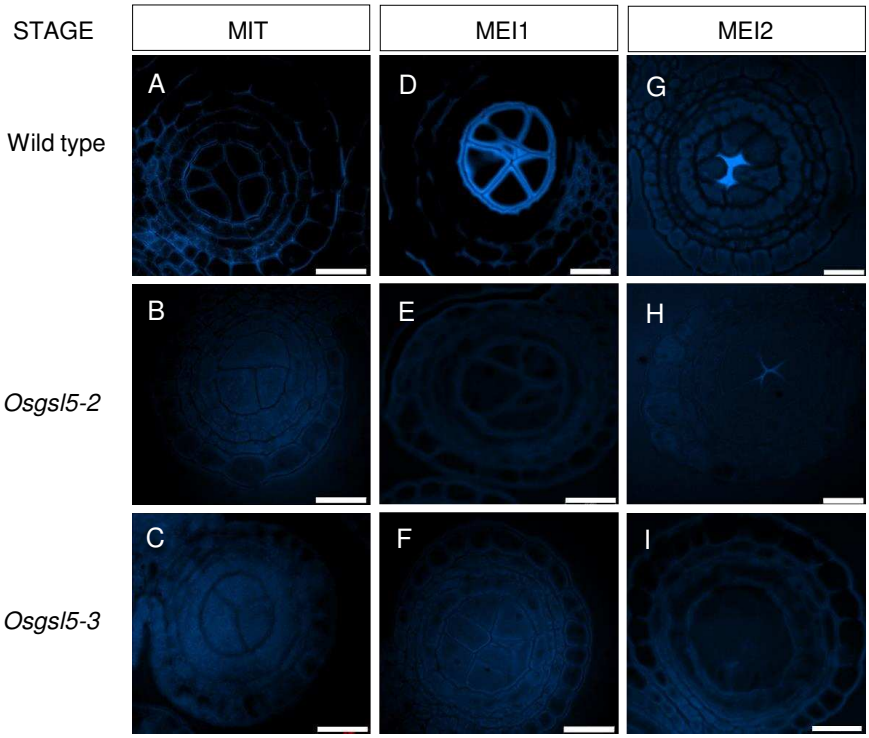

Figure 1: Callose deposition during mitosis-meiosis transition in wild type (WT), *Osgsl5-2* and *Osgsl5-3* in rice anthers. During mitotic sporogenous cell stage (Mit, anther length (AL) =< 0.35mm), callose is not present within the locular space of anthers in WT, *Osgsl5-2* and *Osgsl5-3* (A, D, G). At premeiotic interphase stage (Mei1, AL= 0.35-0.45mm), callose is deposited around the pollen mother cells in WT anther locules but not in *Osgsl5-2* and *Osgsl5-3* anthers (B, E, H). During early meiotic prophase stages (lepto/zygo/pachy, Mei2, AL= 0.5-0.55mm), the callose is deposited at the center of locule in WT anther (C, F, I). Very little to no callose is observed at early prophase stage in *Osgsl5-2* and *Osgsl5-3.* Scale bar=20µm.

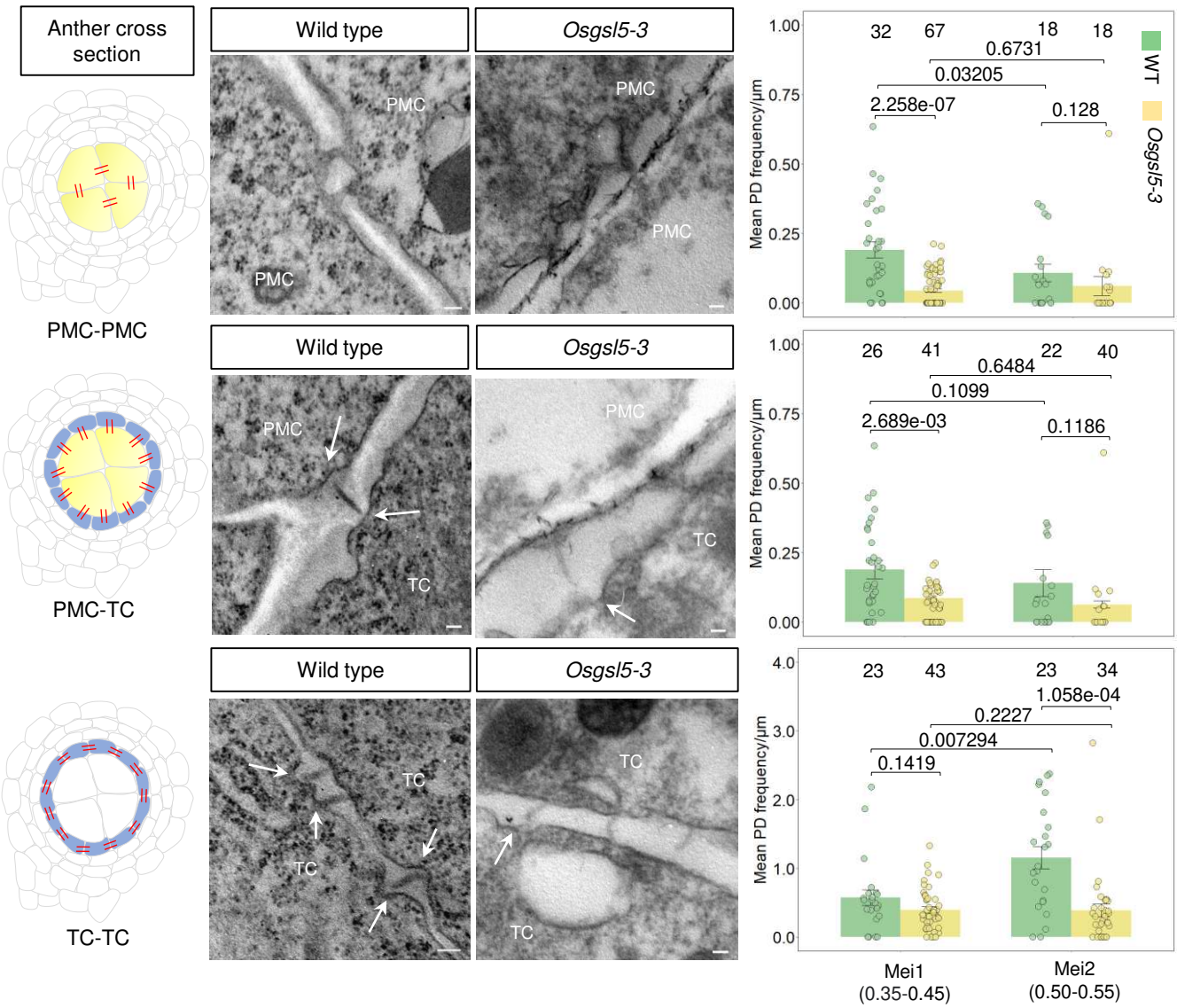

Figure 2: Plasmodesmata frequency is differentially regulated upon entry to meiosis in *Osgsl5-3* mutant anthers. The left cartoons depict cross view of premeiotic anthers (the double lines in red indicate plasmodesmata, PD) showing the different cellular interfaces between pollen mother cells (PMC) and tapetum cells (TC). Middle panels: transmission electron microscopy images showing PD at the three cellular interfaces in wild type and *Osgsl5-3* anthers. Right panels: quantification of PD frequency (#/µm of cell wall) in Mei1 (premeiotic interphase) and Mei2 (early meiosis I) for wild type and *Osgsl5-3* anthers. Values above bars indicate *p* values by Mann Whitney's U Test. Error bars indicate the standard deviation of mean PD frequency/µm of cell wall derived from observations in at least three independent rice florets for both wild type and *Osgsl5-3*. Each dot represents the average PD frequency/µm of cell wall at each interface and stage in an individual rice floret. Values on top show the number of sections quantified for PD frequency at each interface and respective anther stage in wild type and *Osgsl5-3* anthers. Anther lengths (mm) used for Mei1&2 are indicated in the brackets below. Arrows indicate membrane invaginations at PD site. PMC: Pollen Mother Cell, TC: Tapetal Cell. Scale bar= 100nm

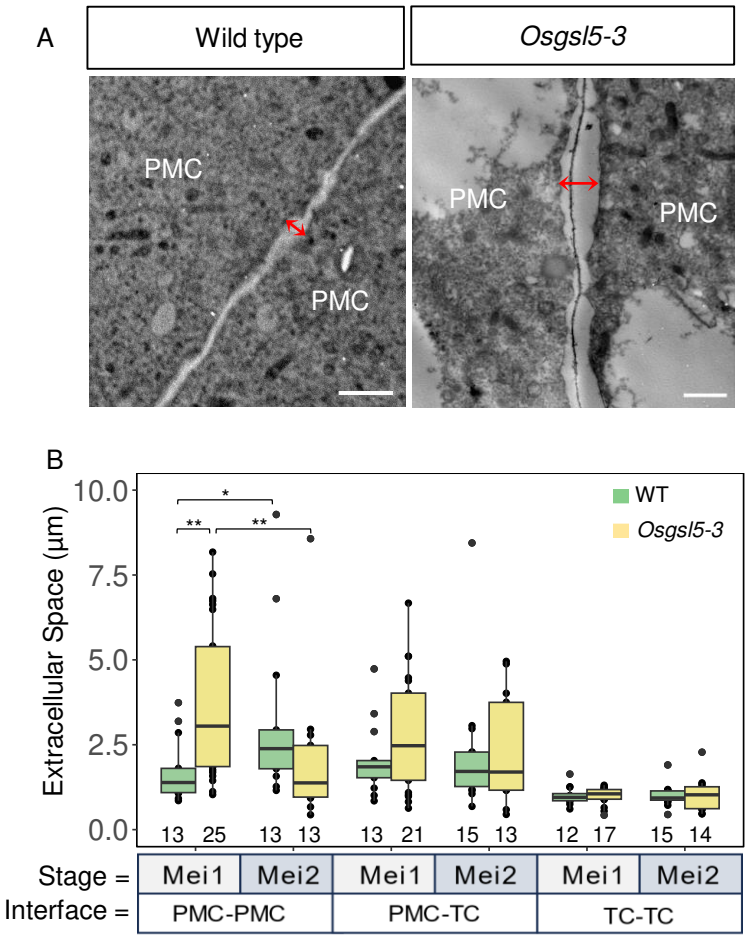

Figure 3: Extracellular distance between adjacent Pollen Mother Cells increases upon entry into meiosis in *Osgsl5-3* anthers. A: Representative electron micrographs showing the Extracellular distance between plasma membranes (red double arrowhead) of two adjacent pollen mother cells in WT and *Osgsl5-3* anthers at Mei1 stage. Dark gray line between cells is the pectin enriched middle lamella (ML). Scale bar = 1µm. B: Comparison of Extracellular distance (µm) at three cellular interfaces between WT and *Osgsl5-3* anthers. All distances are normalized against TC-TC distance. Horizontal line within each box indicates median. Asterisks indicate significant differences (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ by Mann Whitney's U Test). Premeiotic interphase (Mei1) anther = 0.35-0.45mm in length, early meiosis I (Mei2) anther = 0.50-0.55mm. Numbers below the plot correspond to the number of sections observed for each interface and stage in both WT and *Osgsl5-3*.

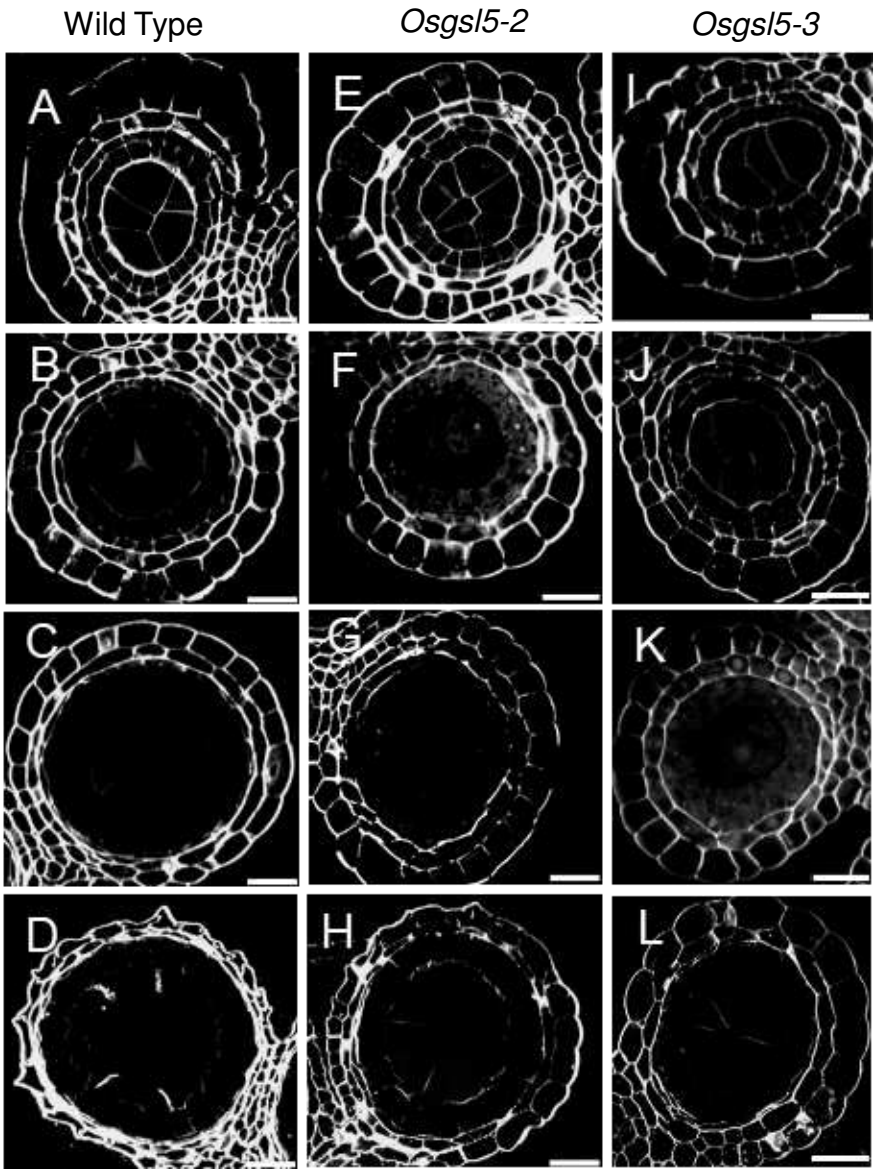

Figure 4. Cellulose accumulation in Wild type (WT), *Osgsl5-2* and *Osgsl5-3* anthers. Renaissance staining revealed cellulose fluorescence in WT, *Osgsl5-2* and Os*gsl5-3* cell walls of pollen mother cells undergoing mitosis (A, E, I), premeiosis interphase (B, F, J), Early-mid prophase I (C, G, K) and at the dyad stage (D, H, L). Scale bar = 20µm**.**

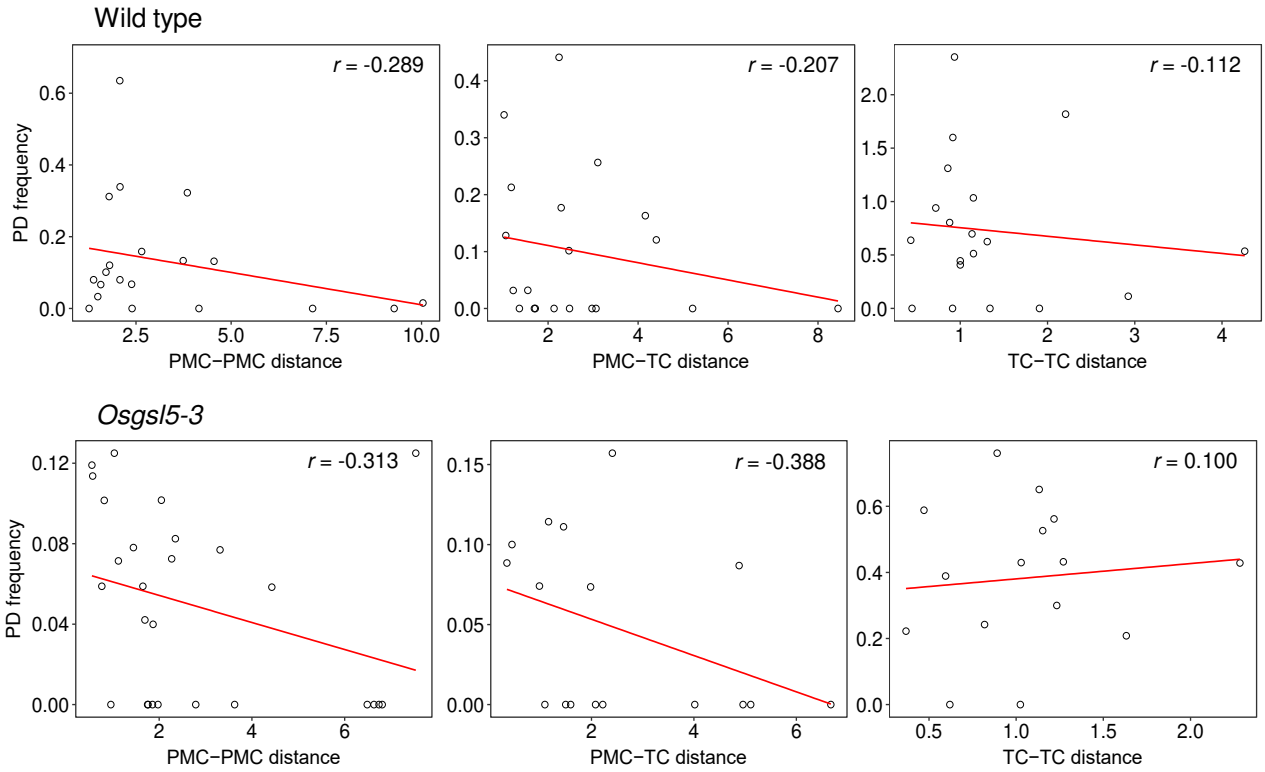

Figure 5: Correlation between Extracellular distance and PD frequency at three cellular interfaces in Wild Type (WT) and *Osgsl5-3* anthers. Scatter plot showing relationship between PMC-PMC (left), PMC-TC (middle), TC-TC (right) distances and PD frequency (#/µm cell wall length) in WT (above) and *Osgsl5-3* (below) anthers respectively. The PD frequency is plotted against the given extracellular distance (µm) at each interface. The correlation coefficient values (r) for each combination of extracellular distance and PD frequency are shown on the plots. The data from both premeiotic interphase and early meiotic-I anthers is used for correlation analyses between PD frequency and extracellular distance.

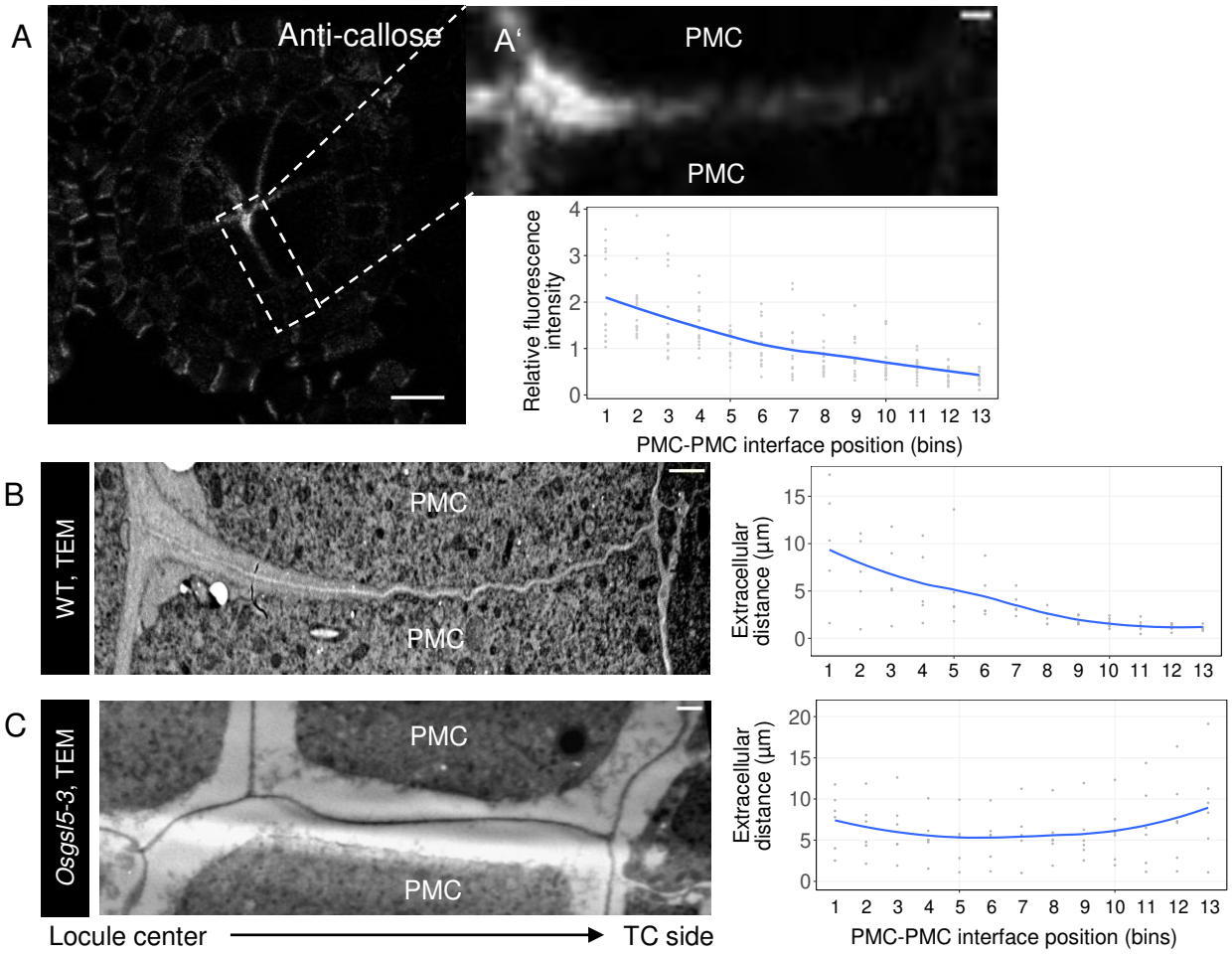

Figure 6: Callose deposition and Extracellular distance in premeiotic Wild type (WT) and *Osgsl5-3* mutant anthers underpin differences in the shaping of pollen mother cells (PMC). A. Image showcasing the callose deposition at the PMC-PMC junction in WT early premeiotic interphase anthers prior to spreading to the PMC-TC interface. Callose is immuno-stained using anti-callose antibody. On the right (A'), the picture shows a zoom in to highlight the differential spreading of callose in the PM-PMC interface. On the bottom graph, fluorescence intensity (as a measure of callose deposition) is quantified along this interface. Below left are TEM section of early premeiotic WT anthers (B) and *Osgsl5-3* mutant anthers (C). Dark gray line between cells is the pectin enriched middle lamella. The graphs on the right show the normalized extracellular distance at PMC-PMC interface. Note the downward curve associated with PMC shaping in WT while extracellular distances remained large but constant across the PMC-PMC interface in the mutant. Scale bar (A) =10µm, (A', B, C) = 1µm.

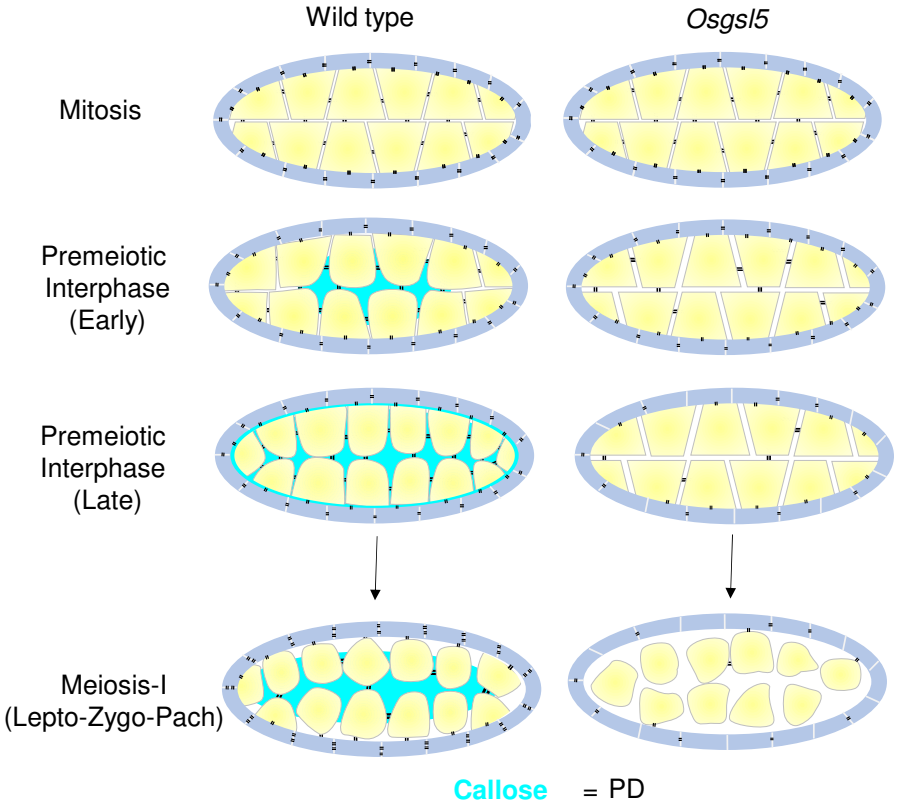

Figure 7. Model representing changes in callose deposition, plasmodesmata connections, extracellular distance and PMC shaping in anther locules. In wild type (WT), during mitosis and premeiotic interphase stage, PD are dense among the anther locules cells. Callose deposits at the central locule between angular shaped WT PMCs during premeiotic interphase (Early) and the PMC shape become curvy at the regions corresponding to the callose deposition sites. At premeiotic interphase (Late) stage, callose completely fills the anther locules leading to PMCs sphericalization at meiosis–I. In turn PD number decreases with the increase in callose between PMC-PMC as they reach early meiosis–I. In callose deficient mutant *Osgsl5*, PD frequency is reduced and extracellular distance (apoplastic spaces) increases (but remained constant along the interface) at all premeiotic and early meiosis stages. The lack of callose is proposed to influence the curvy-shaped PMC corners at premeiotic interphase locule center, delaying sphericalization affecting meiosis.