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

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Abstract

Polysaccharidic mucilage is a widespread plant trait with diverse features, often present around plant structures in contact with the environment, providing numerous functions including protection and adhesion. Seed mucilage is released upon imbibition and therefore can play roles in the early seedling stages, but the evolutionary origins of this trait are unclear. Its presence in several flowering plant species suggests that it was present in their last common ancestor whereas the extreme inter-species morphological and chemical natural diversity suggests multiple origins. Here, we summarize the recent advances on molecular mechanisms and ecological functions underlying this inter- and intra-species natural diversity. A master regulatory complex balancing carbon partitioning in seed appears to be conserved among flowering plants with a sequential evolution of its molecular components. At the intra-species level, a high polymorphism was detected for a few genes in relation to the observed morphological diversity. 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 advances enable drawing a clearer picture of the seed mucilage evolution, the underlying molecular mechanisms, and the associated ecological roles.

Introduction

In the earliest diverging groups of terrestrial plants such as Anthocerotes, mucopolysaccharides, also called mucilage, are produced around organs and have dehydration protection roles during growth and reproduction (Renzaglia, Duff, Nickrent & Garbary 2000). In flowering plants, several kinds of mucilage can be secreted by a wide range of organs such as seeds, fruits, roots, leaves, and stems conferring impressive and diverse physical properties such as viscosity or adherence (Galloway, Knox & Krause 2019). The ability of species to release seed mucilage (SM) upon imbibition is called myxospermy. Its presence and the description of its morphology in several species, and its putative ecological functions, were reported early in a Charles Darwin's letter (Weitbrecht, Müller & Leubner-Metzger 2011) and for over a century by the scientific community. Review of biochemical analysis of SM composition and of deep morphological characterization of the mucilage secretory cells (MSCs) has been performed more recently (Phan & Burton 2018). In addition, several recent reviews mainly focused on the molecular, biochemical and structural characterization of the most studied SM plant model species *Arabidopsis thaliana* have been published (Griffiths & North 2017; Golz *et al.* 2018; Phan & Burton 2018; Šola, Dean & Haughn 2019). SM represents 2-3% of the total seed mass with numerous ecological functions (reviewed in Western 2012; Yang, Baskin, Baskin & Huang 2012c; North, Berger, Saez-Aguayo & Ralet 2014). This gathered knowledge led to several applications in pharmaceutical and food industry taking advantage of mucilage specific physical and chemical properties as well as its easy extraction (Mirhosseini & Amid 2012; Soukoulis, Gaiani & Hoffmann 2018).

In the last 50 years, SM was described in a majority of flowering plants orders associated with the diversity of MSCs and polysaccharidic mixture produced (Phan & Burton 2018). However, within the studied plants orders, several species do not have mucilage or do not extrude it. This raises the question of the evolutionary origins of SM: is it an ancestral trait of flowering plants present in the common ancestor that subsequently greatly diverged or was lost, or has it appeared several times independently? The answer to this evolutionary question rests on solving SM function(s), on standardized morphological description and on molecular studies focused on phylogenetically selected plant species/families. So far, a deep characterization of molecular actors implicated in SM establishment was performed primarily in *A. thaliana* . Comparisons with other species should allow to trackback the evolutionary scenario. However, the occurrence of numerous genes in complex regulatory networks, unequal implications in the trait and their often pleiotropic functions, makes it difficult to clearly identify genes that are committed with the trait. As evolution should conserve the better compromise between selective advantage and metabolic cost, looking at the entire carbon partitioning in seed will help to better understand SM evolution. Seed formation represents a high metabolic cost for the mother plant leading to a tight regulation of carbon partitioning between *e.g.* storage lipids accumulated in the endosperm and embryo, seed coat flavonoid pigments and SM (Song *et al.* 2017). The embryo/endosperm lipids will provide the required nutriments for proper embryo development, while pigments will confer impermeability and radiation protection (Baroux & Grossniklaus 2019). In this review, we first present a quick emphasis on SM and MSC structural diversity. The current state of the literature concerning the evolutionary origin of underlying genes and of their integration with other seed traits are developed in a second part at both intra- and inter- species level. Finally, we present the recent insights on SM ecological functions facing abiotic and biotic constraints as well as SM impact on plant development.

Development, physiology AND diversity of mucilage secretory cells

To properly extrude the SM to the environment, MSCs correspond to the outermost seed coat epidermal cell layer. As exemplified in *A. thaliana* and *Linum usitatissimum* (flax), this specific cell layer is formed during seed development to become a dead layer at the end of the seed maturation (Figure 1; Western 2001; Miart *et al.*2019). These two models illustrate the obvious diversity in the MSC cell wall dynamics during seed development as well as in the various mucilage organizations and extrusion modes. During the MSC development, the*A. thaliana* SM is trapped, with no apparent sub-layering, between the periclinal primary wall and a volcano-shaped polarized secondary wall called columella, whereas in flax, a complex multilayered SM is sequentially deposited in the MSCs during seed development (Figure 1). Beyond these two models, the MSC morphological diversity also occurs among the various species studied over the years (Phan & Burton 2018) as exemplified by the pioneer morphological survey of mature MSCs conducted for 200 Brassicaceae species covering 90 genera (Vaughan & Whitehouse 1971). Upon seeds imbibition, the hydrophilic nature of the polysaccharide mixture absorbs water, becoming a hydrogel swelling outwards. As a result, the SM volume and mass increase 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. The differences also occur in SM extrusion modes despite being less studied (Figure 1). The swelling increase pressure within the MSCs that allows the rupture of primary wall domains occurring either simultaneously in all Arabidopsis MSCs or sequentially in adjacent flax MSCs (Figure 1). This organized explosion is carefully prepared previously during MSC seed development by cell wall polysaccharides differential deposition and modifications. The polysaccharidic-proteinaceous cell wall domain 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 polysaccharidic composition and its internal organization are proposed to play a role in proper MSC opening (Miart *et al.* 2019). A convenient method to attest the presence of SM is pectin staining directly performed on hydrated seeds with ruthenium red or section staining with a generic and polychromatic stain such as toluidine blue (Western 2001). Further characterizations are obtained by immunolabeling with cell wall epitope antibodies directly performed on whole mount hydrated seeds or on sections (Ben-Tov *et al.* 2018), chemical analysis facilitated by the easy extraction of mucilage (Zhao, Qiao & Wu 2017; Poulain, Botran, North & Ralet 2019) or ultrastructural analysis by scanning electron microscopy and atomic force microscopy (Kreitschitz & Gorb 2018; Williams *et al.* 2020). In Arabidopsis, released SM is composed by an adherent layer bound to the seed and a non-adherent layer both enriched in poorly branches type I rhamnogalacturonan (RGI) pectin domains (Figure 1; Macquet *et al.* 2007; Poulain *et al.*2019) while in flax the SM is composed by four contrasted layers enriched in RGI, arabinoxylans and xyloglucans/cellulose, respectively (Figure 1; Kreitschitz & Gorb 2017; Miart *et al.* 2019)). This type of SM layering and variable composition also occurs for 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). SM microstructure also displayed inter-species diversity (Kreitschitz & Gorb 2018), implicating polysaccharide-polysaccharide specific interactions (Yu *et al.*2018) making SM an excellent model for cell wall dynamics understanding for about 20 years.

Interestingly, for species with seeds enclosed in a dry and non-dehiscent fruit called achene such as *Salvia* and*Artemisia* species, mucilage is extruded by the outermost fruit cell layer, namely the achene pericarp, and not by the seed integument. This parallel feature to myxospermy is called myxocarpy, both traits being regrouped under the term of myxodiaspory (Ryding 2001). Those are nice 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 *A. thaliana* and *L. usitatissimum* . Mucilage accumulates in the outer pericarp epidermal cells during 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, additional peculiarity may exist since 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, the traits associated with myxospermy (or more generally with myxodiaspory) are numerous, including MSC structure, SM polysaccharidic composition and structural organization, and mucilage extrusion mode. However, there is not a simple clear-cut distribution of the myxodiasporic/non myxodiasporic traits along the Angiosperms families and even within families (Vaughan & Whitehouse 1971). For this reason, in the following part we will shed light on molecular mechanisms underlying this morphological diversity through inter-species and intra-species comparative studies for SM evolution understanding.

EVOLUTION OF MOLECULAR actors UNDERLYING intra- and INTER-SPECIES SEED MUCILAGE NATURAL VARIABILITY

Twenty years of forward and reverse genetics together with more global approaches have allowed functional characterization of numerous genes involved in SM and MSC physiology in *A. thaliana.* The list currently contains between 82 (Phan & Burton 2018) and 58 genes (Sullivan *et al.* 2019) considering their indirect or direct involvement in SM, respectively. They constitute the continuously evolving MSC toolbox necessary for a proper SM production and release in the model plant (Francoz, Ranocha, Burlat & Dunand 2015; Voiniciuc, Yang, Schmidt, Günl & Usadel 2015). A majority of these genes are transcription factors including upstream master regulators that will be further discussed hereafter, and less characterized regulatory genes whose integration in the gene regulatory network is still puzzling (Golz*et al.* 2018). The other downstream genes of the toolbox are directly responsible of SM synthesis, assembly and secretion, or are involved in secondary cell wall synthesis and epidermal cell differentiation.

Understanding of SM evolution is easier while 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 or recombinant inbred lines from the cross of two varieties (Liu *et al.* 2016; Miart *et al.* 2019), or in*A. thaliana* natural ecotypes (Saez-Aguayo *et al.* 2014; Voiniciuc *et al.* 2016) shows gradient of SM abundance and release efficiency, reaching a complete loss of adherent mucilage extrusion for*A. thaliana* natural populations such as Sha (Macquet *et al.* 2007) or Rak-1 (Saez-Aguayo *et al.* 2014).Interestingly, in both cases the absence of adherent mucilage extrusion does not mean a lack of mucilage synthesis since Rak-1 releases even more non adherent mucilage than Col-0 (Saez-Aguayo *et al.* 2014). All natural mutants characterized for the myxospermy ability are related with polymorphisms concentrated on three downstream genes of the MSC tool box, namely *PMEI6* for Dja, *MUM2* for Sha, and*PRX36* and *MUM2* for Sk-1. These three genes encode enzymes necessary for proper SM extrusion and not SM synthesis or MSCs formation and PMEI6 and PRX36 functions are tightly related (Francoz *et al* . 2019). As non-myxospermic seeds have much better buyoancy efficiency and since Sha habitat is close to a river, seed dispersal by water run-off is one of the SM functions proposed to explain the loss of myxospermy (Macquet *et al.* 2007; 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).

More recent genome wide association studies (GWAS) conducted on *A. thaliana* (Fabrissin *et al.* 2019) and *L. usitatissimum*(Soto-Cerda *et al.* 2018) allowed identifying the statistically most relevant single nucleotide polymorphisms (SNPs) to explain the observed SM phenotype. In *L. usitatissimum,* all SNPs concerned orthologs of previously characterized *A. thaliana* MSC tool box genes that are either direct actors of SM synthesis or modification or regulatory genes that are not master regulators (except for LuTT8 having the lower E-value among the candidate genes) (Soto-Cerda *et al.*2018). In *A. thaliana* , through a very precise and molecular phenotyping, the GWAS revealed only 8 peaks significantly above the huge background of less implicated positions reminding that SM is an extremely polygenic trait (Fabrissin *et al.* 2019). Upon the 8 candidates genes two genes were identified and characterized, one already known to belong to MSC toolbox gene, and a newly implicated in SM (Fabrissin *et al.* 2019). These results highlight the fact that the MSC toolbox starts to be well characterized in *A. thaliana*and can be used to investigate whether functional orthologs are present in other species. It also suggests that the intra-species level, SM selective pressure does not act on the master regulators but rather on their downstream target genes of the toolbox. Reciprocally, the conservation of the top genes of the toolbox will allow to traceback the evolutionary origin of MSC toolbox genes across diverse families.

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 are actors of the seed carbon partitioning regulation (Golz *et al.* 2018; Li, Zhang, Chen, Ji & Yu 2018; Chen & Wang 2019). The combinations of specific MYB and bHLH proteins together with TTG1, a WD40 domain repeats (WDR) protein allows the regulation of each of these traits. It constitutes the MYB-bHLH-WD40 repeat (MBW) regulatory complexes highly conserved across Angiosperms (Zhang & Hülskamp 2019; Zhang, Chopra, Schrader & Hülskamp 2019). It is important to note that (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 (Zhang *et al.* 2019; Figure 2A). In *A. thaliana* , the *ttg1* mutant lacks root hair, trichome, has a reduced level of anthocyanins and proanthocyanins and does not accumulate mucilage in the MSCs (Western 2001). In *A. thaliana* , the phosphorylation of TTG1 by SHAGGY-like kinases 11/12 prevents its interaction with *TT2,* a MYB member of MBW complex, which decreases the transcription of the downstream regulator *GL2* (Li *et al.* 2018; Figure 2A). The consequence for the seeds is the promotion of lipid storage in the embryo at the expense of mucilage and flavonoid pigment synthesis in the seed coat (Li *et al.* 2018; Figure 2A). This regulation is probably responsible of the differential balance between seed lipid and pigment/mucilage contents in two natural *Medicago*species that correlate with *GL2* expression level (Song *et al.* 2017; Figure 2A). However, additional regulation mechanisms through interaction, competition, ubiquitination or epigenetics chromatin modifications may also occur (Xu, Dubos & Lepiniec 2015; Nguyen, Tran & Nguyen 2019). Within the three members of the WDR family existing in angiosperm species, TTG1 is the most recent one, appearing in the common ancestor of seed plants (Airoldi, Hearn, Brockington, Webb & Glover 2019) for the control of epidermal cells differentiation in essentially all organs of plants (Figure 2B). Interestingly, the serine 215 that can be phosphorylated by SK11/12 is conserved across seed plant TTG1 orthologs suggesting an ancestral function for this master regulator allowing switches in carbon flow between the seed coat and the embryo independently of the SM presence (Li *et al.* 2018).

It appears that some proteins belonging to the MBW complex were already present prior the emergence of the vascular plants (Doroshkov, Konstantinov, Afonnikov & Gunbin 2019), and that the control of flavonoid synthesis could be the most ancient trait controlled by this complex. This is coherent with the rescue of anthocyanin synthesis by*PAP1* and *PAP2* angiosperm MYB ortholog genes in the*A. thaliana* double mutant *pap1pap2* and the absence of restoration of trichome differentiation in the *A. thalianagl1* mutant by *GL1* MYB ortholog genes coming from the same species (Zhang & Hülskamp 2019). Since bHLH and MYB proteins have been subjected to numerous and recent duplication events (Doroshkov *et al.* 2019; Sullivan *et al.* 2019), the different combinations of bHLHs and MYBs within MBW complexes have probably been co-opted to control the emergence of other biological process often linked to epidermal cells such as SM (Figure 2B). Indeed, the *A. thaliana*MBW regulatory complex controlling SM and seed coat pigments involves at least 2 bHLHs (TT8-EGL3), 1 MYB (TT2) together with TTG1 (reviewed in (Golz *et al.* 2018). For SM proper establishment there is a need of at least 3 additional MYBs (MYB5-MYB23-MYB61) (Figure 2A). Functional conservation of TTG1 in 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 *MUM4*gene expression (Liu *et al.* 2017). Using a similar trans-complementation approach, the functional conservation of the two bHLHs EGL3 and TT8 has also been demonstrated in all tested angiosperm for all 5 traits (Zhang & Hülskamp 2019). *MYB5* and *TT2*ortholog genes in *M. truncatula* have a conserved function of seed coat pigment and mucilage positive regulation (Liu, Jun & Dixon 2014) suggesting that the pathway regulation in seed through MBW complex dedicated to SM and seed coat pigments is conserved among the Rosids clade (Figure 2B). *A. thaliana GL3* , another bHLH member of the MBW complex known to be implicated in anthocyanin, trichome and root hair, is unable to rescue SM in *gl3 /egl3 /tt8* triple mutant. However, trans-complementation of the same triple mutant with*A. alpina GL3* partially rescues SM (Zhang & Hülskamp 2019) indicating an intra- Brassicaceae divergency between the two proteins coherent with the contrasted morphology of MSCs compared to those of *A. thaliana* (Chopra *et al.* 2014). Similarly, in each Rosid family, few changes can be expected in the SM-related MBW complex and higher divergence in the downstream genes responsible of the observed morphological diversity of MSCs such as in Brassicaceae (Vaughan & Whitehouse 1971). As phylogeny of these multigenic family is difficult to solve and since their association in MBW complexes depend of non-binary competitive interaction (Zhang *et al.* 2019), more studies will be helpful to fully characterize the evolution of this protein complexes. *A. thaliana* NARS1, NARS2 and AP2 are also major regulators in *A. thaliana* controlling proper establishment of seed coat cells layers as well as embryogenesis (Kunieda *et al.* 2008). As they seem to act in parallel to TTG1 and GL2 and to control different downstream genes (Golz *et al.* 2018), investigation of their evolution in Angiosperms would be also very instructive.

Altogether, 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 and control of each traits in different seed zones such as SM in the MSCs.

New insights in ecological function of seed mucilage

Since SM is costly for mother plant metabolism, its presence implies that it displays major functions and that this trait is under a positive selection pressure in the myxospermic/myxodiasporic species. According to SM sticky and hydrophilic properties, the scientific community first investigated its influence in seed dispersal and germination (reviewed in Western 2012; Yang *et al.* 2012c) and more recently moved to the potential interactions between SM and the abiotic and biotic constraints.

4.1. Influence on seed dispersal and germination

A seed adaptation such as SM is expected to have an effect on seed dispersal and germination (Figure 3A). However, between closely relative species, these roles can be completely different making difficult to extend the concept to all myxodiasporic species. Counter-intuitively, SM can be a negative regulator of seed germination in *Leptocereus scopulophilus* (Barrios, Flores, González-Torres & Palmarola 2015) and also for the achene mucilage of *Artemisia monosperma* (Huang & Gutterman 1999a). In *Blepharis persica* , the SM 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 as supposed also improve germination (Figure 3A-1). The pioneer most cited SM 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 that presents a defect in SM extrusion, germination is also delayed suggesting a positive effect of mucilage on germination efficiency rather than on germination rate (Arsovski *et al.* 2009). However, no such phenotypes were obtained in the *mum2* or *myb61* mutants (Saez-Aguayo *et al.* 2014). Thus, the role of SM in *A. thaliana* germination deserves to be deeply explored. For *S. hispanica* , the intact myxocarpic achenes germinates clearly better than the achenes without mucilage (Geneve *et al.* 2017). Interestingly, SM-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),* SM presence increased water uptake but SM removal led to contrasted germination effects (Bhatt, Santo & Gallacher 2016). Therefore, mucilage function in germination seems to be related to water uptake and/or seed permeability to water and possibly to gazes.

Another interesting approach is the study of contrasted SM adaptation occurring within the same species on two seed morphotypes (myxospermic and non-myxospermic seeds). Those species use these dimorphic seeds to improve 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 higher dormancy does not have SM (Lu, Tan, Baskin & Baskin 2010; Toorop *et al.* 2012; Arshad *et al.* 2019) suggesting that myxospermic seeds should germinate without delay. The co-occurrence of SM 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 reaching an optimal place for hydric conditions (Figure 3A-2). Interestingly, *Lunaria annua* which possesses non-myxospermic but flattened and winged seeds shows a surprising use of mucilage by secreting it from the fruit to keep the seeds sticked, waiting for windy conditions to improve long distance dispersal of seed-carrying dry fruits by the wind (Leins, Fligge & Erbar 2018).

4.2. Influence facing abiotic constraints

As SMs constitute highly hydrophilic gels, it is tempting to propose that SM may provide water for the embryo. For *A. thaliana* , SM takes a large amount of water from the environment but sequesters it due to ionic linkages with galacturonic acid residues (Figure 3B-3; Saez-Aguayo *et al.* 2014). Indeed, mutants that have a lack of mucilage, imbibed their seed faster than wild type or mutants with un-released mucilage (Saez-Aguayo *et al.* 2014). However, SM allows fast seed sinking in water as compared to non myxospermic 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 SM can change according to its hydration level influencing at least its dispersal properties (Kreitschitz, Kovalev & Gorb 2015, 2016). In *A. arabicum* , the SM thick fibers emerging upon imbibition are able to conserve their structures and size upon dehydration (Lenser *et al.* 2016) allowing a trade-off of dispersal efficiency for the dry and the imbibed seeds, with wind, water run-off, and buoyancy dissemination ways, respectively (Arshad *et al.* 2019). A more complete understanding of the complex roles of SM in water management would necessitate investigating the SM role in more complex/natural situation 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 3B-4). SM of *Artemisia sphaerocephala* enhances seedling emergence in its sandy environment and reduces plant mortality (Yang, Baskin, Baskin, Liu & Huang 2012a). By adding SM extracted 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* SM addition which links soil particles to increase aggregate stability for at least 30 days in diverse kinds of soil (Figure 3B-4; Di Marsico *et al.*2018). So, SM could improve the soil rheological local environment in agreement with the non-disseminating lifestyle of species such as*A. thaliana* excreting non-adherent mucilage.

4.3. Biotic interactions

Similarly to root border cell mucilage (Knee *et al.* 2001), non-adherent SM constitutes an impressive 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 SM on biotic constraints (Figure 3C). Involvement of SM on the influence of 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 in SM in comparison to *V. dahlia*considerably reducing the plant disease symptoms (Meschke & Schrempf 2010). This “selective media effect” (Figure 3C-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 microorganisms, providing CO_2 and soluble sugars to promote seedling establishment (Yang, Baskin, Baskin, Zhang & Huang 2012b). This promotion 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 seedling from drought and pathogens (Hu *et al.* 2019b). Glomeromycota is one of the groups of fungal species positively impacted by achene mucilage. However, these fungi responsible of arbuscular mycorrhizal symbiosis do not significantly interact with mucilage at the seedling stage, 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 myxospermic species *Raphanus sativus* or in the *A. thaliana gl2* mutant deprived of SM suggesting that protein secretion and SM are independent (Raviv *et al.* 2017).

Nematodes are in close interaction with plants and can have major pathogenic impact on plant development. *A. thaliana* SM contributes to attract root-knot nematodes (Figure 3C-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, this nematode could be a predator of an even more dangerous organism for the plant. *Capsella bursa-pastoris,* a closely relative species of *A. thaliana,* has myxospermic seeds 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 SM increases plant development from germination to young seedling establishment, especially under low nutrient level (Roberts *et al.* 2018). Thus, SM involvement in biotic interactions starts to be uncovered promising new astonishing functions that could impact the plant development in an unexpected manner than the previously characterized functions

CONCLUSIONS AND PERSPECTIVES

Myxodiaspory is a very diverse trait among Angiosperms that shows great variability both at the intra-species and inter-species levels. This astonishing morphological diversity makes it difficult to trace back the evolutionary origin of SM based on this sole trait. The available deeply characterized *A. thaliana* MSC toolbox genes now allows comparison of these molecular actors within a species or between species starting to shed light on this mysterious evolutionary story. The intense selection pressure that undergoes mucilage establishment is applied mainly on downstream genes of the MSC toolbox contrary to the SM-related MBW master regulatory complex that appears to be majoritarily conserved across Angiosperms. From this regulatory complex, mucilage may have evolved several times independently as a highly diverse trait allowing a wide range of ecological functions for the seed of each species facing contrasted environments. The biotic constraints are the least studied point in this field and probably the most promising track to uncover new SM integrated functions related to particular environments.

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FIGURE LEGENDS:

Figure 1: Arabidopsis and flax as model species for seed mucilage secretory cell (MSC) illustrating the diversity of development, intracellular polysaccharidic mucilage organization, and extrusion mechanisms upon water imbibition. 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 at similar scale, the released mucilage layers are not drawn to scale. Note the numerous differences between both models: Major features in *A. thaliana* : presence of a volcano-shaped columella, simultaneous rupture of primary cell wall domain in each MSC cells and 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 2: Evolution of MYB-bHLH-WDR (MBW) complexes regulating spatiotemporal carbon partitioning in *A. thaliana* seeds. (A) The conserved ancestral master regulator TTG1 together with a bHLH and MYB modularity enables to regulate the spatiotemporal specificity of various seed traits including SM production and release (Golz *et al.*2018; Li *et al.* 2018; Chen & Wang 2019). **(B)** Simplified phylogenetic tree of land plants giving an overview of the sequential evolution of the *A. thaliana* MSC toolbox from upstream MBW complex members until downstream directs actors of SM morphology so far characterized in *A. thaliana* (Liu *et al.* 2014, 2017; Li*et al.* 2018; Airoldi *et al.* 2019; Zhang & Hülskamp 2019; Zhang *et al.* 2019).

Figure 3: Global overview of seed mucilage (SM) major functions facing environmental constraints. (A) SM 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)** SM 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)** SM 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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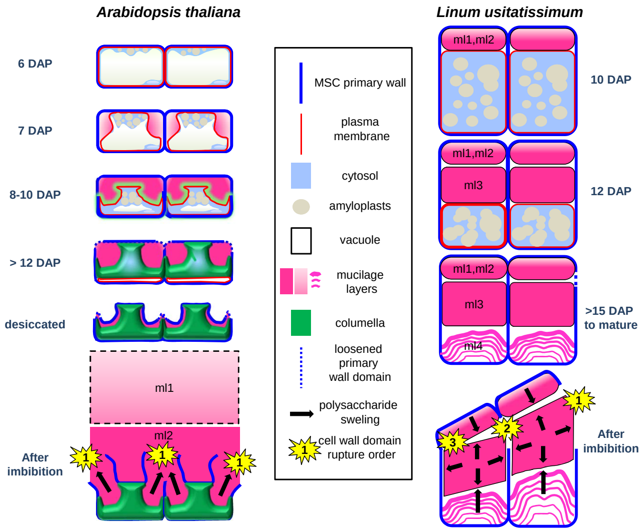

Figure 1: Arabidopsis and flax as model species for seed mucilage secretory cell (MSC) illustrating the diversity of development, intracellular polysaccharidic mucilage organization, and extrusion mechanisms upon water imbibition.

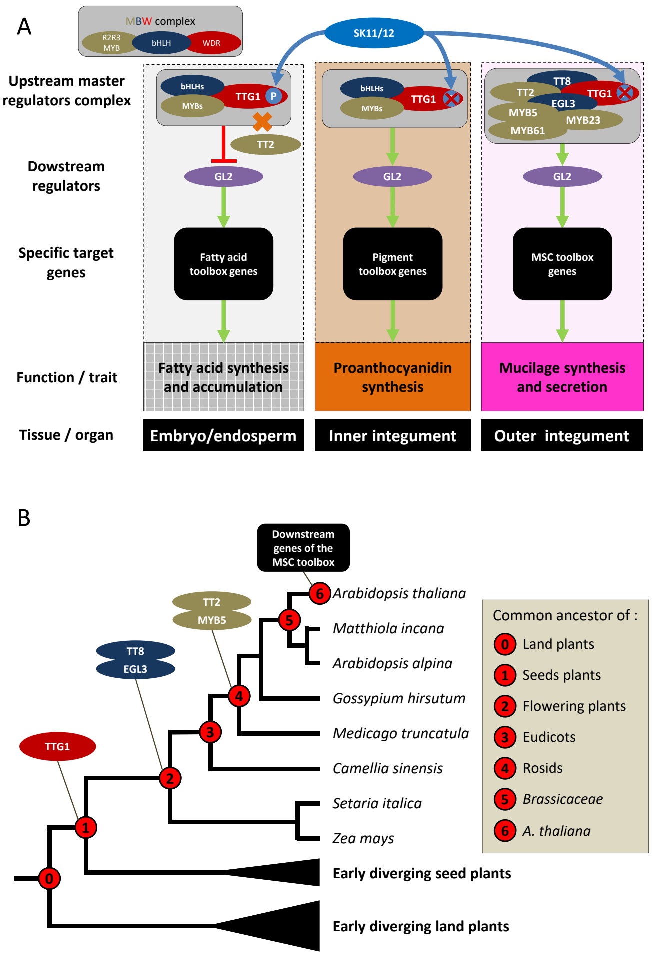

Figure 2: Evolution of MYB-bHLH-WDR (MBW) complexes regulating spatiotemporal carbon partitioning in *A. thaliana* seeds

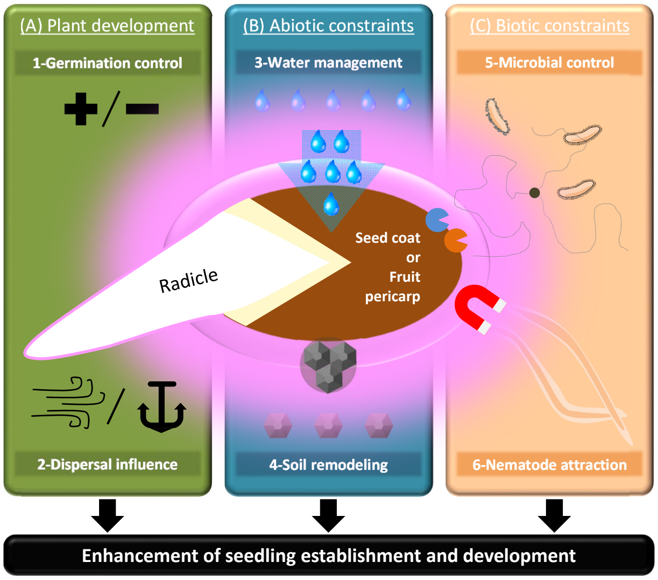

Figure 3: Global overview of seed mucilage major functions facing environmental constraints