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Molecular analysis of endo-β-mannanase genes upon seed imbibition suggest a cross-talk between radicle and micropylar endosperm during germination of *Arabidopsis thaliana*

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The endo-β-mannanase (MAN) family is represented in the Arabidopsis genome by eight members, all with canonical signal peptides and only half of them being expressed in germinating seeds. The transcripts of these genes were localized in the radicle and micropylar endosperm (ME) before radicle protrusion and this expression disappears as soon as the endosperm is broken by the emerging radicle tip. However, only three of these *MAN* genes, *AtMAN5*, *AtMAN7* and especially *AtMAN6* influence the germination time (t_{50}) as assessed by the analysis of the corresponding knock-out lines. The data suggest a possible interaction between embryo and ME regarding the role of MAN during the Arabidopsis germination process.

The Endosperm-limited Seed Germination

The seed phase is the most important stage of the plant life cycle, ensuring species survival. The seed is a viable and autonomous organism which will germinate when internal and environment conditions are suitable.[1] In endospermic seeds, the diploid embryo is surrounded by two covering layers: the triploid endosperm (nutritive tissue, living cells) and the diploid testa (the seed coat, maternal tissue, dead cells). The endosperm of Arabidopsis is constituted by a single thin layer and originated by a cellularization process that begins in the micropylar region and during seed development spreads to the central and chalazal regions.[2]

Seed germination is initiated with water uptake by the dry seed and terminates with the elongation and emergence of the embryonic axis. Physically, germination in Arabidopsis is a two-stage sequential process, where testa rupture is followed by endosperm rupture. Following rupture of the micropylar endosperm (ME) by the elongating radicle, germination is completed.[1,3] This physiological process is tightly controlled by environmental conditions, as well as, by the developmental program of the seed, in which abscisic acid (ABA) and gibberellins (GA) are some of the main hormones involved. Ethylene (ET), nitric oxide (NO), auxins, etc., are also part of the complex network of interacting signals that together with the transcriptional network involved in germination are now being actively investigated.[3-9]

Mannans, Endo-β-mannanases and Seed Germination

Two major forces play antagonistic roles in radicle emergence: the radicle growth potential (i.e., primarily cell elongation),[10] and the mechanical resistance of the covering layers which is likely to be diminished before radicle protrusion by cell-wall (CW) hydrolytic enzymes.[1,4,5]

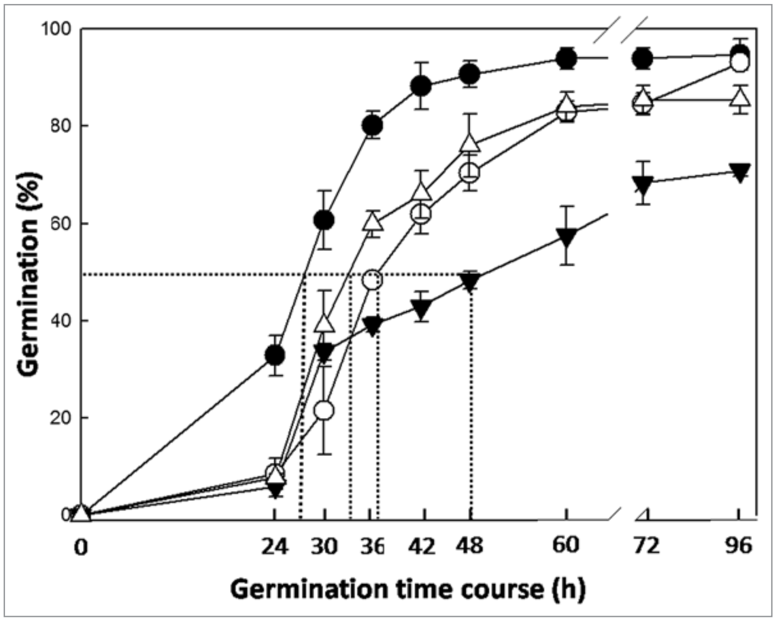

Figure 1. Germination time course of *Arabidopsis thaliana* Wt and T-DNA insertion mutant seeds. Wt: Closed circles (●; t_{50} = 25); K.O. MAN7 (GABI_747H02): Opened triangles (Δ; t_{50} = 34.4); K.O. MAN5 (GABI_707G06): Opened circles (○; t_{50} = 37.5); K.O. MAN6 (Salk_122701): Closed triangles (▼; t_{50} = 48). Data are means ± standard error (SE) of three independent experiments.

In order for the radicle apex to emerge, the growth potential must overcome the mechanical resistance of the endosperm. ABA inhibits endosperm rupture, but not testa rupture, and interacts with ET during Arabidopsis and Lepidium germination.[9,11] Since the weakening of endosperm CW is an important factor for radicle protrusion in seeds of Arabidopsis, it is of great interest to study the genes involved in the dismantling of the endosperm CW structure, which, unlike radicle CW, is rich in mannans,[12] conferring to this tissue a remarkable mechanical resistance. Endo-β-mannanase (MAN) activity has been reported in seeds of several endospermic species before and following radicle emergence.[13] However, although the degradation of mannans is initiated by MAN,[1,14] the role of this enzyme in endosperm weakening is still controversial, to the point that some authors maintain that MAN is not involved, while others conclude that MAN is the main enzyme component of the CW dismantling process.[5,15,16] Taken together all these observations, the consensus appears to be that while MAN is required for endosperm weakening, itself is not enough to allow completion of germination.[5]

Is there a Cross-talk between Radicle and ME in Relation to Endo-β-mannanases during Seed Germination?

In our recent study with T-DNA insertion mutants in *AtMAN* genes, we concluded that the expression of *AtMAN5*, *AtMAN6* and *AtMAN7* in imbibed seeds is important for *A. thaliana* germination, probably by decreasing the mechanical resistance of the ME, thus facilitating the radicle emergence.[18] Germination time course is negatively affected in knock-out (K.O.) mutants of *AtMAN7* and *AtMAN5* and strongly inhibited in K.O. *AtMAN6* (**Fig. 1**). In parallel, we have detected transcripts of *AtMAN5*, *AtMAN6* and *AtMAN7* genes not only in the ME but also in the radicle, thus not ruling out the possibility that the *AtMAN* gene products could be also involved in the radicle cell growth through a transglycosylase activity. This conclusion is a consequence of the fact that the role of MAN as a hydrolase may not be the dominant role in CW of the radicle itself, where mannans are only minor, although important components.[12] MAN expression is high in the vascular elements of the radicle, probably indicating a role in emptying the nascent conducting vessels that will ultimately develop into the vascular systems of xylem and phloem. Another point to be considered is that all the annotated MAN genes in Arabidopsis have predicted signal peptides, an indication that their corresponding proteins will be probably exported into the apoplastic space and could reach the CWs of the ME. Thus, radicle and endosperm MAN synthesis will contribute together to the weakening of the micropylar zones of the endosperm (**Fig. 2**). Remarkably, hormonal cooperation between the embryo and endosperm have been demonstrated in other physiological processes.[19,20] As far as we know, since the radicle of Arabidopsis is also a production site of MAN that can be allocated to the periplasmic space, through the secretion pathway, it is reasonable to postulate a cooperation between embryo and endosperm MAN enzymes in the dismantling of the ME CW thus facilitating radicle protrusion. Our work[18] would be the first data to support such a cross-talk regarding the synthesis of MAN upon germination.

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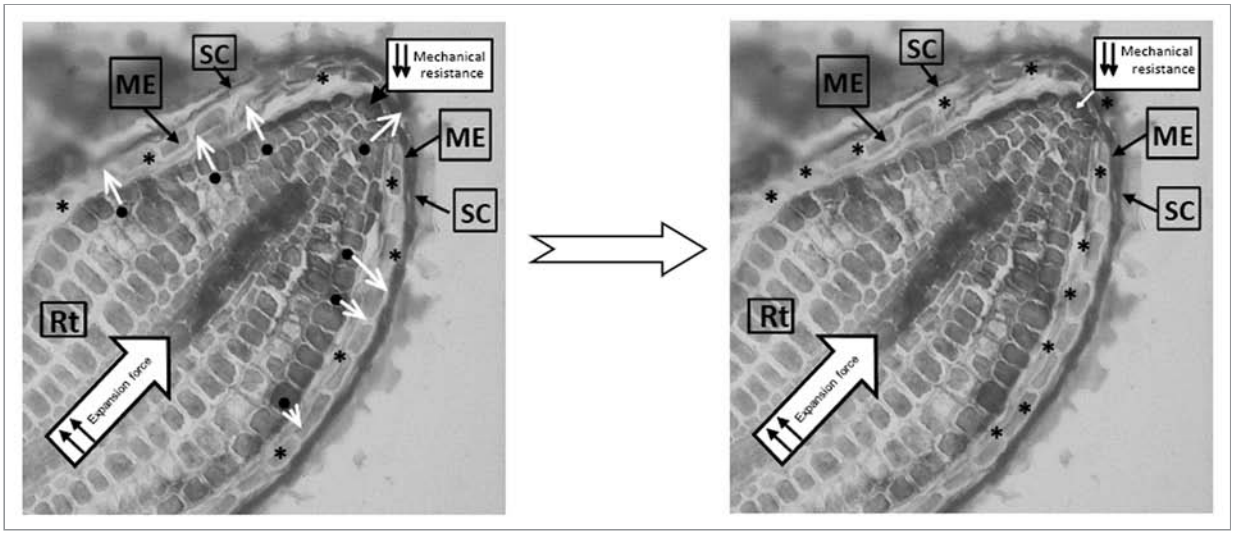

Figure 2. Proposed model of MAN enzyme traffic in the germinating seed. MAN proteins with their signal peptides (SP; ●) are secreted ■ to the apoplastic space where they become active after proteolysis of the SP (*). Root, Rt; micropylar endosperm, ME; and seed coat SC.

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