Seed mucilage evolution: diverse molecular mechanisms generate versatile ecological functions for particular environments

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Abstract

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Abstract

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myxospermy, and their functions in seeds seem to be conserved across Rosids. Historically, the ecological functions of seed mucilage were mostly related to germination and seed dissemination but recently some exosystemic functions were uncovered such as soil micro-organism control and plant establishment support. These recent studies have advanced the understanding of seed mucilage diversity and part of its evolution as well as its ecological functions.

Keys words: seed mucilage, inter-species natural variability, intra-species natural variability, ecological roles, myxospermy, myxocarpy, myxodiaspory, MBW master regulator, MSC toolbox gene evolution

Running head : A radiative evolution of myxospermy for diversified ecological functions

Introduction

Mucopolysaccharides, also called mucilage, are found to be produced in early diverging non-vascular plant groups such as hornworts (e.g. Anthoceros sp.) which extrude it around organs for various functions such as dehydration protection during growth and reproduction (Renzaglia, Duff, Nickrent & Garbary 2000). In flowering plants, several kinds of mucilage with cell wall-like compositions can be secreted by a wide range of organs such as seeds, fruits, roots, leaves, and stems conferring an impressive diversity of physical properties (Galloway, Knox & Krause 2020). The term myxodiaspory designates the ability to extrude mucilage upon imbibition from the seed coat or the fruit pericarp (Ryding 2001; Figure 1). The ability of species to release seed mucilage from the seed coat epidermis is called myxospermy, while the same ability coming from the fruit epicarp outermost cell layer is called myxocarpy (Figure 1). Seed mucilage presence was reported early in a Charles Darwin's letter (Weitbrecht, Muller & Leubner-Metzger 2011). The description of its morphology in several species and its putative ecological functions has drawn the interest of the scientific community for over a century. In the last 50 years, myxodiaspory was described in a majority of angiosperm orders associated with the diversity of mucilage secretory cells (MSCs) and their released polysaccharidic mixtures (reviewed in Phan & Burton 2018). Recent reviews have explored the biochemical composition and cell wall dynamics of MSCs during seed development, and the molecular, biochemical and structural characterization of mucilage, in particular in*Arabidopsis thaliana* (Griffiths & North 2017; Golz *et al.*2018; Phan & Burton 2018; Šola, Dean & Haughn 2019a). Seed mucilage can represent a significant metabolic cost as, for instance, it accounts for 2-3% of the *A. thaliana* seed mass (Macquet, Ralet, Kronenberger, Marion-Poll & North 2007b). In the large array of studied species, this metabolic investment was documented to fulfill numerous ecological functions such as seed protection and seed dispersal by direct physical modification of the local conditions (reviewed in Western 2012; Yang, Baskin, Baskin & Huang 2012c; North, Berger, Saez-Aguayo & Ralet 2014). As mucilage has specific physical and chemical properties and can be easily extracted, several applications were developed in pharmaceutical and food industry, as dietary supplement and biopolymer respectively (Mirhosseini & Amid 2012; Soukoulis, Gaiani & Hoffmann 2018).

Even if myxodiaspory has been described all along the angiosperm phylogenetic tree (Phan & Burton 2018), several species within the studied plant orders do not have mucilage or do not extrude it. It is unclear whether myxodiaspory has one or multiple evolutionary origins and for which reason it was, supposedly, lost in several species. Full answer to these evolutionary questions will require comparative and integrative studies connecting seed mucilage function(s) to precise and standardized morphological descriptions, as well as characterization of the involved molecular actors on species spread across angiosperms. Such rare studies concern a few species and are restricted to a single research field (*e.g.* genetic characterization, physiology and development or ecology). The overall aim of this review is to focus on the under-studied field of myxodiaspory evolution. So far, a deep characterization of the molecular actors implicated in seed mucilage establishment was performed primarily in *A. thaliana* constituting the so-called MSC tool box (Francoz, Ranocha, Burlat & Dunand 2015). Computational analyses should identify orthologous relationships between*A. thaliana* MSC toolbox genes and genes in other species, in turn clarifying the origin of this toolbox, and when it could have started to be associated with myxospermy. However, numerous MSC toolbox genes form a complex regulatory network that has unequal contributions to myxospermy and individual genes can display pleiotropic functions (Golz*et al.* 2018). Therefore, it is very difficult to clearly identify genes committed with myxospermy, merely from computational analyses. Indeed, in *A. thaliana* seeds, a regulatory complex dictates carbon partitioning between storage lipids, flavonoid pigments and the seed mucilage (Song *et al.* 2017). Lipids accumulating in the embryo and the endosperm will provide the required nutriments for proper embryo development, while pigments will confer impermeability and radiation protection (Baroux & Grossniklaus 2019), and the mucilage will bring additional ecological advantages depending on the species.

The objective of this review is to integrate multiple levels of information in a survey on (i) the occurrence and diversity of myxospermy (and more widely of myxodiaspory), (ii) the evolution of the molecular actors involved in this complex trait and (iii) the various ecological functions of seed mucilage. First, we briefly expose seed mucilage and MSC structural diversity mainly illustrated by comparison between the pioneer *A. thaliana* model and the emerging flax model. In the second part, we present the current status of the literature concerning the evolutionary origin of the underlying genes, initially identified in *A. thaliana* . Their integration with other seed traits such as storage lipids and protective pigments are developed focusing on their diversity at both the intra- and inter-species level. In the last part, we discuss recent insights on seed mucilage numerous ecological functions facing abiotic and biotic constraints, as well as its impact on plant development obtained since the last reviewing in 2012 (Western 2012; Yang *et al.* 2012c).

Diversity of myxodiaspory

A convenient method to attest the presence of mucilage and to study mucilage secretory cells (MSCs) is mucilaginous pectin staining performed on hydrated seeds with ruthenium red, or section staining with a generic and polychromatic stain such as toluidine blue (Western 2001). Further characterization is obtained by immunofluorescence with cell wall epitope antibodies directly performed on whole hydrated seeds or on sections (Ben-Tov *et al.* 2018). Polysaccharide chemical analysis can be performed, facilitated by the easy extraction of mucilage (Zhao, Qiao & Wu 2017; Poulain, Botran, North & Ralet 2019). Ultrastructural analysis by scanning electron microscopy and atomic force microscopy was also reported (Kreitschitz & Gorb 2018; Williams *et al.* 2020).

MSCs correspond to the outermost seed coat epidermal cell layer enabling mucilage extrusion to the environment (Figures 1, 2A). As exemplified in*A. thaliana* and *Linum usitatissimum* (flax), this specific cell layer is differentiated during seed development to become a dead layer at the end of the seed maturation (Figure 2; Western 2001; Miart*et al.* 2019). These two model species illustrate the diversity in the MSC cell wall dynamics during seed development as well as in the various mucilage organization patterns and extrusion modes.

During the MSC development, the *A. thaliana* seed mucilage is trapped, with no apparent sub-layering, between the outer periclinal primary wall and a volcano-shaped polarized secondary wall called columella (Figure 2). In flax, a complex multilayered mucilage is sequentially deposited in the MSCs and becomes visible after extrusion (Figure 2). Beyond these two models, the MSC morphological diversity is also illustrated in the various species studied over the years (Phan & Burton 2018), as exemplified by the pioneer morphological survey of mature MSCs conducted on 200 Brassicaceae species covering 90 genera (Vaughan & Whitehouse 1971).

Upon seed imbibition, sequential events occur within seconds in all species. First, the hydrophilic nature of the polysaccharide mixture constituting the mucilage allows water absorption. This induces a mucilage swelling pressure breaking the MSC primary cell wall (Figure 2). Finally, this leads to mucilage extrusion outwards the seed. As a result, the seed mucilage volume and mass increase up to 75-fold in*Capsella bursa-pastoris* (Deng, Jeng, Toorop, Squire & Iannetta 2012) and 5.5-fold in *Henophyton deserti* (Gorai, El Aloui, Yang & Neffati 2014), respectively. However, subtle differences occur in seed mucilage extrusion modes contributing to the diversity of the trait (Figure 2). Indeed, the rupture of peculiar primary wall domains occurs either simultaneously in all Arabidopsis MSCs or sequentially in adjacent flax MSCs (Figure 2). This organized explosion is carefully prepared earlier during MSC seed development by differential cell wall polysaccharide deposition and localized modifications. The polysaccharidic-proteinaceous molecular scaffold enabling the *A. thaliana* primary wall domain loosening starts to be uncovered (Kunieda*et al.* 2013; Saez-Aguayo *et al.* 2013; Francoz *et al.* 2019). In flax, the MSC polysaccharidic composition and its internal organization are proposed to play a role in proper MSC opening (Miart *et al.* 2019).

The structure and polysaccharidic composition of seed mucilage directly contribute to the observable diversity of myxospermy. In Arabidopsis, the released mucilage is separated between an adherent layer bound to the seed and a non-adherent layer, both enriched in poorly branched type I rhamnogalacturonan (RGI) pectin (Figure 2; Macquet *et al.*2007a; Poulain *et al.* 2019). In flax, the mucilage is composed by four contrasted layers enriched in RGI, arabinoxylans and xyloglucans/cellulose, respectively (Figure 2; Kreitschitz & Gorb 2017; Miart *et al.* 2019). This type of seed mucilage variable layering and composition also exists in other species such as for example,*Lepidium perfoliatum* (Huang, Wang, Yuan, Cao & Lan 2015),*Neopallasia pectinata* (Kreitschitz & Gorb 2017) or*Plantago ovata* (Tucker *et al.* 2017; Yu *et al.*2017). The comparison among multiple species has demonstrated the diversity of seed mucilage microstructure observed by scanning electron microscopy (Kreitschitz & Gorb 2018). Studying seed mucilage allowed characterization of polysaccharide-polysaccharide specific interactions (Yu *et al.* 2018) making seed mucilage an excellent model for cell wall dynamics understanding (Arsovski, Haughn & Western 2010).

Interestingly, for myxocarpous species such as *Salvia* and*Artemisia* species, the mucilage is extruded by the outermost fruit cell layer, named the achene (non-dehiscent fruit) pericarp, and not by the seed integument (Ryding 2001). These are clear examples of evolutionary convergences leading to the similar differentiation into MSCs of different types of outer cells facing the environment. Indeed, the *Salvia hispanica* (chia) achene mucilage and MSCs show interesting parallels with myxospermous species such as *A. thaliana* and *L. usitatissimum* . Mucilage accumulates in the outer pericarp epidermal cells during chia seed development. After extrusion, the mucilage remains indirectly attached to the seed via the inner pericarp-seed tegument contact (Geneve, Hildebrand, Phillips, Al-Amery & Kester 2017). Finally, an additional peculiarity may exist in*Medicago truncatula* and *M. orbicularis* , the cell wall of the endosperm forms a mucilage gel between the seed coat and the embryo (Song *et al.* 2017). Therefore, myxodiaspory is a trait that encompasses multiple levels of diversity, including MSC structure, mucilage extrusion mode, and mucilage polysaccharidic composition and structural organization. However, there is no simple clear-cut distribution of the myxodiasporous/non myxodiasporous traits along the angiosperm families and even within families as exemplified for Brassicaceae (Vaughan & Whitehouse 1971). For this reason, in the following part, we will shed light on molecular mechanisms underlying this morphological diversity through intra-species and inter-species comparative studies for seed mucilage evolution understanding.

Evolution of the molecular actors underlying the intra- and inter species myxospermy natural variability

3.1. The MSC tool box genes may be separated between upstream regulators and downstream actors

Twenty years of forward and reverse genetics together with more global approaches have allowed functional characterization of numerous genes involved in seed mucilage and MSC physiology in *A. thaliana*.Recent reviews reported 54 genes (Francoz *et al.* 2015) and 82 genes (Phan & Burton 2018). Since then, 12 additional genes have been characterized (Li, Zhang, Chen, Ji & Yu 2018; Shimada *et al.*2018; Takenaka *et al.* 2018; Voiniciuc *et al.* 2018; van Wijk *et al.* 2018; Kunieda, Hara-Nishimura, Demura & Haughn 2019; Šola *et al.* 2019b; Yang *et al.* 2019; Wang *et al.*2019; Fabrissin *et al.* 2019) bringing the list to 94 genes so far. They constitute the continuously growing MSC toolbox that participate to a proper seed mucilage production and release in Arabidopsis (Francoz *et al.* 2015; Voiniciuc, Yang, Schmidt, Günl & Usadel 2015). A majority of these genes are transcription factors including well characterized upstream master regulators that will be further discussed hereafter, and less characterized regulatory genes whose position in the gene regulatory network is still puzzling (Golz*et al.* 2018). The other downstream genes of the toolbox mostly encode direct actors responsible of seed mucilage synthesis, assembly and secretion (Francoz *et al.* 2015).

3.2. The intra-species myxospermy natural variability reveals a strong selection pressure targeted on a few downstream MSC tool box genes directly involved in mucilage synthesis and release

Detecting and understanding the effects of selection pressure on the MSC toolbox genes should be easier when considering the intra-species rather than the inter-species natural variability, because changes are still relatively recent on the evolutionary scale time and are scarcer. Indeed, the natural diversity occurring in *L. usitatissimum*cultivars (Liu *et al.* 2016; Miart *et al.* 2019), or in*A. thaliana* natural ecotypes (Saez-Aguayo *et al.* 2014; Voiniciuc *et al.* 2016) shows a gradient of mucilage abundance and myxospermy efficiency. This can reach a complete loss of mucilage extrusion for *A. thaliana* natural populations such as Sha (Macquet *et al.* 2007a) or the loss of adherent mucilage extrusion for Rak-1 (Saez-Aguayo *et al.* 2014). Interestingly, the absence of adherent mucilage extrusion does not necessarily mean a lack of mucilage synthesis since Rak-1 releases even more non adherent mucilage than Col-0 (Saez-Aguayo *et al.* 2014). All characterized mutation in natural *A. thaliana* populations related to myxospermy converge to three downstream genes of the MSC tool box, namely*PECTINMETHYLESTERASE INHIBITOR6* (*PMEI6*) for Dja,*MUCILAGE-MODIFIED2/BETA-GALACTOSIDASE6* (*MUM2/BGAL6*) for Sha, and *PEROXIDASE36* (*PRX36*) and *MUM2/BGAL6* for Sk-1. This represents a low number of genes as compared to the MSC toolbox size and considering the nearly 300 studied natural populations (Saez-Aguayo *et al.* 2014; Voiniciuc *et al.* 2016). This strongly suggests that downstream MSC toolbox genes undergo strong selection pressure, but also that myxospermy is a fast-evolving trait as exemplified by the seven independent *MUM2/BGAL6* natural mutants found in only two different geographical area (Saez-Aguayo *et al.*2014). *PMEI6* , *MUM2/BGAL6* and *PRX36* encode enzymes necessary for proper seed mucilage hydration and extrusion (Dean*et al.* 2007; Kunieda *et al.* 2013; Saez-Aguayo *et al.* 2013) and PMEI6 and PRX36 functions are tightly and sequentially related (Francoz *et al.* 2019). As non-myxospermous seeds have a much better buyoancy efficiency and since Sha habitat is close to a river, seed dispersal by water run-off is one of the seed mucilage functions proposed to explain the loss of myxospermy (Macquet *et al.* 2007a; Saez-Aguayo *et al.* 2014). Unfortunately, no clear association can be established between the natural population habitats and their mucilage phenotypes (Voiniciuc *et al.* 2016) but the selection pressure toward a mucilage disappearance highlights its ambivalent function for myxospermous species.

More recently, genome wide association studies (GWAS) conducted on*A. thaliana* (Fabrissin *et al.* 2019) and *L. usitatissimum* (Soto-Cerda *et al.* 2018) allowed the identification of the statistically most relevant single nucleotide polymorphisms (SNPs) explaining the observed seed mucilage phenotype. In*L. usitatissimum,* out of the seven loci implicated in mucilage content, five are orthologs of previously characterized *A. thaliana* MSC tool box genes that are downstream direct actors of seed mucilage synthesis or modification (Soto-Cerda *et al.* 2018). In*A. thaliana* , through a very precise and molecular phenotyping, GWAS revealed only 8 peaks significantly above the high background of less implicated positions, pointing to seed mucilage being a polygenic trait (Fabrissin *et al.* 2019). Upon the 8 candidates, two genes were identified, one already known to belong to the MSC toolbox and another one characterized as implicated in the production of seed mucilage pectin content (Fabrissin *et al.* 2019). These results highlight the fact that the MSC toolbox starts to be well characterized in *A. thaliana* and that it can be used to investigate whether functional orthologs are present in other species. It also suggests that at the intra-species level, the myxospermy selective pressure mostly conserved the upstream master regulators but altered their downstream target genes of the MSC toolbox. Hopefully, this conservation of the upstream genes of the toolbox regulation network will allow to study the evolutionary origin of part of the MSC toolbox genes across angiosperms.

3.3. The angiosperm inter-species myxospermy variability involves a conserved master regulatory complex

In *A. thaliana* , some of the master regulators belonging to the MSC toolbox also regulate the formation of trichomes and root hairs (Jones & Dolan 2012), the flavonoid biosynthesis (anthocyanidins and proanthocyanidins) (Lloyd *et al.* 2017), and the seed carbon partitioning (Golz *et al.* 2018; Li *et al.* 2018; Chen & Wang 2019). The combinations of specific MYB and bHLH transcription factors together with TRANSPARENT

TESTA GLABRA 1 (TTG1), a WD40 domain repeats (WDR) transcription factor allows the regulation of each of these traits. They constitute the MYB-bHLH-WD40 repeat (MBW) regulatory complexes (Figure 3). It is important to note that, to our knowledge, (i) TTG1 is common to all aforementioned traits, (ii) each bHLH protein is involved in the regulation of two or more traits and (iii) each MYB mostly controls only one trait (Chen & Wang 2019; Zhang, Chopra, Schrader & Hülskamp 2019; Figure 3). Accordingly, the *A. thaliana ttg1* mutant lacks root hairs and trichomes, has a reduced level of anthocyanins and proanthocyanins and does not accumulate mucilage in the MSCs (Galway *et al.* 1994; Walker*et al.* 1999; Western 2001). In wild type *A. thaliana* , the phosphorylation of TTG1 by SHAGGY-like kinases 11/12 (SK11:12) prevents its interaction with TRANSPARENT TESTA2/MYB123 (TT2/MYB123)*,* a MYB member of the MBW complex, decreasing the transcription of the downstream regulator *GLABRA2* (*GL2*) (Li *et al.*2018); Figure 3). The consequence for the seeds is the promotion of lipid storage in the embryo at the expense of mucilage and flavonoid pigment production in the seed coat (Li *et al.* 2018; Figure 3). This differential regulation of *GL2* is probably responsible for the differential balance between seed lipid and pigment/mucilage contents in two natural Medicago species that correlates with *GL2*expression level (Song *et al.* 2017; Figure 3). However, additional regulation mechanisms through interactions, competitions, post-translational or epigenetic modifications may also occur (Xu, Dubos & Lepiniec 2015; Nguyen, Tran & Nguyen 2019). Altogether, this probably explains how a few molecular actors control several functions.

Land plant phylogenetic analysis of the WD40 domain repeats (WDR) transcription factor family reveals that TTG1 appeared in the common ancestor of angiosperms and gymnosperms (seed plants). It has undergone a duplication at this node and acquired new functions for control of epidermal cells differentiation in essentially all organs of plants, in addition to its circadian clock ancestral function (Airoldi, Hearn, Brockington, Webb & Glover 2019, Figure 4). Interestingly, the serine 215 that can be phosphorylated by SK11/12 is conserved across seed plant TTG1 orthologs, suggesting that this ancestral regulation for this master regulator allowing switches in carbon flow between the seed coat and the embryo is independent of the presence of seed mucilage (Li*et al.* 2018; Airoldi *et al.* 2019).

The bHLH and MYB proteins have been subjected to numerous and recent duplication events (Sullivan *et al.* 2019; Doroshkov *et al.*2019). Their different combinations within several MBW complexes have probably been co-opted to control the emergence of new biological process often linked to epidermal cells such as seed mucilage establishment (Figure 4). Indeed, the *A. thaliana* MBW regulatory complex controlling seed mucilage and seed coat pigments involves at least two bHLHs (TT8-EGL3), one MYB (TT2) together with one WDR (TTG1) (reviewed in (Golz *et al.* 2018). For the proper establishment of myxospermy, there is a need of at least three additional MYBs (MYB5-MYB23-MYB61) (Penfield 2001; Matsui, Hiratsu, Koyama, Tanaka & Ohme-Takagi 2005; Li *et al* . 2009, Figure 3). The functional conservation of TTG1 in the mucilage production and release has been demonstrated in the two Brassicaceae species *Matthiola incana*(Dressel & Hemleben 2009) and *Arabis alpina* (Chopra *et al.* 2014), and in *M. truncatula* (Pang *et al.* 2009). Orthologs of TTG1 from *Camellia sinensis* (Liu *et al.*2018b) or even from the monocotyledonous species *Setaria italica*can restore mucilage wild type phenotype of *A. thalianattg1* mutant through the recovery of *GL2* and *MUCILAGE MODIFIED4/RHAMNOSE BIOSYNTHESIS 2* (*MUM4/RHM2)* gene expression (Liu *et al.* 2017). This functional conservation together with clear orthologous relationships give more confidence for the presence of TTG1 in the most recent common ancestor of seed plants (Figure 4). Using a similar trans-complementation approach, the functional conservation of the two bHLH proteins EGL3 and TT8 have also been demonstrated in all tested angiosperms for all MBW complex-associated traits (root hair, trichome, anthocyanin and proanthocyanin pigments and mucilage) (Zhang & Hülskamp 2019). Additionally, the phylogeny of these two genes is well resolved at the angiosperm level, with the *Amborella trichopoda* ortholog gene branching early for each gene (Doroshkov*et al.* 2019, Figure 4). In *A. thaliana,* the three bHLH genes, *EGL3* , *GL3* and *MYC1* (root hair regulators) originated from an ancestral bHLH gene after a triplication within Brassicaceae (Doroshkov *et al.* 2019, Figure 4). Brassicaceae, as many other plant clades have undergone a whole-genome duplication (Mabry*et al.* 2019). Thus, it will be not surprising to find several duplication events at the Brassicaceae node for other genes belonging to the MSC toolbox, especially for multigene family such as bHLHs and MYBs. These duplicated genes then functionally diverged as shown by the unexpected partial rescue of seed mucilage by the transcomplementation of *A. alpina GL3 in the A. thaliana gl3 /egl3 /tt8* triple mutant (Zhang & Hülskamp 2019). Since the Arabidopsis GL3 is not able to complement the mucilage phenotype in the same triple mutant, this indicates an intra-Brassicaceae divergence between both *GL3* genes from their last common ancestor, in agreement with the contrasted morphology of*A. alpina* MSCs as compared to those of *A. thaliana* (Chopra*et al.* 2014). Similarly, in each Rosid family, few changes can be expected in the myxospermy-related MBW complex, and the more the genes are in the downstream part of the network (*e.g.* bHLHs), the higher the divergence will be responsible of the observed morphological diversity of MSCs, such as in Brassicaceae (Vaughan & Whitehouse 1971).

Concerning MYB genes, *MYB5* and *TT2* ortholog genes in*M. truncatula* have a conserved function because they positively regulate seed coat pigment and mucilage (Liu, Jun & Dixon 2014), suggesting that the MBW complexes dedicated to seed mucilage and seed coat pigments are conserved among the Rosids species (Figure 4). Furthermore, the identification by genome wide association study (GWAS) of seven genes implicated in the seed mucilage content in flax, all orthologous to *A. thaliana* MSC toolbox genes (Soto-Cerda *et al.* 2018), show a conservation in sequences and functions between the two species, even for some downstream genes. As their last common ancestor is at the basis of the Rosid clade, this reinforces the hypothesis of shared ancestral origin of myxospermy in the entire Rosid clade (Figure 4). However, in its current form, the MYB family phylogeny is not sufficiently well resolved to comfort this hypothesis, due to the small size and the great variability of MYB genes (Doroshkov *et al.* 2019). Since the phylogeny of this multigene family is difficult to solve and since bHLH and MYB association in MBW complexes depend of non-binary competitive interaction (Zhang *et al.* 2019) and of post translational modifications (Li *et al.* 2018), more studies will be helpful to fully characterize the evolution of the MBW complexes.

These results suggest that during seed plant evolution, TTG1 first appeared to balance carbon flow in seed tissues. It progressively interacted with bHLH members allowing more regulatory functions through a ternary complex modularity, with numerous and versatile recruitment of MYB members for deeper specialization to control each trait in different seed zones such as seed mucilage in the MSCs. A remaining dark area concerning the mucilage evolution is located between the Rosids and*A. thaliana* . This could be solved by comparative studies between Brassicaceae species closely related to *A. thaliana* such as*Camelina sativa* , or at a larger scale by comparing the two emerging models for MSCs flax and *A. thaliana* .

Altogether, the *A. thaliana* MSC toolbox genes seem to have undergone an asymmetrical selection pressure comparing the great conservation of the MBW complex during evolution, to the strong and rapid changes of downstream direct actors existing in intra-species variability. In the following part, we present the newly identified putative ecological functions of myxodiaspory that can be helpful to understand why it can confer a selective advantage and to which kind of constraints.

New insights in ecological function of seed mucilage

Considering that seed mucilage establishment is costly for the mother plant metabolism, its presence implies that it probably displays major functions and that this trait is under a positive selection pressure in the myxodiasporous species. According to mucilage adherent and hydrophilic properties, the scientific community first investigated its influence on seed dispersal and germination (reviewed in Western 2012; Yang *et al.* 2012c), and more recently looked for potential interactions between seed mucilage and the abiotic and biotic constraints.

4.1. Influence on seed dispersal and germination

A seed adaptation such as myxospermy is expected to influence seed dispersal and germination (Figure 5A). However, these roles can be completely different between closely related species, making it difficult to extend the concept to all myxodiasporous species. Counter-intuitively, mucilage can be a negative regulator of seed germination in *Leptocereus scopulophilus* (Barrios, Flores, González-Torres & Palmarola 2015) and also for the achene germination of *Artemisia monosperma* (Huang & Gutterman 1999a). In*Blepharis persica*

, the seed mucilage could block oxygen transfer under water excess and then prevent germination (Witztum, Gutterman & Evenari 1969). This function was regularly re-emphasized (last time in Gorai *et al.* 2014) though never fully demonstrated. However, mucilage can also improve germination (Figure 5A-1). The pioneer most cited seed mucilage function in *A. thaliana* was a positive role during germination under osmotic stress conditions, considering the polyethylene glycol (PEG)-dependent decrease of germination rate observed for *myb61* , *gl2* and *ttg1* mutants (Penfield 2001). On another MSC toolbox downstream gene mutant, a defect in seed mucilage extrusion induce a delayed germination, suggesting a positive effect of mucilage on germination efficiency rather than on germination rate (Arsovski *et al.* 2009). However, more recently no such phenotypes were obtained in the *mum2* or *myb61* mutants (Saez-Aguayo *et al.* 2014). Thus, the role of seed mucilage in*A. thaliana* germination deserves to be deeply explored. For*S. hispanica* , the intact myxocarpous achenes germinate better than the achenes without mucilage (Geneve *et al.* 2017). Interestingly, seed mucilage-dependent better germination phenotypes are obtained with PEG application and not with salt at equal osmotic potential (Geneve *et al.* 2017). Upon five desert species (*Lavandula subnuda, Lepidium aucheri, Boerhavia elegans, Plantago ciliata and Plantago amplexicaulis),* the seed mucilage presence increased water uptake but its removal led to contrasted germination effects (Bhatt, Santo & Gallacher 2016). Therefore, the mucilage function in germination seems to be related to water uptake and/or seed permeability to water and possibly to gazes.

Some studies took advantage of the fact that two seed morphotypes occur within the same species (myxospermous and non-myxospermous seeds). These species use these dimorphic seeds to improve species persistence and dispersion (Liu, Wang, Tanveer & Song 2018a). For three Brassicaceae species having characterized dimorphic seeds for myxospermy, namely*Diptyocharpus strictus, Capsella bursa-pastorisi and Aethionema arabicum,* the seed morphotype with the higher dormancy are not myxospermous (Lu, Tan, Baskin & Baskin 2010; Toorop *et al.* 2012; Arshad *et al.* 2019) suggesting that myxospermous seeds should germinate without delay. The co-occurrence of seed mucilage and wings on seeds of *D. strictus* (Lu *et al.* 2010) and *Henophyton deserti* (Gorai *et al.* 2014) questions whether antitelochory and anemochory are opposite or can be complementary. Antitelochory prevents seed dispersion far from the mother plant while anemochory favors wind-driven dissemination under dry conditions until the seed encounters water and stops its dispersion. Combination of both traits can give a powerful advantage by an efficient dispersal until an optimal place for hydric conditions is reached (Figure 5A-2). Interestingly, *Lunaria annua* shows a surprising use of mucilage by secreting it from the inner surface of the fruit to keep the four seeds sticked to it, even after its dehiscence, allowing a differential dispersion between the two halves of the fruit and their two attached seeds by the wind (Leins, Fligge & Erbar 2018).

4.2. Influence facing abiotic constraints

As seed mucilage constitutes highly hydrophilic gels, it is tempting to propose that it may provide water for the embryo. For *A. thaliana* , the seed mucilage takes a large amount of water from the environment but sequesters it through ionic linkages with galacturonic acid residues (Figure 5B-3; Saez-Aguayo *et al.* 2014). Indeed, mutants that have a lack of mucilage, imbibed their seed faster than the wild type or mutants with un-released mucilage (Saez-Aguayo *et al.* 2014). However, the seed mucilage allows fast seed sinking in water as compared to non myxospermous seeds that can float on water surface for longer time and even germinate on it. Achenes of *Artemisia sphaerocephala* germinate and float better when their mucilage was removed (Huang & Gutterman 1999b). Interestingly, adhesive and frictional properties of the seed mucilage can change according to its hydration level influencing at least its dispersal properties (Kreitschitz, Kovalev & Gorb 2015, 2016). In *A. arabicum* , the seed mucilage thick fibers emerging upon imbibition are able to conserve their structure and their size upon dehydration (Lenser *et al.*2016) allowing a dispersal efficiency compromise, for the re-dried seeds, for wind, water run-off and buoyancy dissemination ways, as compared to never imbibed seeds and fully imbibed seeds (Arshad *et al.* 2019). A more complete understanding of the complex roles of seed mucilage in water management would necessitate investigating its role in more natural situations such as succession of wetting and drying cycles, or flooding following a long drying period.

Soil physical properties can have a major impact on water availability and root penetration (Figure 5B-

4). The myxocarpy of *Artemisia sphaerocephala* enhances seedling emergence in its sandy environment and reduces plant mortality (Yang, Baskin, Baskin, Liu & Huang 2012a). By adding seed mucilage extract from *C. bursa pastoris* , soil rheological properties are modified particularly for hydraulic conductivity retaining water for longer time (Deng *et al.* 2014). A similar effect is provoked by *S. hispanica* seed mucilage addition, which links soil particles to increase aggregate stability for at least 30 days in diverse kinds of soil (Figure 5B-4; Di Marsico*et al.* 2018). Therefore, seed mucilage could locally improve the soil rheological in agreement with the non-disseminating lifestyle of species such as *A. thaliana* excreting non-adherent mucilage.

4.3. Biotic interactions

Similar to root border cell mucilage (Knee *et al.* 2001), non-adherent seed mucilage constitutes a significant amount of polysaccharides released in the environment. Taking into consideration the importance of micro-organisms in plant physiology along their development and their omnipresence around plant organs, this metabolic investment could indicate an involvement of seed mucilage in dealing with biotic constraints (Figure 5C). Its influence on microbial community for the plant was shown in *A. thaliana* using the bacteria *Streptomyces lividans* that inhibits germination and growth of the pathogenic fungus *Verticillium dahlia* that causes the verticillium wilt. When both micro-organisms are co-inoculated on seeds, *S. lividans* has a better proliferation within the seed mucilage in comparison to *V. dahlia* and considerably reduced the plant disease symptoms (Meschke & Schrempf 2010). This "selective media effect" (Figure 5C-5) promoting microbial hyphae development was further illustrated with *Salvia hispanica* achene mucilage and*Colletotrichum graminicola* fungi (Geneve *et al.* 2017). The desert plant *Artemisia sphaerocephala* achene mucilage was shown to be degraded by micro-organisms, providing CO_2 and soluble sugars to promote seedling establishment (Yang, Baskin, Baskin, Zhang & Huang 2012b). This beneficial effect was recently explained in the same species through the mucilage positive effect on soil microbial community composition and diversity to favor fungal-bacterial interaction and soil enzyme activities, protecting young seedlings from drought and pathogens (Hu *et al.* 2019b). Glomeromycota is one of the groups of fungal species positively impacted by achene mucilage. However, the positive effect on seedling growth of these fungi responsible for arbuscular mycorrhizae (mutualistic symbiosis with plants) do not show significant combined positive effect with mucilage, acting probably independently to enhance seedling establishment (Hu*et al.* 2019a). Active stress-associated enzymes, such as nuclease, protease, and chitinase, are secreted from the seed coat of several species even in seeds several decades old (Raviv *et al.*2017). However, this ability to secrete proteins is conserved in the non myxospermous species *Raphanus sativus* or in the *A. thaliana gl2* mutant deprived of seed mucilage suggesting that protein secretion and seed mucilage are independent (Raviv *et al.*2017).

Nematodes are in close interaction with plants and can have major pathogenic impact on plant development. *A. thaliana* seed mucilage contributes to attract root-knot nematodes (Figure 5C-6) with the additional requirement of seed-surface carbohydrates and proteins (Tsai*et al.* 2019). Considering the parasitic nature of those nematodes, this attraction is more probably due to the nematode adaptation rather than a plant adaptation. Conversely, nematodes could be predators of even more dangerous organisms for plants. *Capsella bursa-pastoris,* a closely relative species of *A. thaliana,* has myxospermous seeds that are also able to attract nematodes (Roberts, Warren & Provan 2018). Surprisingly, it seems to be a case of protocarnivory because the massive death of trapped nematodes in seed mucilage increases plant development from germination to young seedling establishment, especially under low nutrient level (Roberts *et al.* 2018). Thus, seed mucilage involvement in biotic interactions starts to be uncovered revealing astonishing functions that could affect the plant development in unexpected manners as compared to the previously characterized functions

Conclusions and perspectives

Myxodiaspory is a macroscopic seed trait that results from a surprising diversity of microscopic features among angiosperms, at both the intra-species and inter-species levels. This striking microscopic morphological diversity makes it difficult to trace back the evolutionary origin of seed mucilage based only on morphology. The available deeply characterized *A. thaliana* mucilage secretory cell (MSC) toolbox genes now allow comparison of these molecular actors within a species or between species, which starts to shed light on this mysterious evolutionary story. On the one hand, the intense selection pressure that mucilage establishment undergoes appears to be mainly applied on a few downstream genes of the MSC toolbox as illustrated in non myxospermous *A. thaliana* natural populations. On the other hand, the seed mucilage-related MBW upstream master regulatory complex appeared sequentially during seed plants evolution and is notably conserved across Rosids. From this regulatory complex, mucilage may have evolved several times independently as a combination of highly diverse traits including two MSC epidermal origins, various patterns of MSC cell wall dynamics, various mucilage polysaccharidic composition and sub-layering patterns or several modes of mucilage release. In turn, this diversity of traits probably contributes to the wide range of ecological functions observed in each species that face contrasted environments. The biotic and abiotic constraints are the least studied points in this field and probably the most promising tracks to uncover new seed mucilage integrated functions related to particular environments.

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FIGURE LEGENDS:

Figure 1: Developmental scheme comparing the two epidermal origins of mucilage secretory cells enabling mucilage release in myxospermous vs myxocarpous species. Note that the different layers are not drawn to scale and that more than one seed per fruit is commonly found in myxospermous species.

Figure 2: Arabidopsis and flax as model species for seed mucilage secretory cell (MSC) development illustrating the diversity of cell wall dynamics, intracellular polysaccharidic mucilage organization, and extrusion mechanisms upon water imbibition. (A) conceptual seed cross section wide view and magnification illustrating the localization of seed mucilage secretory cells (MSCs) **(B)**Kinetics of MSC development in the historical model *Arabidopsis thaliana* on the left (adapted from (Western 2001; Francoz *et al.*2019), and in the emerging model *Linum usitatissimum* on the right (adapted from (Miart *et al.* 2019). The 30-50 µm wide cells from both species are drawn to similar scale, the released mucilage layers are not drawn to scale. Note the numerous differences between both models: Major features in *A. thaliana* : highly complex cell wall dynamics with the presence of a volcano-shaped columella, the simultaneous rupture of primary cell wall domain in each MSC cells and the distinction between adherent mucilage (am) and non-adherent mucilage layers (nam). Major features in *L. usitatissimum* : sequential synthesis of four highly different mucilage layers (m1 to m4) and sequential rupture of primary wall domains expending from cell to cell. DAP=day after pollination

Figure 3: The MYB-bHLH-WDR (MBW) complexes regulate the spatiotemporal carbon partitioning in *Arabidopsis thaliana* seeds through a complex modularity. The conserved ancestral master regulator WDR member (TTG1) and the phosphorylation status of a conserved serine (P), together with a bHLH and MYB modularity of competitive interaction enables the regulation of the spatiotemporal specificity of various seed traits including mucilage production and release (Golz *et al.*2018; Li *et al.* 2018; Chen & Wang 2019).

Figure 4: Simplified phylogenetic tree of land plants giving an overview of the sequential evolution of the *A. thaliana*MSC toolbox using published phylogenomic, transcomplementation and functional characterization studies . Note that the members of the MBW complex controlling the myxospermy trait in *A. thaliana*sequentially evolved and were complete at the Rosid node, and that the downstream enzymatic actors of the MSC tool box appear to be specific to*A. thaliana* . Species names on the tree leaves are the ones use in the reviewed studies. EGL3* is the ancestor gene of AtEGL3, AtGL3 and

AtMYC1 that were later duplicated and diverged within the Brassicaceae family. TTG1 phosphorylation site (P) seems to be conserved together with the gene itself. The phylogenomic, transcomplementations and functional characterizations studies used to build this Figure come from Dressel & Hemleben 2009; Pang *et al.* 2009; Chopra *et al.*2014; Liu *et al.* 2014, 2017, 2018b; Li *et al.* 2018; Airoldi *et al.* 2019; Zhang *et al.* 2019; Doroshkov *et al.* 2019; Zhang & Hülskamp 2019.

Figure 5: Global overview of seed (or fruit) mucilage major ecological functions facing environmental constraints. (A) Seed mucilage may influence plant development through (1) positive or negative impact on germination upon inappropriate condition, depending on the species, or (2) seed dispersal in relation to conferred seed physical properties such as sinking ability or soil anchoring**(B)** Seed mucilage is modified by abiotic conditions in close environment through (3) regulation of water flux and water availability as well as (4) soil rheological remodelling properties. **(C)**Seed mucilage is involved in biotic interactions though (5) direct or indirect influence on microbial community establishment around the seed and the future plant and (6) attraction ability of nematodes.

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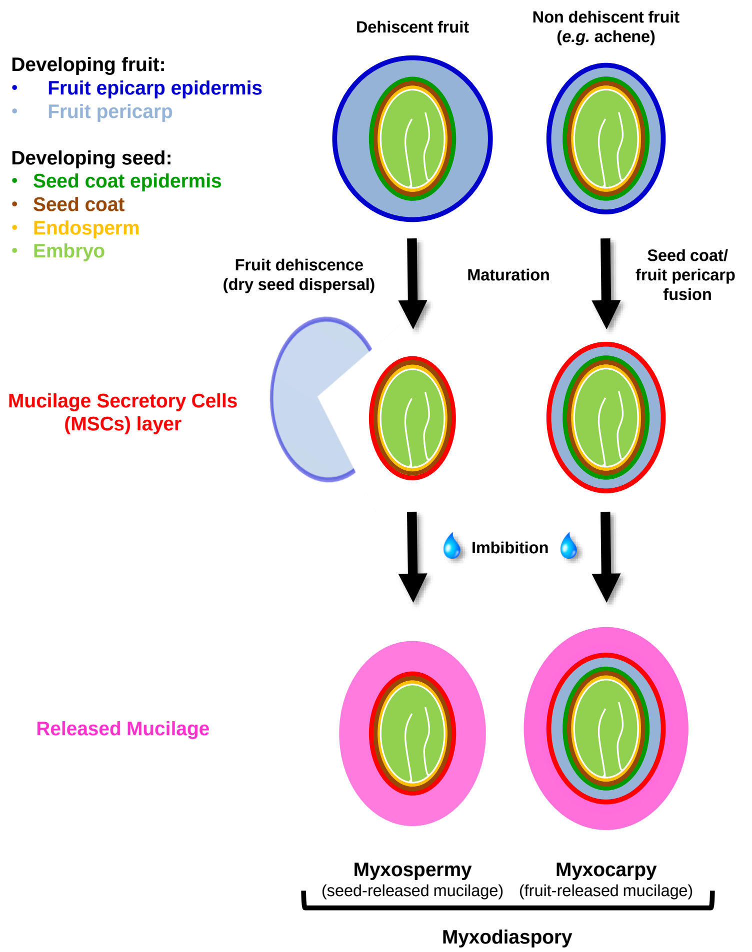

Figure 1: Developmental scheme comparing the two epidermal origins of mucilage secretory cells enabling mucilage release in myxospermous vs myxocarpous species..

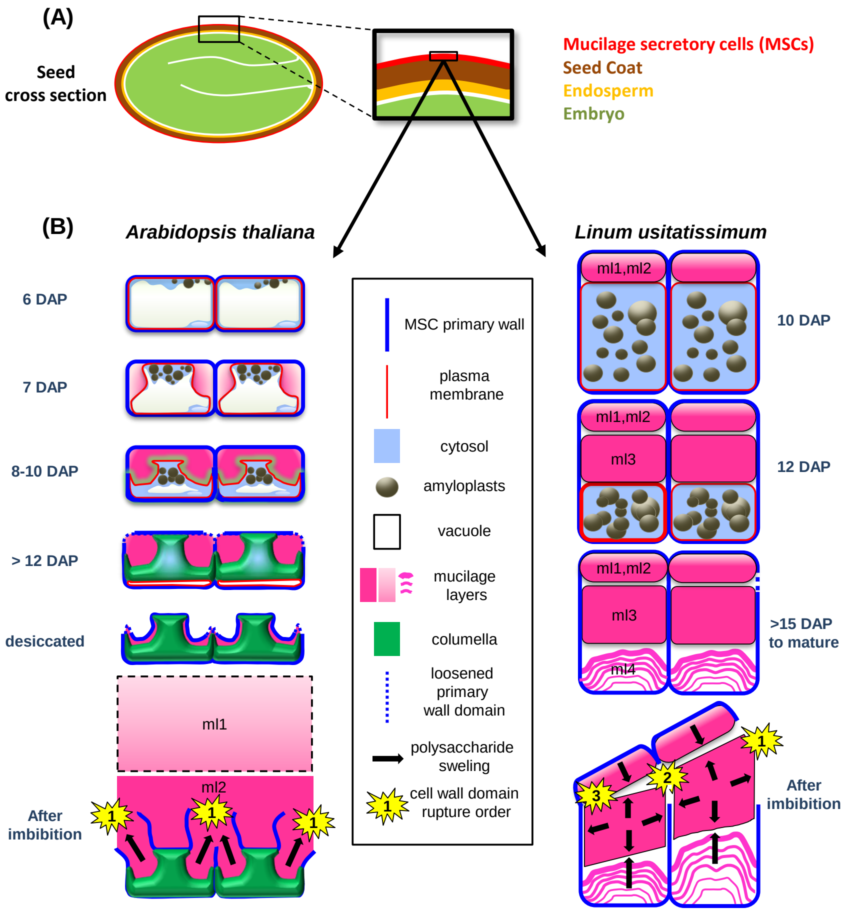

Figure 2: Arabidopsis and flax as model species for seed mucilage secretory cell (MSC) development illustrating the diversity of cell wall dynamics, intracellular polysaccharidic mucilage organization, and extrusion mechanisms upon water imbibition.

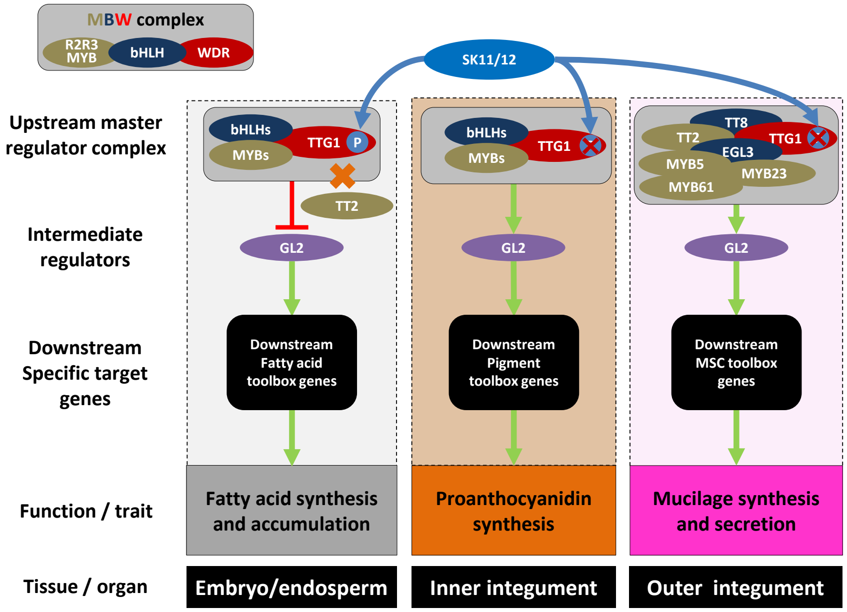

Figure 3: The MYB-bHLH-WDR (MBW) complexes regulate the spatiotemporal carbon partitioning in *Arabidopsis thaliana* seeds through a complex modularity.

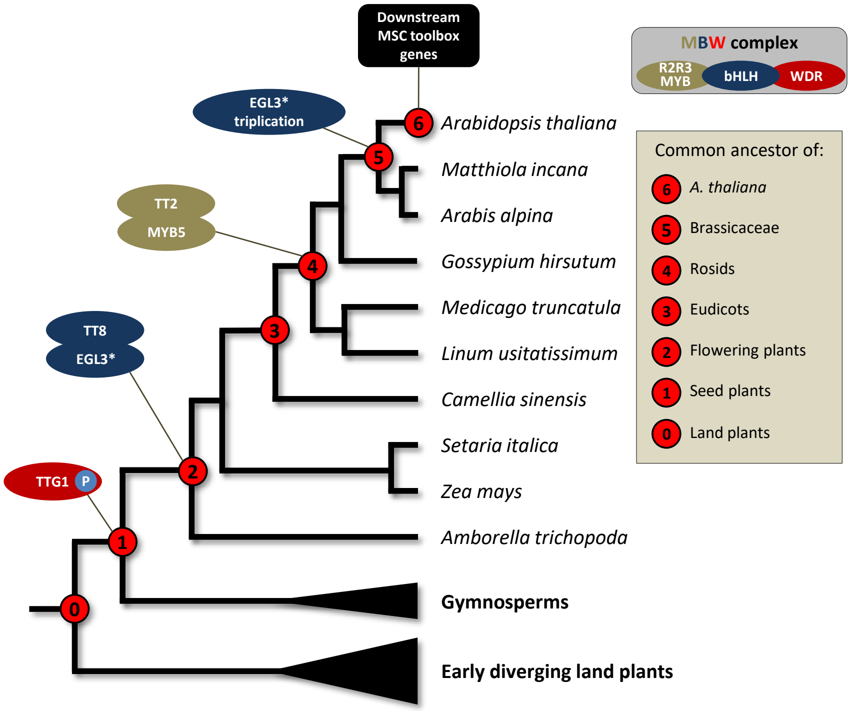

Figure 4: Simplified phylogenetic tree of land plants giving an overview of the sequential evolution of the *A. thaliana* MSC toolbox using published phylogenomic, transcomplementation and functional characterization studies.

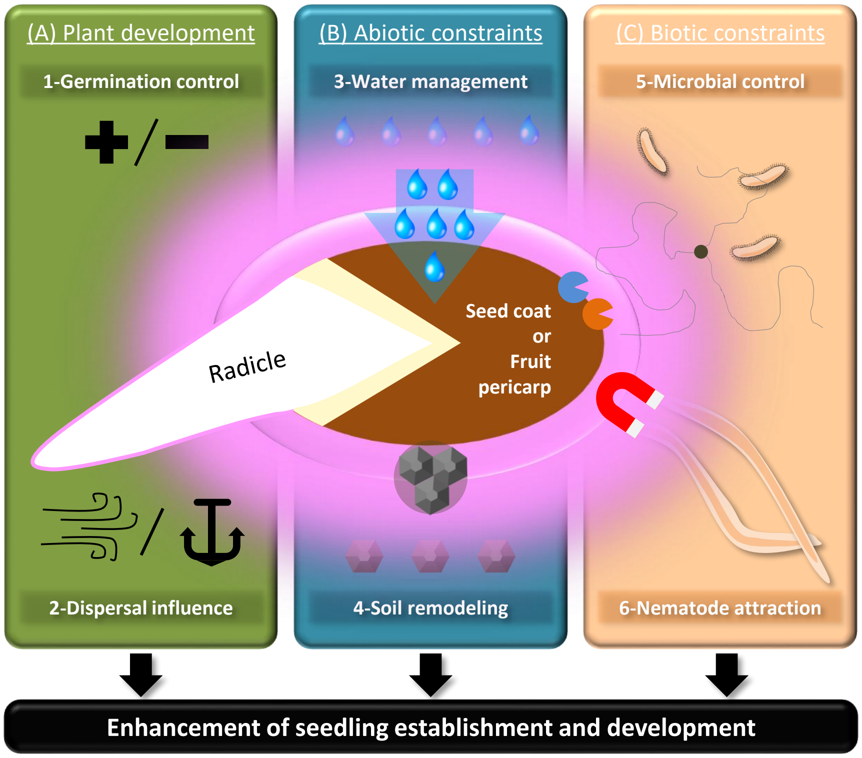

Figure 5: Global overview of seed (or fruit) mucilage major ecological functions facing environmental constraints.