The role of cytokinin during ovule development in *Arabidopsis thaliana*

DIPARTIMENTO DI BIOSCIENZE

UNIVERSITÀ DEGLI STUDI DI MILANO

Giada Callizaya Terceros, Lucia Colombo

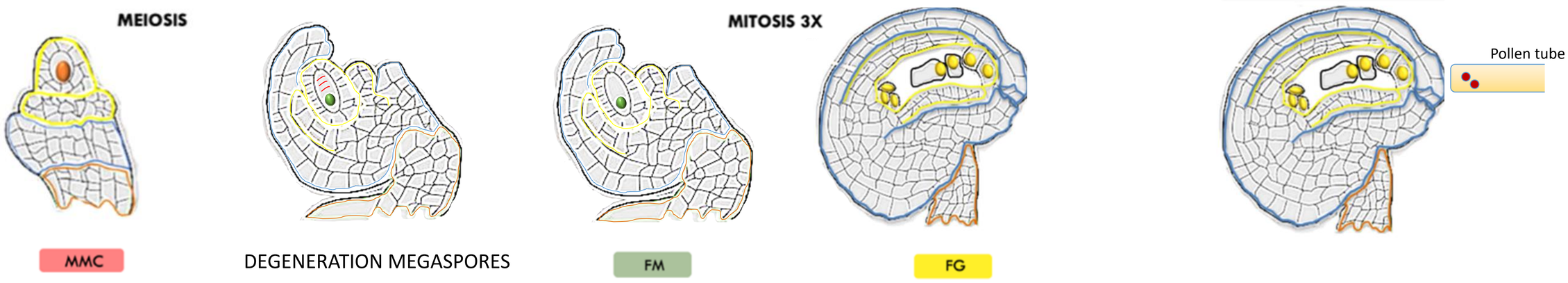
Dipartimento di Bioscienze, Università degli Studi di Milano, via Celoria 26, 20133 Milano

ABSTRACT

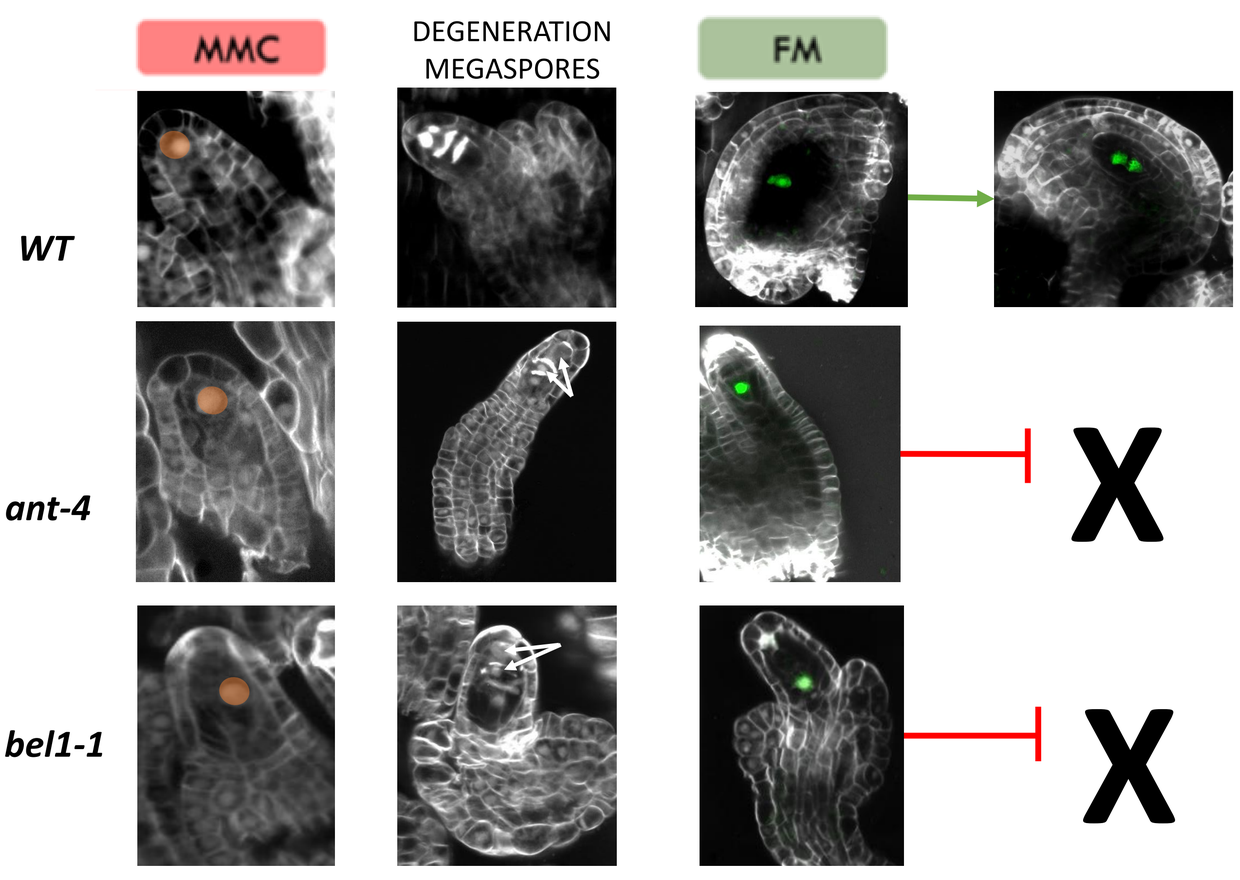
Sexual reproduction allows plants to produce seeds and to propagate themselves. In Angiosperms this process occurs between two haploid individuals: the female (embryo sac) and the male (pollen grain) gametophytes. In Angiosperm, the gametophytes origin by two subsequently processes: the sporogenesis and the gametogenesis. During the female sporogenesis a single differentiated somatic cell, called Megaspore Mother cell (MMC) divides by meiosis and gives rise to four megaspore. Three of them degenerate and the only one surviving becomes the Functional Megaspore (FM). This cell, then, undergoes gametogenesis which consists of three rounds of mitosis at the end of which the seven-celled mature female gametophyte is formed.

Several mutants defective in cytokinin (CK) biosynthesis and/or signalling exhibit a reduced female fertility, suggesting that cytokinins have a conserved role in regulating ovule development (1) and/or reproduction. It is well known that cytokinin control the expression of two transcription factors, *BELL1 (BEL1)* and *AINTEGUMENTA* (*ANT*) (2,3). The *bel1-1* and *ant-4* mutants exhibit female sterility due to alteration in gametogenesis (4,5). Therefore, they result good candidates for studying the role of cytokinin in the control female germline development. We characterized by morphological analyses both mutants to assess the altered gametogenesis and trying to exploit the role of cytokinin in the control of gametogenesis. Regarding the fertilization process, it has been shown that cytokinin could control the degeneration of synergid cells, which is required for a correct accomplishment of fertilization process. The *vdd-1/+* mutant is defective in synergid degeneration (6). In particular, in half *vdd-1/+* ovules, the promoter of *CKX7* (cytokinin oxidase/hydrogenase, responsible for cytokinin degradation) isn't active (7), suggesting that a correct physiological level of cytokinin is required for the synergid degeneration. Therefore, we are trying to increase the level of cytokinin within synergid cells to mimic the *vdd-1/+* phenotype.

OVULE DEVELOPMENT AND FERTILIZATION PROCESS IN *ARABIDOPSIS THALIANA*

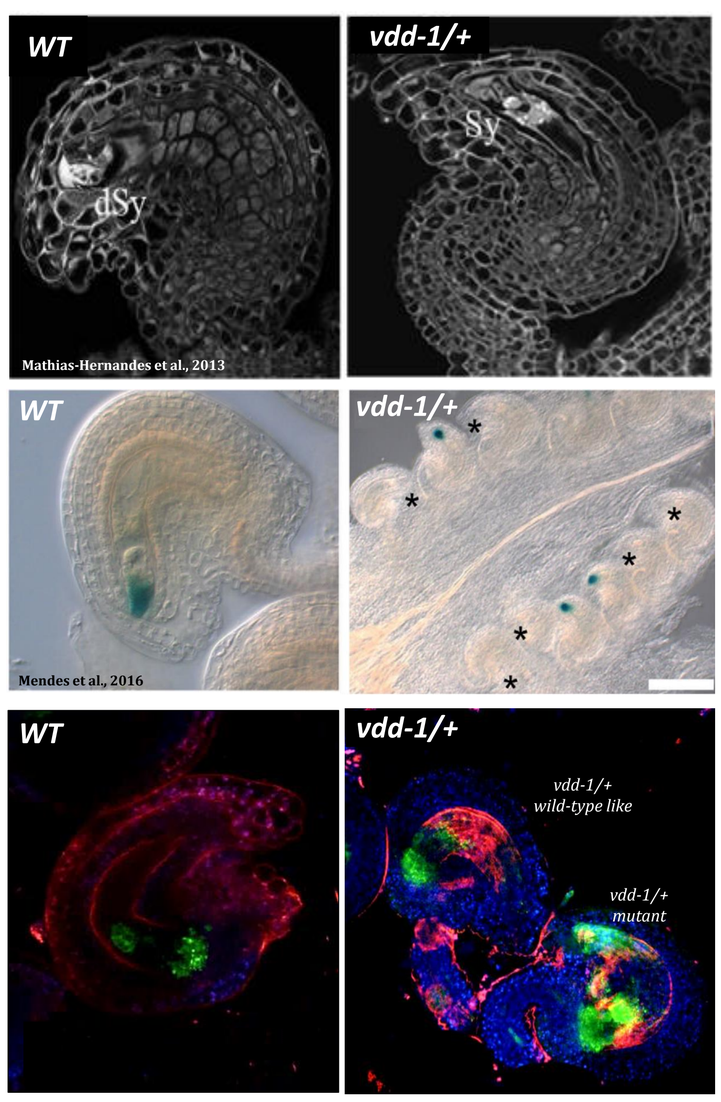


IN BOTH *bel1-1* and *ant-4* THE GAMETOGENESIS DOESN'T OCCUR



PHYSIOLOGICAL LEVELS OF CYTOKININ ARE REQUIRED FOR DEGENERETATION OF SYNERGID CELLS LEADING TO A SUCCESFULL FERTILIZATION

FERTILIZATION



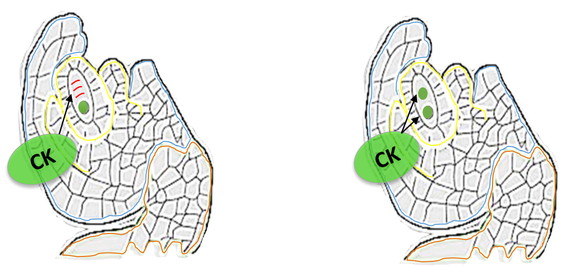
In ***vdd-1/+*** SYNERGID DEGENERATION IS IMPAIRED

pCKX7::nlsGUS IS EXPRESSED IN HALF *vdd-1/+* OVULES

CYTOKININ SIGNALLING (***TCSn::GFP***) IS ALTERED IN *vdd-1/+* OVULES

ONGOING EXPERIMENTS

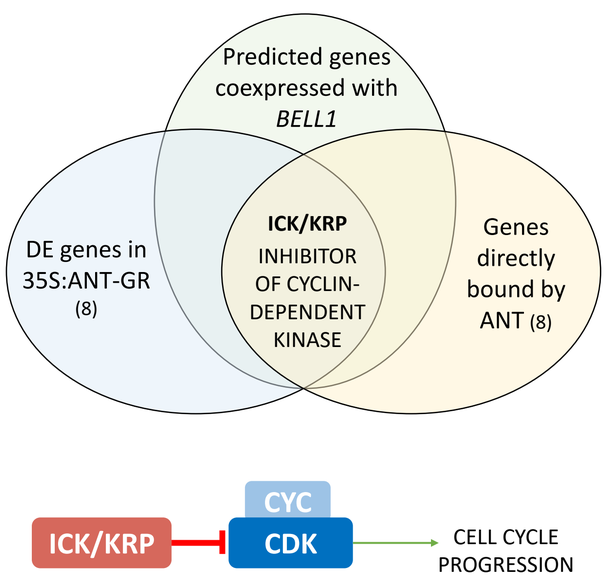
1. TRY TO RECOVER bel1-1 AND ant-4 PHENOTYPES BY GIVING CK TO ALLOW DEGENERATION OF MEGASPORE AND/OR PROGRESSION IN GAMETOGENESIS.



DEGENERATION OF MEGASPORES

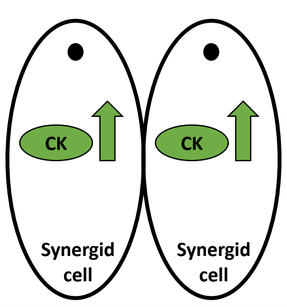
PROGRESSION OF GAMETOGENESIS

2. LOOKING FOR PUTATIVE TARGETS



ONGOING EXPERIMENTS

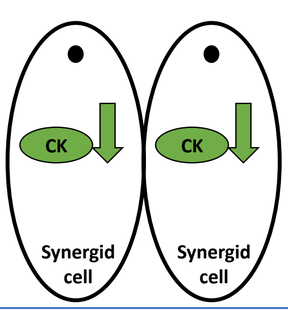
1. INCREASE LEVEL OF CYTOKININ WITHIN SYNERGD CELLS - *pDD2::IPT1*



pDD2 IS SYNERGID SPECIFIC

IPT1 IS INVOLVED IN CYTOKININ BIOSYNTHESIS

2. COMPLEMENTATION TEST *pDD2::CKX7* in *vdd-1/+*



pDD2 IS SYNERGID SPECIFIC

CKX7 IS INVOLVED IN CYTOKININ DEGRADATION

REFERENCES

1. Cheng, C.Y.; Mathews, D.E.; Schaller, G.E.; Kieber, J.J. Cytokinin-dependent specification of the functional megaspore in the Arabidopsis female gametophyte. *Plant J.* **2013**, *73*, 929–940.
2. Bencivenga, S.; Simonini, S.; Benková, E.; Colombo, L. The transcription factors BEL1 and SPL are required for cytokinin and auxin signaling during ovule development in Arabidopsis. *Plant Cell.* **2012**, *24*, 2886–97.
3. Randall, R.S., Miyashima S., Blomster T., Zhang J., Elo A., Karlberg A., Immanen J., Nieminen K., Lee J.Y., Kakimoto T., Blajecka K., Melnyk C.W., Alcasabas A., Forzani C., Matsumoto-Kitano M., Mähönen A.P., Bhalerao R., Dewitte W., Helariutta Y., Murray J.A. AINTEGUMENTA and the D-type cyclin CYCD3;1 regulate root secondary growth and respond to cytokinins. *Biol Open.* **2015**; 4,1229-36.
4. Robinson-Beers, K.; Pruitt, R.E.; Gasser, C.S. Ovule Development in Wild-Type Arabidopsis and Two Female-Sterile Mutants. Plant Cell. 1992; 4,1237-1249.
5. Klucher, K.M., Chow H., Reiser L., Fischer R.L. The AINTEGUMENTA gene of Arabidopsis required for ovule and female gametophyte development is related to the floral homeotic gene APETALA2. Plant Cell. 1996; 8,137-53.
6. Luis Matias-Hernandez, Raffaella Battaglia, Francesca Galbiati, Marco Rubes, Christof Eichenberger, Ueli Grossniklaus, Martin M. Kater, Lucia Colombo, VERDANDI Is a Direct Target of the MADS Domain Ovule Identity Complex and Affects Embryo Sac Differentiation in Arabidopsis , The Plant Cell, Volume 22, Issue 6, June 2010, Pages 1702–1715
7. Mendes MA, Guerra RF, Castelnovo B, Silva-Velazquez Y, Morandini P, Manrique S, Baumann N, Groß-Hardt R, Dickinson H, Colombo L. Live and let die: a REM complex promotes fertilization through synergid cell death in Arabidopsis. Development. 2016 Aug 1;143(15):2780-90.
8. Krizek BA, Blakley IC, Ho YY, Freese N, Loraine AE. The Arabidopsis transcription factor AINTEGUMENTA orchestrates patterning genes and auxin signaling in the establishment of floral growth and form. Plant J. 2020 Jul;103(2):752-768.