Seed Science Research (2012) **22**, 1–25
doi:10.1017/S0960258511000249

REVIEW ARTICLE

The sticky tale of seed coat mucilages: production, genetics, and role in seed germination and dispersal

Tamara L. Western*

Biology Department and Developmental Biology Research Initiative, McGill University, 1205 avenue Docteur Penfield, Montreal, QC H3A 1B1, Canada

(Received 8 August 2011; accepted after revision 16 August 2011; first published online 26 September 2011)

Abstract

The production of hydrophilic mucilages by the seed coat or pericarp, which are released upon seed hydration, is a commonly found adaptation in angiosperms, known as myxodiaspory. These are composed primarily of pectins and hemicelluloses that undergo substantive swelling upon hydration. Synthesized in the Golgi apparatus and secreted to an apoplastic space via secretory vesicles, mucilages can also contain cellulose microfibrils or cellulosic fibres that are synthesized at the plasma membrane in association with microtubules. Investigation of mucilage production in, and differentiation of, the mucilage secretory cells of the genetic model plant *Arabidopsis thaliana* has identified a number of regulatory genes and enzymes involved in pectin synthesis and secretion, *in muro* pectin modification and secondary cell wall synthesis. Studies of the role of mucilages in both a number of species and in Arabidopsis mutants affected in its production suggest that they have multiple ecological roles. These include facilitation of seed hydration, mediation of germination under waterlogged conditions, prevention of seed dispersal or predation by adherence to soil, and promotion of seed dispersal by attachment to animals. The precise role of mucilages appears to be dependent on species and their environmental context.

Keywords: Arabidopsis, germination, mucilage, pectin, seed coat, seed dispersal, testa

*Correspondence
Fax: 1-514-398-5069
Email: tamara.western@mcgill.ca

Introduction

Double fertilization in angiosperms leads not only to the commencement of embryogenesis and development of the nutritive triploid endosperm, but also the differentiation of the surrounding ovule integuments to become the mature seed coat. Changes to the coat include specializations to enable protection of the embryo as well as to facilitate dispersal and eventual germination. These can include mechanical reinforcement through the synthesis of secondary cell walls that may be impregnated with impermeable polymers such as lignin or suberin, or synthesis of secondary metabolites often visible as pigments (e.g. polyphenolics) that may aid in protection from pathogens. Another common specialization is the deposition of a hydrophilic, pectinaceous mucilage in the seed coat epidermis or surrounding pericarp developed from ovary tissue (i.e. fruit) (Grubert, 1974; Fahn, 1982; Boesewinkel and Bouman, 1984). The production of seed mucilage, known as myxospermy, occurs in a broad range of plants, from the Acanthaceae to the Brassicaceae to the Linaceae to the Plantaginaceae, while myxocarpy (fruit mucilage) occurs in families such as the Asteraceae, Lamiaceae and Poaceae (Grubert, 1974; Fahn, 1979; Grubert, 1981; Ryding, 2001; Kreitschitz, 2009). Mucilage is deposited into the apoplast of epidermal cells during differentiation of the seed coat or pericarp, and is released in response to seed hydration to form a water-containing, gel-like capsule surrounding the seed. Myxodiaspory (mucilage production by the seed dispersal unit, i.e. seed coat or pericarp) has been proposed to play a number of roles, including facilitation of seed hydration, regulation of germination by affecting oxygen entry into the seed, and mediation of seed dispersal through adhesion to soil or animal vectors (Grubert, 1974; Fahn, 1982; Ryding, 2001; Kreitschitz, 2009).

In this review, I will focus on the structure and synthesis of seed and pericarp mucilages, and their roles in seed hydration, germination and dispersal. Further, I will attempt to integrate the recent detailed work on seed coat mucilage of the genetic model plant, *Arabidopsis thaliana* (Arabidopsis; Brassicaceae) with literature on other species. For simplicity, throughout this review, I will refer generally to both seed epidermis-derived and pericarp exocarp-derived mucilage as seed coat mucilage; species that are myxocarpous are noted in Table 1 and will be mentioned in the text as necessary.

Occurrence and structure of seed mucilage

Mucilage, often referred to as slime, is a 'catch-all' term for polysaccharides (mainly pectins or hemicelluloses) or proteoglycans (e.g. heavily glycosylated arabinogalactan proteins) secreted by the plant during development. This is to contrast with gums, which are similar exudates, but are released after wounding and pathogenic cell wall degradation (Frey-Wyssling, 1976). In addition to being present in seed coats, mucilages are also secreted from the root cap, where they are thought to lubricate root extension through the soil or to promote interactions with ions and/or microbes in the soil, and in the transmitting tract, where they provide a medium for pollen tube growth (Esau, 1977; Fahn, 1982). While seed coat, root and transmitting-tract exudates are all considered to be mucilages, they have different compositions, as demonstrated for Arabidopsis seed coat versus root mucilage (Willats *et al.*, 2001). Seed coat mucilages are found in a broad range of angiosperms, including the Acanthaceae, Amaranthaceae, Asteraceae, Bombacaceae, Boraginaceae, Brassicaceae, Cistaceae, Cucurbitaceae, Dilleniaceae, Euphorbiaceae, Fabaceae, Goodeniaceae, Hydrocaritaceae, Lamiaceae, Linaceae, Lythraceae, Malvaceae, Nyctaginaceae, Onograceae, Orchidaceae, Plantaginaceae, Poaceae, Polemoniaceae, Rosaceae, Rutaceae, Sapindaceae, Solanaceae, Urticaceae, Voilaceae and Zygophyllaceae (see Fig. 1 for examples and Table 1 for a complete list) (Kraemer, 1898; Mühlethaler, 1950; Harper and Benton, 1966; Edwards, 1968; Witztum *et al.*, 1969; Young *et al.*, 1970; Swarbrick, 1971; Vaughan and Whitehouse, 1971; Young and Evans, 1973; Grubert, 1974; Bouman, 1975; Bushway *et al.*, 1981; Buth and Ara, 1981; Fahn, 1982; Schnepf and Deichgräber, 1983a, b; Gowda, 1984; Garwood, 1985; Panigrahi, 1986; Sharma and Koul, 1986; Eskin, 1992; Oomah *et al.*, 1995; Ibrahim *et al.*, 1997; Duletiae-Lauševiae and Marin, 1998; Huang and Gutterman, 1999; Ryding, 2001; Lobova *et al.*, 2003; Zeng *et al.*, 2004; Diederichsen *et al.*, 2006; Müller *et al.*, 2006; Fitch *et al.*, 2007; Hamilton *et al.*, 2007; Iglesias-Fernández *et al.*, 2007; Kreitschitz and Vàlles, 2007; Huang *et al.*, 2008; Jordaan, 2008; Thapliyal *et al.*, 2008; Kreitschitz, 2009; Kreitschitz *et al.*, 2009; Inceer, 2011). For a detailed listing of species in 100 families with seed coat mucilage as well as references up to 1981, see Grubert (1974, 1981).

The polysaccharide and acidic qualities of mucilages make them extremely hydrophilic, such that they hydrate rapidly in the presence of water, forming hydrogels or possibly even superabsorbent polymers (Frey-Wyssling, 1976; Fahn, 1982; Zwieniecki *et al.*, 2001; Zohuriaan-Mehr and Kabiri, 2008). This enables seed coat mucilage not only to break through the containing primary cell walls of epidermal cells to surround the seed, but also to hold water around the seed. Seed coat mucilages are often characterized by the presence of the pectin rhamnogalacturonan I (RG I), which has an alternating backbone of 1 → 2-linked rhamnose and 1 → 4-linked galacturonic acid, with varying degrees of 1 → 5-linked arabinose, 1 → 3-linked galactose and/or arabinogalactan side-chains linked to the rhamnose residues (Caffall and Mohnen, 2009). Hemicelluloses such as arabinoxylan, a 1 → 4-linked xylan backbone with arabinose substitutions (Lerouxel *et al.*, 2006), are also prevalent. Some of the best characterized seed coat mucilages are those of Arabidopsis (see below), yellow mustard *Sinapis alba* (*aka Brassica hirta* (Brassicaceae)), basils (*Ocimum* spp.; Lamiaceae), linseed flax (*Linum usitatissimum*; Linaceae) and plantain/psyllium (*Plantago* spp.; Plantaginaceae) (Table 1). While Arabidopsis mucilage is primarily composed of unbranched RG I (Goto, 1985; Western *et al.*, 2000, 2004; Penfield *et al.*, 2001), flax mucilage has branched RG I and the hemicellulose arabinoxylan (Anderson and Lowe, 1947; Muralikrishna *et al.*, 1987; Cui *et al.*, 1994b; Fedeniuk and Biliaderis, 1994; Warrand *et al.*, 2003; Naran *et al.*, 2008). *S. alba* seed coat mucilage, like that of Arabidopsis and other characterized *Brassica* species, is pectinaceous and contains both RG I and homogalacturonan (HG) (Hirst *et al.*, 1965; Vose, 1974; Woods and Downey, 1980; Siddiqui *et al.*, 1986; Van Caeseele *et al.*, 1987; Cui *et al.*, 1993, 1994a; Wu *et al.*, 2009). The basil species' seed mucilage can resemble Arabidopsis mucilage, but with arabinogalactan or galactan side-chains (*Becium filamentosum* and *Ocimum adscendens*, respectively) (Anjaneyalu *et al.*, 1984; Khan *et al.*, 1987), or contain arabinoxylan, like flax seed mucilage (*Ocimum gratissimum* and *Ocimum basilicum*) (Anjaneyalu *et al.*, 1983; Khan *et al.*, 1987; Razavi *et al.*, 2009). *O. basilicum* seed mucilage has also been suggested to contain a glucomannan (Razavi *et al.*, 2009). *Plantago* spp. seed mucilages are less well-defined in terms of specific polysaccharides, but appear to contain both arabinoxylan and pectin (Anderson and Fireman, 1935; Tomoda *et al.*, 1981; Sharma and Koul, 1986; Guo *et al.*, 2009).

In addition to their pectin and hemicellulose compositions, seed mucilages have been classified by

https://doi.org/10.1017/S0960258511000249 Published online by Cambridge University Press

Table 1. Partial survey of myxodiaspory in angiosperms, including role in germination and dispersal

Family	Species	Mucilage structure[a]	Mucilage composition	Demonstrated role in germination or dispersal
Acanthaceae	*Blepharis persica* (1,2), *Ruellia* spp. (3), *Ruellia strepens, Hygrophila spinosa, Barleria macrostegia,* other spp. (4)	Seed coat of long mucilaginous hairs (*B.p.*, *R.s.*, *H.s.*, *B.m.*) (1–4); spiral hairs embedded in cellulosic mucilage (3); mucilage cells or hairs on part or whole seed (4)	Unesterified galacturonic acid (1) (suggested by effect of Ca^{2+} on mucilage swelling/germination)	Swollen mucilage acts as oxygen barrier and inhibits germination in excess water, e.g. flooding (*B.p.*) (1)
Aceraceae	*Acer* spp. (4)	n.a.[c]	n.a.	n.a.
Adoxaceae – P[b]	*Adoxa moschatellina* (46)	n.a.	n.a.	n.a.
Araceae	Several spp. (4)	n.a.	n.a.	n.a.
Amaranthaceae – P	*Spinacia oleracea* (5)	n.a.	n.a.	Swollen mucilage acts as oxygen barrier and inhibits germination in excess water, e.g. waterlogged soils (5)
Asteraceae – P	*Artemisia sphaerocephala* (6,7), *A. ordosica* (6), *A. monosperma, Artemisia* spp., *Neopallasia pectinata* (8), *Helipterum craspedioides, Helichrysum cassinianum* (9), *Matricaria* spp. (10), *Chrysanthemum nivellei, Matricaria lamellata*, many spp. (4)	Cellulosic mucilage (8,10) (staining); mucilage cells or hairs (4,9); mucilage cells or hairs on part or whole seed (*C.n.*) (4); some have spiral cellulosic hairs embedded in mucilage (*M.l.*) (4)	Galactomannan, arabinoxylan (*A.s.*) (7)	Seed hydration and germination in stressful environments (*A.s.*, *H.c.*) (7,9); aids seed priming through embryo repair (*A.s.*, *A.o.*) (6)
Bombacaceae	*Cavanillesia platanifolia* (11)	n.a.	n.a.	Seedling development and prevention of wilting (11)
Boraginaceae (– P)	*Cordia* spp. (4), members of Subfamily Hydrophyllaceae (4)	*Hydrophyllum* spp. have spiral cellulosic hairs embedded in mucilage (4)	n.a.	n.a.
Brassicaceae	*Arabidopsis thaliana* (12–21), *Brassica campestris* (26–29), *B. juncea* (29), *B. napus* (26,29), *B. hirta/Sinapis alba* (30–38), *Brassica* spp. (39–41), *Sisymbrium altissimum* (42), *S. officinale* (43), *Descurainia pinnata, Lepidium perfoliatum* (42), *L. sativum* (34,44,45), *Capsella bursa-pastoris* (32), *Carrichtera annua, Anastatica heirochuntica* (46), *Diptychocarpus strictus* (47), *Camelina sativa* (48), *Sinapis arvensis* (49), *Lesquerella perforata, L. stonensis* (50), *Iberis pectinata, Conringia orientalis, Aethionema arabicum*, many spp. (4,51,52)	Cellulosic mucilage divided into outer diffuse layer and inner adherent layer, cellulosic/radial cell wall rays extending into hydrated mucilage (*A.t.*, *C.s.*) (4,12,17)[d]; cellulosic mucilage (*B.h./S.a.*, *L.s.*) (32,44)[d]; swollen intact cells form conical masses (*C.o.*, *A.a.*) (4)[d]; spirals of cellulosic material (4)	Outer layer primarily branched rhamnogalacturonan I, inner layer also contains cellulose, arabinans, galactans, homogalacturonan with varying degrees of methylesterification (*A.t.*) (12,14, 16,17–19,21); homogalacturonic acid and arabinose main components with rhamnose also present (*B.c.*, *B.j.*, *B.n.*) (26,28); rhamnogalacturonan I, homogalacturonan, arabinans, cellulose (*B.h./S.a.*) (26,31,35–38)	Seed hydration and germination (*A.t.*) (16,22–25); aid germination through water rention and soil particle contact (*C.a.*, *A.h.*, *D.s.*, *L.s.*, *C.s.*, *S.a.*) (46–48); inhibit germination (*L.p.*, *L.s.*) (50)
Caprifoliaceae	*Symphoricarpus rivularis* (51)	n.a.	n.a.	n.a.
Caricaceae	*Carica* spp. (4)	n.a.	n.a.	n.a.

Table 1. *Continued*

Family	Species	Mucilage structure[a]	Mucilage composition	Demonstrated role in germination or dispersal
Chenopodiaceae	*Chenopodium* spp. (4)	n.a.	n.a.	n.a.
Cistaceae	*Helianthemum apeninum, Helianthemum* spp. (4,51), other spp. (4)	Spiral cellulosic hairs embedded in mucilage (4); epidermal and subepidermal mucilage cells (*H.a.*) (4)	n.a.	n.a.
Connaraceae	*Cnestis, Rourea* and *Roueopsis* spp. (4)	n.a.	n.a.	n.a.
Convolvulaceae	*Cuscuta* spp. (4)	n.a.	n.a.	n.a.
Cucurbitaceae	*Ecballium elaterium*, many spp. (4)	Some species have mucilaginous hairs (4); mucilage cells contain threads of cellulose (*E.e.*) (4)	n.a.	n.a.
Dilleniaceae	*Dillenia indica* (53)	n.a.	n.a.	Does not promote or inhibit germination (53)
Ericaceae	*Vaccinium* spp. (4)		n.a.	n.a.
Euphorbiaceae	*Euphorbia falcata*, *Euphorbia* spp. (4,51,52), other spp. (4)	Spiral cellulosic hairs embedded in mucilage (*E.f.*) (4)	n.a.	n.a.
Fabaceae	*Colophospermum mopane* (54), many spp. (4)	Can have subcuticular mucilage stratum (4), cellulosic mucilage (*C.m.*) (54)	n.a.	n.a.
Gesneriaceae	*Codonanthe gracilis, Columnea picta* (4)	n.a.	n.a.	n.a.
Goodeniaceae	*Selliera radicans* (4)	Mucilage cells on seed margins (4)	n.a.	n.a.
Hydrocaritaceae	*Hydrocharis morsus-ranae*, several spp. (4)	Some species have mucilaginous hairs or cellulosic spirals (*H.m.*) (4)	n.a.	n.a.
Hydrostachyaceae	*Hydrostachys* spp. (4)	n.a.	n.a.	n.a.
Irvingiaceae	*Klaindoxa* spp. (4)	n.a.	n.a.	n.a.
Juncaceae	*Juncus* spp., *Luzula* spp. (4,51)	n.a.	n.a.	n.a.
Lamiaceae – P[e]	*Becium filamentosum* (55), *Ocimum adscendens* (56), *O. gratissimum* (55,57), *O. basilicum* (55,58), *Hyptis suaveolens* (59), *Salvia polystachya* (60), *S. columbariae* (61), *S. horminum* (4), *S. hispanica*, *Salvia* spp. (44,51,52), *Thymus* spp., *Prunella* spp. (51,52), Subfamily Nepetoideae (62,63), many spp. (4)	Cellulosic mucilage (*O.b.*) (44)[d]; mucilage can have embedded straight or spiral thickenings (*S.h.*) (4,62,63)[d]; mucilage cells on part of or whole seed (4)	Rhamnogalacturonan I with arabinogalactan side-chains (*B.f.*) (55); rhamnogalacturonan with galactose (*O.a.*) (56); xylan backbone with arabinose, galacturonan, rhamnose and galactose (*O.g.*, *O.b.*) (55,57,58), plus glucomannan (*O.b.*) (58); acidic methylglucuronoxylan with fucose and neutral polysaccharide including glucose, galactose and mannose (*H.s.*) (59); major components are xylose, arabinose, rhamnose (60)	Mucilage attaches sand coat so not eaten (*S.c.*) (61)
Lentibulariaceae	*Ultricularia* spp. (4)	n.a.	n.a.	n.a.

Table 1. *Continued*

Family	Species	Mucilage structure[a]	Mucilage composition	Demonstrated role in germination or dispersal
Linaceae	*Linum usitatissimum* (4,30,34,44,64–72), *Linum* spp. (4,51), *Hesperolinon* spp., *Radiola* spp. (4)	'True' slime (44); mucilage divided into large outer diffuse layer and thin inner adherent layer (*L.u.*) (72)[d]	Arabinoxylan and rhamnogalacturonan I (66–68,70,72)	Aid germination through water rention and soil particle contact (*L.u.*) (48)
Lythraceae	*Rotala* spp., *Ammannia* spp., *Nesaea* spp., *Hionanthera* spp. (73), many spp. (4)	Mucilage hairs invaginations within epidermal cells that push out when wet (4,73)	n.a.	n.a.
Malvaceae	*Glossostemon bruguieri* (74), *Firmiana affinis*, *Scaphium* spp., *Sterculia* spp. (4)	n.a.	Primarily rhamnose and galacturonic acid, plus glucuronic acid, xylose, mannose, arabinose (74)	n.a.
Martyniaceae	*Craniolaria integrifolia* (4)	n.a.	n.a.	n.a.
Moraceae – P	*Ficus awkeotsang* (4)	n.a.	n.a.	n.a.
Nyctaginaceae[f]	*Oxybaphus nyctagineus*, many spp. (4)	Subepidermal cells on part of anthocarp (*O.n.*) (4)	n.a.	n.a.
Onagraceae	*Oenothera* spp. (4,52)	n.a.	n.a.	n.a.
Orchidaceae	*Renanthera* spp. (4)	Spiral cellulosic hairs embedded in mucilage (4)		
Oxalidaceae	*Oxalis* spp. (4)	n.a.	n.a.	n.a.
Pedaliaceae	*Sesamum* spp. (4)	n.a.	n.a.	n.a.
Plantaginaceae	*Plantago ovata* (75,76), *P. psyllium* (4,48,77), *P. major* (48), *Plantago* spp. (4,34,51,52,76), *Veronica* spp. (51,52)	'True' slime (34,44); radial elongation of mucilage cells (*P.p.*) (4)	Xylose, arabinose, aldobiuronic acid; arabinoxylan (*P.o.*) (76,77); arabinoxylans, presence of galacturonic acid and major effect of Ca^{2+} on gelling suggests presence of pectins (*P.p.*) (78,79)	Aid germination through water rention and soil particle contact (*P.m.*) (48)
Poaceae – P	*Eragrostis pilosa* (80), *Sporobolus cryptandrus*, many spp. (4)	'True' slime (72); some have subepidermal mucilage layers (*S.c.*) (4)	n.a.	n.a.
Podostemaceae	Many spp. (4)	n.a.	n.a.	n.a.
Polemoniaceae	*Collomia* spp. (81), *C. grandiflora* (4,82), *Phlox drummondi*, *Gilia* spp. (51), many spp. (4)	Spiral hairs embedded in cellulosic mucilage (4,74)	n.a.	n.a.
Rosaceae	*Cydonia oblonga* (30,44,83), Subfamily Maloideae (4)	Cellulosic mucilage (42,75)	n.a.	n.a.
Rutaceae	*Citrus garrawayi* (84), many spp. (4)	Epidermal fibres covered with mucilage (84)	n.a.	n.a.
Sapindaceae	*Magonia glabrata* (4)	Subepidermal cells (4)	n.a.	n.a.
Saxifragaceae	*Ribes* spp. (4)	n.a.	n.a.	n.a.
Scrophulariaceae	Many spp. (4)	n.a.	n.a.	n.a.
Solanaceae	*Nicandra physalodes*, *Salpichroa origanifolia* (51), *Solanum viarum* (85), many spp. (4)	n.a.	n.a.	n.a.

Table 1. *Continued*

Family	Species	Mucilage structure[a]	Mucilage composition	Demonstrated role in germination or dispersal
Urticaceae – P	*Cecropia* spp. (86), many spp. (4)	n.a.	n.a.	Mucilage influences dispersal by bats (86)
Voilaceae	*Viola* spp. (4,51)	n.a.	n.a.	n.a.
Zygophyllaceae	Many spp. (4)	*Zygophyllum* spp. have spiral hairs embedded in mucilage (4)	n.a.	n.a.

[a]Notable characteristics if available; [b]P = mucilaginous pericarp; [c]n.a. = not available; [d]See Fig. 1; [e]Pericarp of nutlets; [f]Anthocarps; [g]See Grubert (1981) for comprehensive list of species with mucilage.

References: (1) Witztum *et al.* (1969); (2) Gutterman *et al.* (1973); (3) Schnepf and Deichgräber (1983b); (4) Grubert (1974); (5) Heydecker and Orphanos (1968); (6) Huang *et al.* (2008); (7) Yang *et al.* (2010); (8) Kreitschitz and Vàlles (2007); (9) Mott (1974); (10) Inceer (2011); (11) Garwood (1985); (12) Goto (1985); (13) Beeckman *et al.* (2000); (14) Western *et al.* (2000); (15) Windsor *et al.* (2000); (16) Penfield *et al.* (2001); (17) Willats *et al.* (2001); (18) Western *et al.* (2004); (19) Macquet *et al.* (2007a); (20) McFarlane *et al.* (2008); (21) Young *et al.* (2008); (22) Rautengarten *et al.* (2008); (23) Arsovski *et al.* (2009a); (24) Arsovski *et al.* (2009b); (25) Panikashvili *et al.* (2009); (26) Vose (1974); (27) Van Caeseele *et al.* (1981); (28) Van Caeseele *et al.* (1987); (29) Eskin (1992); (30) Kraemer (1898); (31) Hirst *et al.* (1965); (32) Bouman (1975); (33) Woods and Downey (1980); (34) Fahn (1982); (35) Siddiqui *et al.* (1986); (36) Cui *et al.* (1993); (37) Cui *et al.* (1994a); (38) Wu *et al.* (2009); (39) Vaughan and Whitehouse (1971); (40) Buth and Ara (1981); (41) Zeng *et al.* (2004); (42) Young *et al.* (1970); (43) Iglesias-Fernández *et al.* (2007); (44) Mühlethaler (1950); (45) Müller *et al.* (2006); (46) Gutterman and Shem-Tov (1997); (47) Lu *et al.* (2010); (48) Harper and Benton (1966); (49) Edwards (1968); (50) Fitch *et al.* (2007); (51) Swarbrick (1971); (52) Young and Evans (1973); (53) Thapliyal *et al.* (2008); (54) Jordaan (2008); (55) Khan *et al.* (1987); (56) Anjaneyalu *et al.* (1984); (57) Anjaneyalu *et al.* (1983); (58) Razavi *et al.* (2009); (59) Gowda (1984); (60) Bushway *et al.* (1981); (61) Fuller and Hay (1983); (62) Duletiae-Lauševiae and Marin (1998); (63) Ryding (2001); (64) Anderson and Lowe (1947); (65) Boesewinkel (1980); (66) Muralikrishna *et al.* (1987); (67) Fedeniuk and Biliaderis (1994); (68) Cui *et al.* (1994b); (69) Oomah *et al.* (1995); (70) Warrand *et al.* (2003); (71) Diederichsen *et al.* (2006); (72) Naran *et al.* (2008); (73) Panigrahi (1986); (74) Ibrahim *et al.* (1997); (75) Hyde (1970); (76) Sharma and Koul (1986); (77) Tomoda *et al.* (1981); (78) Anderson and Fireman (1935); (79) Guo *et al.* (2009); (80) Kreitschitz *et al.* (2009); (81) Hsiao and Chuang (1981); (82) Schnepf and Deichgräber (1983a); (83) Abeysekera and Willison (1988); (84) Hamilton *et al.* (2007); (85) Srinivas *et al.* (1998); (86) Lobova *et al.* (2003).

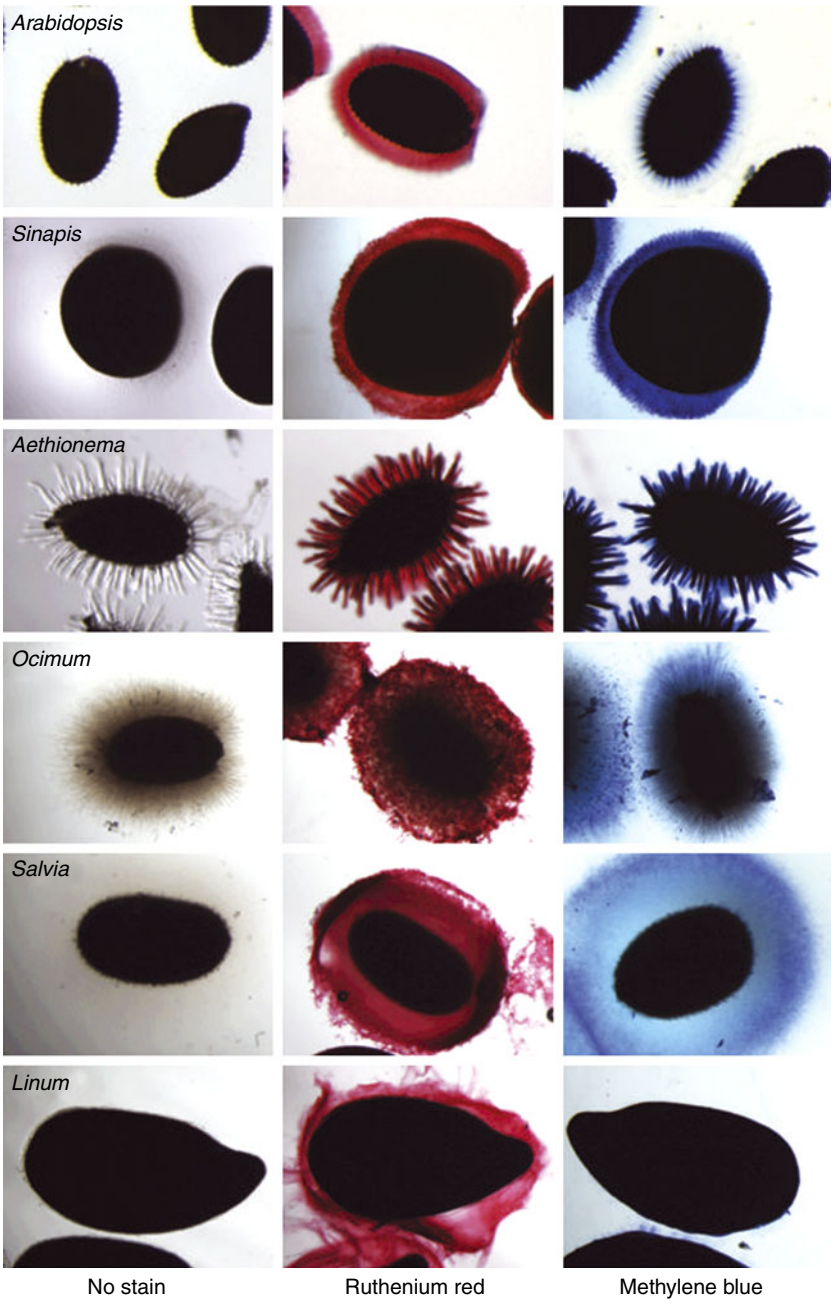

Figure 1. Mucilage staining for pectin and cellulose in various species (for a colour version of this figure see online at http://journals.cambridge.org). Hydrated seeds were photographed without staining, incubated with 0.1% w/v ruthenium red to stain pectin (Western *et al.*, 2000) or with 0.1% w/v methylene blue to stain glycans (primarily cellulose) (Kreitschitz and Vàlles, 2007). Species are *Arabidopsis thaliana, Sinapis alba, Aethionema arabicum* (Brassicaceae), *Ocimum basilicum, Salvia hispanica* (Lamiaceae) and *Linum usitatissimum* (Linaceae). The same magnification was used within each species. Arabidopsis columellae are apparent without staining; ruthenium red highlights the inner, adherent mucilage layer, while methylene blue stains columellae and cellulose rays within the inner mucilage layer. *Sinapis alba* has a thick layer of mucilage containing cellulosic fibres. *Aethionema arabicum* epidermal cells elongate with hydration, but remain intact, containing both mucilage and cellulose. *Ocimum basilicum* has a thick layer of mucilage containing many cellulosic fibres. *Salvia hispanica* has copious mucilage with fine cellulosic threads. *Linum usitatissimum* has pectinaceous mucilage without cellulose.

the presence or absence of dispersed cellulose microfibrils, as detected through techniques such as histochemical staining, polarized light microscopy ('Maltese-cross effect') and electron microscopy (Mühlethaler, 1950; Vaughan and Whitehouse, 1971; Frey-Wyssling, 1976; Schnepf and Deichgräber, 1983a, b; Willats *et al.*, 2001; Kreitschitz and Vàlles, 2007). Cellulosic slimes have been identified in a number of

https://doi.org/10.1017/S0960258511000249 Published online by Cambridge University Press

species, including many of the Brassicaceae (e.g. Arabidopsis, *S. alba*, *Lepidium sativum*; for a survey, see Vaughan and Whitehouse, 1971), plus members of the Acanthaceae, Asteraceae, Fabaceae, Laminaceae, Polemoniaceae and Rosaceae (Fig. 1) (see Table 1 for details) (Mühlethaler, 1950; Grubert, 1974; Fahn, 1982; Schnepf and Deichgräber, 1983a; Abeysekera and Willison, 1988; Kreitschitz and Vàlles, 2007). Grant suggests that cellulosic mucilage is an example of colloidally dispersed cellulose that is solubilized through its interaction with pectins, and notes that *S. alba* seed mucilage contains 50% cellulose by weight (Grant *et al.*, 1969). The presence of cellulose has been suggested to prevent mucilage from being washed away from the seed and has led to cellulosic mucilage sometimes being classified as secondary-cell-wall-like (Fahn, 1982). Mucilages lacking cellulose are known as 'true slimes' or primary-cell-wall-like (Mühlethaler, 1950; Fahn, 1982), and examples are seeds of the Linaceae, Plantaginaceae and Poaceae (Fig. 1) (Mühlethaler, 1950; Fahn, 1982; Kreitschitz *et al.*, 2009).

Detailed analysis of Arabidopsis seed mucilage, using a combination of chemical, histochemical and immunological methods, has revealed not only that its composition is much more complex than originally thought, but that the extruded mucilage has structural domains. Hydration of seeds in the pectin stain ruthenium red, with and without agitation, demonstrated the presence of two layers: an outer, diffuse layer that is easily extracted by shaking in water or dilute chelators, and an inner, dense layer that is strongly adherent to the seed and can only be removed with enzymatic digestion or harsh chemical treatments (Figs 1 and 2A) (Western *et al.*, 2000; Macquet *et al.*, 2007a; Huang *et al.*, 2011; Walker *et al.*, 2011). The outer, soluble layer can be easily characterized by high pressure liquid chromatography (HPLC) or gas chromatography/mass spectrometry (GC/MS), and is primarily composed of unbranched RG I (Penfield *et al.*, 2001). In contrast, the inner, adherent layer has been characterized by histochemical and antibody staining to contain a complex mixture of RG I with and without arabinan and galactan side-chains, HG with varying amounts of methyl-esterification, cellulose and the hemicellulose xyloglucan (Fig. 2B) (Willats *et al.*, 2001; Western *et al.*, 2004; Macquet *et al.*, 2007a; Young *et al.*, 2008). The strong attachment of this complex polysaccharide layer to the seed correlates well with the finding that cellulose promotes mucilage adhesion to the seed (Harpaz-Saad *et al.*, 2011; Sullivan *et al.*, 2011). A similar organization of mucilage into layers has been observed in flax seeds, the mucilage of which contains hemicellulose rather than cellulose (Naran *et al.*, 2008).

In addition to diffuse cellulose embedded in pectinaceous mucilage, a number of species have obvious cellulosic threads or thick fibres projecting from their epidermal cells (Fig. 1). Often these fibres take on a spiral conformation that stretches and elongates upon mucilage hydration and release [e.g. *Artemisia* spp. (Asteraceae), *Euphorbia falcata* (Euphorbiaceae), *Salvia horminum* (Lamiaceae), *Collomia grandiflora* (Polemoniaceae), members of the Acanthaceae, Boraginaceae, Brassicaceae, Cistaceae, Cucurbitaceae, Orchidacea and Zygophyllaceae] (Grubert, 1974; Hsiao and Chuang, 1981; Schnepf and Deichgräber, 1983a; Kreitschitz and Vàlles, 2007). The secondary cell wall inclusions that are deposited internal to the mucilage in Arabidopsis and other Brassicaceae, known as columellae, could be considered as thickened, truncated cellulosic fibres because their production during development is similar (Figs 1, 2D–E; see below) (Vaughan and Whitehouse, 1971; Schnepf and Deichgräber, 1983a, b; McFarlane *et al.*, 2008). Mucilage cells can also take the form of conical cells or long hairs that extrude varying amounts of mucilage [e.g. *Blepharis persica, Ruellia strepens* (Acanthaceae), *Aethionema arabicum* (Brassicaceae), *Hydrocharis morsus-ranae* (Hydrocaritaceae), some species of the Asteraceae, Cucurbitaceae and Lythraceae] (Witztum *et al.*, 1969; Gutterman *et al.*, 1973; Grubert, 1974; Schnepf and Deichgräber, 1983b; Panigrahi, 1986), or consist of only a few cells or hairs in specific regions of the seed [e.g. *Chrysanthemum nivellei, Artemisia* spp. (Asteraceae) and members of the Acanthaceae, Goodeniaceae, Lamiaceae] (Grubert, 1974; Kreitschitz and Vàlles, 2007). Finally, while most seed coat and pericarp mucilage is derived from the seed or fruit epidermis, some species synthesize and release mucilage to surround the seed from subepidermal cells alone or in combination with epidermal cells [e.g. *Helianthemum apeninum* (Cistaceae), *Oxybaphus nyctagineus* (Nyctaginaceae), *Sporobolus cryptandrus* (Poaceae)] (Grubert, 1974).

Cell biology of mucilage production

Mucilage secretory cell (MSC) differentiation has been studied extensively in Arabidopsis by both light and transmission electron microscopy (TEM) (Beeckman *et al.*, 2000; Western *et al.*, 2000; Windsor *et al.*, 2000; McFarlane *et al.*, 2008; Young *et al.*, 2008). Pollination triggers cell growth driven by vacuolar expansion, following which a phase of extensive mucilage production occurs. Mucilage is secreted into the junction between the radial and the tangential cell walls on the apical face of the seed coat epidermal cells (Fig. 2C–E). This deposition results in the production of a ring-shaped mucilage pocket between the apoplast and the primary cell wall on the outer face of the cell (Fig. 2C). Mucilage secretion is coupled to the shrinking of the vacuole to the bottom of the cell

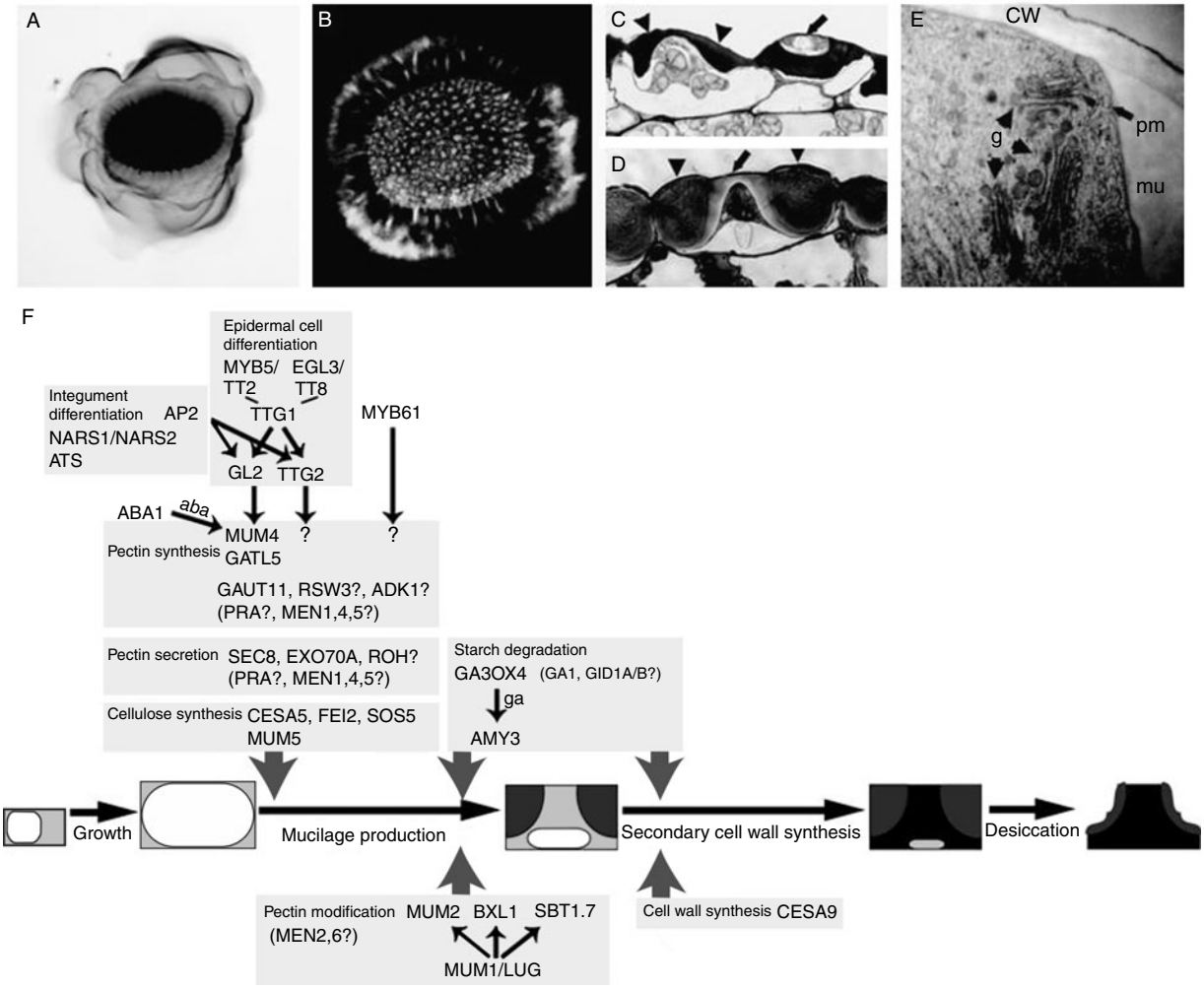

Figure 2. Arabidopsis mucilage secretory cell (MSC) differentiation. (A) Seed staining of wild-type Arabidopsis seed in ruthenium red without shaking. Note the outer, cloudy layer of mucilage as well as the denser, adherent layer directly surrounding the seed. (B) Whole-seed immunofluorescence staining of wild-type Arabidopsis seed with the anti-RG I antibody CCRC-M36. Staining of the mucilage capsule surrounding the seed represents the RG I in the inner, adherent mucilage layer. The seed surface is counterstained with propidium iodide, revealing the hexagonal-shaped MSCs with their central columellae. (C, D) Sections of resin-embedded developing seeds stained with toluidine blue. (C) A seed 7 days post-anthesis (DPA) showing mucilage-containing apoplastic pockets (arrowheads) at the junction between the outer tangential and radial cell walls. The cell on the right, cut at an oblique angle, shows mucilage forming a ring around the cytoplasmic column (arrow). (D) A seed 10 DPA showing final accumulation of mucilage (arrowheads) surrounding the columella (arrow). At this stage, the secondary cell wall is in the process of being deposited around the cytoplasm. (E) Transmission electron micrograph of a 7 DPA seed at the top of the cytoplasmic column, showing the appression of the cytoplasmic column to the primary cell wall at the top of the cell (cw). Mucilage (mu) accumulates in an apoplastic space between the plasma membrane (pm) and cell wall. Several Golgi stacks (g, stacks indicated with arrows) are apparent, along with associated secretory vesicles. (F) Cartoon of the different stages of mucilage secretory cell development with the activity of known genes indicated in terms of basic role and approximate developmental stage of activity. Gene names in bold, large font are those with known regulatory interactions; positive regulation is indicated by black arrows between genes, hormones are noted in lowercase. Genes in brackets are those whose exact function is still to be determined. See the text for details of gene function.

and formation of a volcano-shaped cytoplasmic column that sits under the mucilage pocket. Mucilage production is followed by the synthesis of a cellulosic cell wall to surround and eventually occlude the cytoplasmic column, resulting in a volcano-shaped columella (Fig. 2D) (Beeckman *et al.*, 2000; Western *et al.*, 2000; Windsor *et al.*, 2000). Seed hydration results in rapid mucilage release via breakage of the upper portion of the radial cell wall that is not reinforced by the presence of the columella. Within 1 min, hydrated Arabidopsis seeds are surrounded by a gel-like capsule of swollen mucilage (Arsovski *et al.*, 2009a).

https://doi.org/10.1017/S0960258511000249 Published online by Cambridge University Press

Cell wall remnants remain attached to the tip of the columella and contribute to cellulosic 'rays' observed in the inner layer of mucilage (Western *et al.*, 2000; Macquet *et al.*, 2007a).

Throughout Arabidopsis MSC differentiation, starch granules (amyloplasts) are evident and undergo corresponding changes in size. The starch granules are apparent after cell growth and prior to mucilage synthesis, but become enlarged and prominent as mucilage production commences. As the secondary cell wall of the columella forms, the starch granules shrink, suggesting that their role is to provide sugar for the production of the secondary cell wall, rather than for mucilage synthesis. A corresponding accumulation and reduction of starch granules occurs in the subepidermal palisade cells, which lack mucilage but form a secondary cell wall in parallel with the MSCs (Western *et al.*, 2000; Windsor *et al.*, 2000). Further support for starch depletion being correlated with secondary cell wall synthesis comes from close examination of both starchless (*phosphoglucomutase1*) and *starch excess1* mutants, which have altered columella shape (Windsor *et al.*, 2000).

The ultrastructural details of mucilage production have been studied in Arabidopsis using high-pressure freezing and cryofixation to preserve the endomembrane system (Fig. 2E) (McFarlane *et al.*, 2008; Young *et al.*, 2008). Mucilage synthesis in the Golgi apparatus was confirmed through immunogold staining with an RG I-specific antibody (CCRC-M36) raised from Arabidopsis mucilage. Immunostaining also shows association of mucilage with the trans-Golgi network (TGN) and secretory vesicles, suggesting that mucilage is transported to the apoplast via secretory vesicles (McFarlane *et al.*, 2008; Young *et al.*, 2008). Prior to mucilage synthesis, the Golgi stacks have long, thin cisternae and the associated TGN is relatively small. During mucilage synthesis, however, the Golgi stacks have cisternae with compressed centres and swollen margins, as well as an enlarged TGN surrounded by large numbers of secretory vesicles that also are throughout the cytoplasm (Fig. 2E) (Young *et al.*, 2008). Further, during mucilage synthesis, the number of Golgi stacks approximately doubles, as demonstrated both by counting of fluorescently labelled stacks in live cells and of fixed stacks in electron micrographs. Comparison of the Golgi stacks in a mutant making a reduced amount of mucilage [*mucilage-modified4* (*mum4*)/*rhamnose synthase2* (*rhm2*)] with wild-type cells revealed that, while the doubling of Golgi stacks is developmentally programmed, the altered morphology of the Golgi stacks (compressed cisternal lumens, swollen margins, increased TGN) during mucilage synthesis results from the production of copious amounts of mucilage (Young *et al.*, 2008). During mucilage production, the Golgi stacks are evenly distributed throughout the cytoplasm, rather than accumulating near the site of the growing mucilage pocket. This observation is in contrast to other examples of polar cell-wall secretion in plant cells, such as the formation of the cell plate and cell wall deposition in tip-growing cells (e.g. root hairs, pollen tubes, trichomes), where the Golgi stacks are concentrated near the site of secretion (Jurgens, 2005; Cole and Fowler, 2006; Campanoni and Blatt, 2007; Young *et al.*, 2008).

The contribution of the cytoskeleton to mucilage secretion and MSC differentiation in Arabidopsis has also been investigated via TEM and fluorescence microscopy (McFarlane *et al.*, 2008). Ultrastructural studies revealed a preponderance of cortical microtubules (MTs) lining the mucilage pocket. This organization of the MTs surrounding the zone of secretion at the constricted part of the cytoplasmic column was also observed using fluorescently labelled anti-tubulin antibodies. To further investigate a role for MTs in the regulation of mucilage secretion and/or the shaping of the cytoplasmic column, the temperature-sensitive *mor1-1* mutant, which has disrupted MT organization, was employed (McFarlane *et al.*, 2008). There were no gross differences in morphology of the MSCs or the organization of the Golgi apparatus or secretory vesicles in *mor1-1* seeds grown under restrictive conditions. Mucilage release, however, was impaired in 40% of *mor1-1* seeds grown under restrictive temperatures, versus only 22% for wild-type seeds. The release defect and lack of gross changes to the mucilage pocket or columella shape in *mor1-1* mutants suggests a role for MTs in establishing the physical properties of mucilage, but their exact function is unclear (McFarlane *et al.*, 2008). Unlike MTs, actin is not specifically localized in MSCs (McFarlane *et al.*, 2008). The use of chemical treatments to disrupt MT and actin could reveal more about the role of these cytoskeletal elements in MSC differentiation and mucilage production, but such treatments have been unsuccessful (McFarlane *et al.*, 2008).

In addition to the work in Arabidopsis, seed coat mucilage synthesis has been studied at the ultrastructural level using chemical fixation and TEM in *Brassica campestris*, *Plantago ovata*, *Cydonia oblonga* (Rosaceae), *Solanum viarum* (Solanaceae), *C. grandiflora* and *Ruellia* spp. (Hyde, 1970; Van Caeseele *et al.*, 1981; Schnepf and Deichgräber, 1983a, b; Abeysekera and Willison, 1988; Srinivas *et al.*, 1998). Similar to Arabidopsis, a prominent feature of mucilage production in each case is the appearance of large numbers of swollen Golgi stacks and associated secretory vesicles that are apparent as mucilage accumulates in the apoplastic space of the seed coat epidermal cells. In studies of *P. ovata*, *S. viarum* and *B. campestris*, the authors also note the accumulation of large starch granules which are then lost as mucilage accumulates. Similar results have also been noted at

the light microscopy level for flax, suggesting that starch is providing sugar for mucilage synthesis (Hyde, 1970; Boesewinkel, 1980; Van Caeseele *et al.*, 1981; Srinivas *et al.*, 1998). While these species have a reduction in cytoplasm and/or vacuole that accompanies mucilage production, none of them has cellulosic fibres or inclusions similar to the columella of Arabidopsis.

Collomia spp. and *Ruellia* spp. epidermal cells have both cellulosic mucilage with dispersed cellulose microfibrils and cellulosic inclusions. *Collomia* spp. have well-defined spiral secondary cell wall thickenings that are contiguous with the wall at the base of the cell – these are seen to expand when the cells are wetted and mucilage is released (Hsiao and Chuang, 1981; Schnepf and Deichgräber, 1983a). During differentiation of *Ruellia* spp. seed coats, the epidermal cells elongate into hairs that have spiral fibres embedded in the mucilage ('mucilage strands') that appear to be cellulosic (Schnepf and Deichgräber, 1983b). The differentiation of *C. grandiflora* and several *Ruellia* species were followed at the ultrastructural level by Schnepf and Deichgräber (1983a, b), who focused on the production of both 'elemental fibres' (cellulose microfibrils) and the cellulosic inclusions. In both cases, they found an association between the location and orientation of cellulose microfibrils with those of a number of MTs at the plasma membrane. Consideration of their results suggests a similar role in cellulose production for the MTs identified lining the mucilage pockets in Arabidopsis.

Genetics of mucilage production and MSC differentiation in Arabidopsis

Pectin synthesis and secretion

The presence of seed coat mucilage in Arabidopsis is dispensable under laboratory conditions, allowing the isolation of mutants affecting mucilage production and MSC differentiation (Table 2 and Fig. 2F) (Western *et al.*, 2001). Mutations in genes affecting the synthesis or secretion of mucilage have been identified through the lack of its release upon seed hydration, and fall under two categories: those that lack mucilage altogether and those with reduced mucilage production. Complete lack of mucilage synthesis has been correlated with defects in post-fertilization differentiation of the ovule outer integument, the epidermal layer of which is the MSCs. *apetala2 (ap2)* single- and *nac regulated seed morphology1* (*nars1*) *nars2* double-mutants lack differentiation of the MSCs and subtending palisade cells after the growth phase of seed development (Bowman and Koornneef, 1994; Debeaujon *et al.*, 2000; Western *et al.*, 2001; Kunieda *et al.*, 2008; Molina *et al.*, 2008). Both also exhibit seed shape defects: *ap2* seeds are heart-shaped, with rectangular seed epidermal cells, while *nars1 nars2* seeds are shrivelled with irregular-shaped cells. Embryo defects in *nars1 nars2* suggest delayed development, which could also explain the seed coat phenotype (Kunieda *et al.*, 2008). All three genes encode transcription factors, with *AP2* encoding an AP2-type protein that plays roles in a number of developmental pathways, including flower and ovule development (Jofuku *et al.*, 1994). *NARS1* and *NARS2* encode NAC family proteins (Kunieda *et al.*, 2008). In addition to *AP2, NARS1* and *NARS2*, mutations in *ABERRANT TESTA SHAPE* (*ATS*) also affect mucilage production. *ATS* encodes a KANADI family transcription factor responsible for the specification of ovule integument layers. *ats* mutant seeds are heart-shaped and have only a single integument, the epidermal MSCs of which make a reduced amount of mucilage (Léon-Kloosterziel *et al.*, 1994; McAbee *et al.*, 2006).

The best-characterized reduced-mucilage mutant is *mum4/rhm2*. MUM4/RHM2 is a rhamnose synthase required for the production of UDP-L-rhamnose, a substrate for the production of the pectin RG I (Usadel *et al.*, 2004; Western *et al.*, 2004; Oka *et al.*, 2007). *mum4/rhm2* mutants make less than half the wild-type amount of both rhamnose and galacturonic acid, the two components of the RG I backbone, and are unable to release mucilage unless treated with a metal chelator to weaken the primary cell wall. The smaller mucilage pockets of *mum4/rhm2* seeds are correlated with a flattened columella, demonstrating the role of mucilage accumulation in the shaping of this secondary cell wall. *MUM4/RHM2* is a member of a three-gene family and is specifically upregulated at the time of mucilage production (Usadel *et al.*, 2004; Western *et al.*, 2004). *MUM4/RHM2* transcription in MSCs is regulated by several pleiotropic transcription factors: AP2, TRANSPARENT TESTA GLABRA1 (TTG1), GLABRA2 (GL2), TRANSPARENT TESTA2 (TT2), TT8, ENHANCER OF GLABRA3 (EGL3) and MYB5. With the exception of *ap2* mutants, which lack mucilage completely, single or double mutants of the other regulators of *MUM4/RHM2* all exhibit a similar phenotype to *mum4* (Koornneef, 1981; Bowman and Koornneef, 1994; Debeaujon *et al.*, 2000; Zhang *et al.*, 2003; Western *et al.*, 2004; Gonzalez *et al.*, 2009; Li *et al.*, 2009). The WD40 repeat protein TTG1 is an epidermal cell differentiation factor that acts in multiple tissues through complexes with bHLH and MYB transcription factors. In the MSCs, TTG1 interacts with the bHLH proteins EGL3 and TT8, and the R2R3 MYBs MYB5 and TT2 to upregulate the homeobox transcription factor *GL2* (Rerie *et al.*, 1994; Walker *et al.*, 1999; Gonzalez *et al.*, 2009; Li *et al.*, 2009). GL2, in turn, upregulates *MUM4/RHM2* transcription, though it is not known if it interacts directly with the *MUM4/RHM2* promoter. AP2 also affects the transcription of

https://doi.org/10.1017/S0960258511000249 Published online by Cambridge University Press

Table 2. Genes acting in mucilage production and mucilage secretory cell differentation in Arabidopsis

Gene	AGI number	Protein	Proposed role in mucilage production	Germination	Reference
Regulation of outer integument differentiation					
APETALA2 (AP2)	At4g36920	AP2 TF	Regulation of outer integument differentiation	Slightly reduced dormancy	1–6
ABERRANT TESTA SHAPE (ATS)	At5g42630	Kanadi-family TF	Regulation of ovule integument development	Slightly reduced dormancy	6–8
NAC REGULATED SEED MORPHOLOGY1 (NARS1)/ NAC2	At1g52880	NAC-like TF	Regulation of seed coat and embryo development	n.d.	9
NARS2/NAM	At3g15510	NAC-like TF	Regulation of seed coat and embryo development	n.d.	9
Mucilage synthesis and secretion					
MUCILAGE-MODIFIED4 (MUM4)/RHAMNOSE SYNTHASE2(RHM2)	At1g53500	UDP-L-rhamnose synthase	Pectin biosynthesis (RG I)	Slow germination on water	3,4,10–12
GALACTURONOSYLTRANSFERASE11 (GAUT11)	At1g18580	Glycosyltransferase (GT8)	Pectin biosynthesis (HG?)	n.d.	13
GAUT1-LIKE 5 (GATL5)	At1g02720	Glycosyltransferase (GT8)	Pectin biosynthesis (RG I?)	n.d.	14,15
RADIAL SWELLING3 (RSW3)	At5g63840	Glucosidase II active in N-glycan processing in ER	Pectin biosynthesis?	n.d.	16
SEC8	At3g10380	Subunit of exocyst	Pectin secretion	n.d.	17
EXO70A1	At5g03540	Subunit of exocyst	Pectin secretion	n.d.	17
ROH1	At1g63930	DUF793, interactor with EXO70A1	Regulator of pectin secretion?	n.d.	17
TRANSPARENT TESTA GLABRA1 (TTG1)	At5g24520	WD40 repeat protein	Regulator of mucilage synthesis	Reduced dormancy and reduced germination on osmoticum	2,3,4,6,7, 18–20
MYB5	At3g13540	R2R3 MYB TF	Regulator of mucilage synthesis	n.d.	21,22
TRANSPARENT TESTA2 (TT2)	At5g35550	R2R3 MYB TF	Regulator of mucilage synthesis	Reduced dormancy	6,21
TRANSPARENT TESTA8 (TT8)	At4g09820	bHLH TF	Regulator of mucilage synthesis	Reduced dormancy	6,23
ENHANCER OF GLABRA3 (EGL3)	At1g63650	bHLH TF	Regulator of mucilage synthesis	n.d.	23
GLABRA2 (GL2)	At1g79840	Homeobox TF	Regulator of mucilage synthesis	Slightly increased dormancy and reduced germination on osmoticum	2,3,4,6,18,24
TTG2	At2g37260	WRKY TF	Regulator of mucilage synthesis	n.d.	4,25
MYB61	At1g09540	R2R3 MYB TF	Regulator of mucilage synthesis	Slow germination on water and reduced germination on osmoticum	4,18
ABSCISIC ACID1 (ABA1)	At5g67030	Zeaxanthin epoxidase	ABA biosynthesis, hormone regulation of mucilage synthesis	Non-dormant	26
PRAIRIE (PRA)		Unknown	?	n.d.	27

Table 2. *Continued*

Gene	AGI number	Protein	Proposed role in mucilage production	Germination	Reference
MUM ENHANCER1 (MEN1)		Unknown	?	Slow germination on water (*men1 mum4* double mutant)	12
MEN4		Unknown	?	Slow germination on water	12
MEN5		Unknown	?	Slow germination on water (*men5 mum4* double mutant)	12
Mucilage structure and modification					
MUM5		Unknown	Mucilage structure	n.d.	3,28–30
MUM3/CELLULOSE SYNTHASE5 (CESA5)	At5g09870	Cellulose synthase subunit	Mucilage structure and secondary cell walls (cellulose synthesis)	n.d.	3,28–30,45
SALT OVERLY SENSITIVE5 (SOS5) (FLA4)	At3g46550	GPI-anchored fasciclin-type AGP	Mucilage structure (cellulose synthesis?)	n.d.	30,31
FEI2	At2g35620	Leucine-rich receptor kinase	Mucilage structure (cellulose synthesis?)	n.d.	30,31
MUM2	At5g63800	β-Galactosidase	Mucilage/primary cell wall modification	n.d.	3,32,33
AtBXL1	At5g49360	Bifunctional β-xylosidase/ α-arabinofuranosidase	Mucilage/primary cell wall modification	Slow germination on agar medium	34
AtSUBTILASE1.7 (AtSBT1.7)	At5g67360	Serine protease (subtilase)	Negative regulator of pectin methylesterases in mucilage/primary cell wall	Reduced germination on osmoticum	35
ADENOSINE KINASE1 (ADK1)	At3g09820	Adenosine kinase	Mucilage methylation	n.d.	36
MUM1/LEUNIG_HOMOLOG (LUH)	At2g32700	Groucho/TUP1 family co-repressor	Regulation of mucilage/primary cell wall modification	n.d.	3,37–39
LEUNIG (LUG)	At4g32551	Groucho/TUP1 family co-repressor	Regulation of mucilage/primary cell wall modification	n.d.	37,39
MEN2		Unknown	?	Slow germination on water (*men2 mum4* double mutant)	12
MEN6		Unknown	?	Slow germination on water men6 *mum4* double mutant)	12
Other MSC differentiation					
CELLULOSE SYNTHASE A9 (CESA9)	At2g21770	Cellulose synthase subunit	MSC cell morphology and secondary cell wall synthesis	n.d.	40,41
CELLULOSE SYNTHASE A2 (CESA2)	At4g39350	Cellulose synthase subunit	MSC cell morphology and secondary cell wall synthesis	n.d.	41
DEFECTIVE IN CUTICULAR RIDGES (DCR)	At5g23940	Soluble BAHD acyltrans-ferase,	Seed coat cutin synthesis, seed coat structure	Reduced germination on osmoticum	42

Table 2. *Continued*

Gene	AGI number	Protein	Proposed role in mucilage production	Germination	Reference
GIBBERELLIN-3 OXIDASE4 (AtGA3OX4)	At1g80330	GA-3-oxidase	Promotion of starch degradation during MSC differentiation	n.d.	43
GIBBERELLIN-INSENSITIVE DWARF 1A (AtGID1a)	At3g05120	Gibberellin receptor	MSC cell morphology	No germination in triple mut with *gid1a gid1b gid1c* unless remove seed coat	44
AtGID1b	At3g63010	Gibberellin receptor	MSC cell morphology	No germination in triple mut with *gid1a gid1b gid1c* unless remove seed coat	44
GA1	At4g02780	*ent*-copalyl diphosphate synthetase 1	GA synthesis, regulation of MSC differentiation	No germination without added GA or removal of seed coat	44
MOR1	At2g35630	MAP215 MT-associated protein	Mucilage composition? Guidance of cellulose synthesis for columella?	n.d.	45

ABA, abscisic acid; AGP, arabinogalactan protein; ER, endoplasmic reticulum; GA, gibberellic acid; GPI, glycophosphatidylinositol; GT8, glycosyltransferase family 8; HG, homogalacturonan; MSC, mucilage secretory cell; MT, microtubule; n.d., not determined; TF, transcription factor.

(1) Jofuku *et al.* (1994); (2) Bowman and Koornneef (1994); (3) Western *et al.* (2001); (4) Western *et al.* (2004); (5) Molina *et al.* (2008); (6) Debeaujon *et al.* (2000); (7) Léon-Kloosterziel *et al.* (1994); (8) McAbee *et al.* (2006); (9) Kunieda *et al.* (2008); (10) Usadel *et al.* (2004); (11) Oka *et al.* (2007); (12) Arsovski *et al.* (2009b); (13) Caffall *et al.* (2009); (14) Kong *et al.* (unpublished data); (15) Kong *et al.* (2011); (16) Burn *et al.* (2002); (17) Kulich *et al.* (2010); (18) Penfield *et al.* (2001); (19) Walker *et al.* (1999); (20) Koornneef (1981); (21) Gonzalez *et al.* (2009); (22) Li *et al.* (2009); (23) Zhang *et al.* (2003); (24) Rerie *et al.* (1994); (25) Johnson *et al.* (2002); (26) Karssen *et al.* (1983); (27) Western (2006); (28) Macquet *et al.* (2007a); (29) Sullivan *et al.* (2011); (30) Harpaz-Saad *et al.* (2011); (31) Xu *et al.* (2008); (32) Dean *et al.* (2007); (33) Macquet *et al.* (2007b); (34) Arsovski *et al.* (2009a); (35) Rautengarten *et al.* (2008); (36) Moffatt *et al.* (2002); (37) Walker *et al.* (2011); (38) Huang *et al.* (2011); (39) Bui *et al.* (2011); (40) Stork *et al.* (2010); (41) Mendu *et al.* (2011); (42) Panikashvili *et al.* (2009); (43) Kim *et al.* (2005); (44) Iuchi *et al.* (2007); (45) McFarlane *et al.* (2008).

MUM4/RHM2 indirectly through upregulation of *GL2*, but does so independently of the TTG1–bHLH–MYB complex (Western *et al.*, 2004). *TTG2*, another downstream gene of AP2 and the TTG1–bHLH–MYB complex, also affects mucilage production in parallel with *GL2*. A final regulator of mucilage synthesis is *MYB61*, which appears to act through a parallel pathway to that regulated by *AP2* and *TTG1* (Fig. 2F) (Penfield *et al.*, 2001; Johnson *et al.*, 2002). Both *ttg2* and *myb61* mutants exhibit reduced mucilage phenotypes similar to that of *mum4/rhm2* (Penfield *et al.*, 2001; Johnson *et al.*, 2002).

The use of microarray analyses to look for genes co-regulated with *MUM4/RHM2* by TTG1 and AP2 identified the glycosyl transferase 8 family (GT8) member *GALACTURONOSYLTRANSFERASE-LIKE5* (*GATL5*) (Y. Kong, A.A. Abdeen, J. Schafhauser, T.L. Western and M.G. Hahn, unpublished data). Similar to *mum4/rhm2*, *gatl5* mutants have reductions in both rhamnose and galacturonic acid, though less severe. The predicted function of GATL5 as a galacturonosyl transferase (Kong *et al.*, 2011) in combination with this phenotype suggests that it acts directly in RG I synthesis. Another pectin biosynthetic enzyme implicated in mucilage production is the putative glycosyltransferase GALACTURONOSYLTRANSFERASE11 (GAUT11). *gaut11* mutants can have somewhat reduced mucilage; however, unlike *mum4/rhm2* and *gatl5* mutants, only galacturonic acid content is affected (Caffall *et al.*, 2009). This result suggests that GAUT11 acts in the production of HG in the adherent layer of mucilage.

Recent studies of mutants for two subunits of the exocyst, *sec8* and *exo70a1*, revealed reduced mucilage and flattened columellae similar to those in *mum4/rhm*2 mutants (Kulich *et al.*, 2010). The exocyst is a plasma-membrane-associated secretory vesicle tethering complex (Zhang *et al.*, 2010) and is the first component of the secretory system identified to have a significant effect on mucilage accumulation. A yeast two-hybrid screen to identify exocyst interacting proteins, using EXO70A1 as bait, identified several proteins, including ROH1. While a loss of function T-DNA insertion of *ROH1* (*roh1-d*) had no phenotype, two regulatory region insertions that resulted in increased *ROH1* transcription (*roh1-p* and *roh1-e*) had reduced mucilage. *ROH1* encodes a DUF793 protein and has been hypothesized to act as a negative regulator of secretion (Kulich *et al.*, 2010).

To identify further genes involved in mucilage production and MSC differentiation, the reduced mucilage phenotype of *mum4/rhm2* seeds was used as the basis for a genetic enhancer–supressor screen. Eight *mum enhancers* (*men*) were identified, including new mutant alleles of the known reduced mucilage gene *myb61* and the cell wall modification gene *mum2* (see below), and six novel genes (Arsovski *et al.*, 2009b). Microscopic and chemical analyses suggested that *MEN1, MEN4* and *MEN5* affect the amount of mucilage produced, while *MEN2* and *MEN6* affect mucilage release upon seed hydration (Arsovski *et al.*, 2009b). Other genes with significant effects on mucilage production are *PRAIRIE* and the glucosidase II *RADIAL SWELLING3* (Burn *et al.*, 2002; Western, 2006). Cloning and/or further analyses of these genes will allow elucidation of their exact roles regarding mucilage synthesis and secretion.

A final gene affecting mucilage production is the hormone abscisic acid (ABA) biosynthetic gene *ABSCISIC ACID1* (*ABA1*). The mucilage layer surrounding *aba1* mutant seeds can be reduced as much as 50% and is correlated with the maternal dose of ABA received by seeds mid-way through their development. Mucilage production can be stimulated by ABA, as demonstrated by both the rescue of *aba1* mutants and increased mucilage production of wild-type plants treated with ABA (Karssen *et al.*, 1983). Interestingly, increased *MUM4/RHM2* transcription is induced in seedlings and developing seeds in response to ABA treatment, suggesting that it may regulate mucilage production through the amount of UDP-L-rhamnose substrate available for RG I synthesis (Hoth *et al.*, 2002; Usadel *et al.*, 2004) (U.K. Divi and T.L. Western, unpublished data).

Mucilage structure and modification

A screen for mutants specifically affected in mucilage staining, which uses the pectin dye ruthenium red, identified not only the reduced mucilage mutant *mum4*, but also mutants affected in mucilage staining/composition (*mum3, mum5*) and in mucilage release upon seed hydration (*mum1, mum2*) (Table 2, Fig. 2F) (Western *et al.*, 2001). *mum3* and *mum5* mutants stained in ruthenium red, with shaking, appear to have no, or reduced, mucilage surrounding the seed; however, when stained without shaking, substantial amounts of mucilage are observed (Western *et al.*, 2001). Further studies of *mum5* using both staining and chemical analyses revealed alterations to the mucilage structure suggesting that the inner adherent layer is reduced while the outer soluble layer is increased, i.e. an increased proportion of *mum5* mucilage is soluble RG I (Macquet *et al.*, 2007a; Harpaz-Saad *et al.*, 2011; Sullivan *et al.*, 2011). Presumably this change results from altered cross-linkages between pectins and/or cellulose found within the inner mucilage layer (Western *et al.*, 2001; Macquet *et al.*, 2007a). Recently, mutants for the cellulose synthase subunit CELLULOSE SYNTHASE5 (CESA5), the leucine-rich receptor kinase FEI2 and the glycophosphatidylinositol-anchored fasciclin-like arabinogalactan protein SALT OVERLY SENSITIVE5 (SOS5) were found to

https://doi.org/10.1017/S0960258511000249 Published online by Cambridge University Press

have similar mucilage phenotypes of increased soluble mucilage paired with decreased adherent mucilage (Harpaz-Saad *et al.*, 2011; Mendu *et al.*, 2011; Sullivan *et al.*, 2011). Sequencing and genetic complementation tests have demonstrated that the *mum3* mutant is an allele of *CESA5* (Sullivan *et al.*, 2011). The identification of *cesa5* mutants with reduced adherent mucilage supports the hypothesis that the inner layer of adherent mucilage is attached and organized through pectic interaction with cellulose (Willats *et al.*, 2001; Macquet *et al.*, 2007a). *FEI2* and *SOS5* likely regulate cellulose synthesis in the MSCs, as they have been implicated to do in seedling roots (Xu *et al.*, 2008; Harpaz-Saad *et al.*, 2011). Because *MUM5* is not allelic to any of the other genes affecting this process, its molecular role still remains to be elucidated (B. Forward and T.L. Western, unpublished data).

mum1 and *mum2* are members of a group of mutants that make wild-type quantities of mucilage, but are defective in mucilage release upon seed hydration (Western *et al.*, 2001). Other genes include *AtBETA-XYLOSIDASE1* (*AtBXL1*) and *AtSUBTILASE1.7* (*AtSBT1.7*) (Rautengarten *et al.*, 2008; Arsovski *et al.*, 2009a). *mum2* mucilage has increased galactose, arabinose and RG I branchpoints (Dean *et al.*, 2007; Macquet *et al.*, 2007b). This altered structure is correlated with an inability of *mum2* mucilage to swell appreciably even in cut cells unless treated with sodium carbonate (Dean *et al.*, 2007). Cloning of *MUM2* revealed that it encodes a β-galactosidase that is implicated in the trimming of RG I side-chains such as terminal galactose, galactans and arabinogalactans (Dean *et al.*, 2007; Macquet *et al.*, 2007b). In contrast, *bxl1* mutants have slow and patchy mucilage release that is correlated with increased pectic arabinans in both extracted mucilage and MSC cell walls. *AtBXL1* encodes a bifunctional β-xylosidase/α-arabinofuranosidase that acts as an arabinofuranosidase during MSC development (Arsovski *et al.*, 2009a). Analysis of mutants for both of these genes reveals not only the need for the trimming of RG I side-chains to enable mucilage release, but also the effect of pectin side-chain modification on developmental processes.

Unlike *MUM2* and *AtBXL1*, *AtSBT1.7* has an indirect effect on pectin structure. *sbt1.7* mutants fail to release mucilage unless treated with cation chelators that weaken pectin gel structure by disruption of calcium bridges between galacturonic acid residues (Rautengarten *et al.*, 2008). Methylation analysis of extracted *sbt1.7* mucilage revealed decreased methylesterification, which was reflected by altered binding of antibodies to methylesterified HG. Quantification of pectin methylesterase (PME) activity throughout seed development found increased PME activity late in seed development compared with wild type. *AtSBT1.7* encodes a subtilisin-like serine protease (subtilase) and has been hypothesized to regulate *in muro* pectin de-esterification in mucilage and/or the outer primary cell wall of MSCs by degradation of PMEs or activation of PME inhibitors via limited proteolysis (Rautengarten *et al.*, 2008). Similar to MUM2 and AtBXL1, AtSBT1.7 activity is necessary for sufficient mucilage swelling to drive its release and/or weakening of the outer primary cell wall. Interestingly, decreased pectin methylesterification was also detected in antisense lines for *ADENOSINE KINASE1 (ADK1)*, a gene encoding an enzyme involved in the salvage synthesis of adenine monophosphate from adenosine and ATP (Moffatt *et al.*, 2002). *adk1*-deficient lines are impaired in *S*-adenosyl-L-methionine (SAM)-dependent methylation reactions, including those taking place in the Golgi apparatus to methylate HG. While *adk1*-deficient lines can have significant decreases in pectin methylesterification even below those in *sbt1.7* mutants, they have normal mucilage release, suggesting that localization or developmental timing of de-esterification is important (Moffatt *et al.*, 2002; Rautengarten *et al.*, 2008).

mum1 mutants have a similar phenotype to *mum2*, where lack of mucilage swelling is coupled with increases in galactose, arabinose, RG I branching and HG methylesterification. Close examination of *mum1* versus *mum2* mutants also revealed thickening of the radial cell walls in both lines (Huang *et al.*, 2011; Walker *et al.*, 2011). *MUM1* encodes LEUNIG_HOMOLOG (LUH), a transcriptional co-repressor in the Groucho/Tup1 family (Bui *et al.*, 2011; Huang *et al.*, 2011; Walker *et al.*, 2011). *LUH* is a close homologue of the developmental regulator *LEUNIG (LUG).* The latter forms a co-repression complex with SEUSS or a SEUSS-LIKE protein that partners with specific DNA-binding proteins to regulate flower, embryo, leaf and vascular development. *LUH* acts partially redundantly with *LUG*, both in the seed coat and in the regulation of embryo and flower development (Sitaraman *et al.*, 2008; Bui *et al.*, 2011; Walker *et al.*, 2011). Expression analysis of *MUM2* in *mum1/luh* mutants revealed reduced *MUM2* transcript, suggesting that a role of *MUM1/LUH* is to upregulate *MUM2* during MSC differentiation. Transformation of *mum1/luh* mutants with $35S_{pro}$*::MUM2* and LUH_{pro}*::MUM2* resulted in partial rescue of mucilage extrusion, both confirming the hypothesis that *MUM2* is downstream of *MUM1/LUH* and suggesting other targets during MSC differentiation (Bui *et al.*, 2011; Huang *et al.*, 2011; Walker *et al.*, 2011). Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) of *AtBXL1* and *AtSBT1.7* in developing *mum1/luh* seeds also revealed decreased transcription, suggesting integrated positive regulation of mucilage/MSC cell wall modifications by *MUM1/LUH* (Fig. 2F) (Huang *et al.*, 2011).

Columella production and other aspects of seed coat differentiation affecting MSCs

When viewed with scanning electron microscopy or with fluorescent dye, the MSCs on the surface of the Arabidopsis seed coat are visible as a regular array of hexagonal cells with thickened radial cell walls and raised round or oval 'plateaus' in the centre of each cell, representing the top of the volcano-shaped columella (Fig. 2B). Mutants for the cellulose synthase subunit CESA9 exhibit a disorganized seed coat, including defects in columella and radial cell wall patterning, and reduced radial cell wall height (Stork *et al.*, 2010). While *cesa9* mutant seeds are smaller with decreased cellulose compared to wild-type seeds, they show no difference in mucilage composition or release. Together these data suggest a role for *cesa9* in production of the secondary radial cell walls and columella (Stork *et al.*, 2010). Recently, redundant roles in secondary cell wall development in the MSCs have also been identified for CESA2 and CESA5 (Mendu *et al.*, 2011). The defects in secondary cell wall synthesis in *cesa9* mutants are associated with increases of other polymers, including neutral monosaccharides in pectins and hemicelluloses, and lipid polyester monomers that are normally associated with cutin and/or suberin. Compensative changes in polymer production are common in cell wall biosynthetic mutants (Stork *et al.*, 2010). Another potential example of compensative cell wall changes that affect MSCs is that of mutants for the cutin biosynthetic gene *DEFECTIVE IN CUTICULAR RIDGES* (*DCR*) (Panikashvili *et al.*, 2009). *dcr* mutants, in addition to having substantial decreases in several cutin monomers in the seed, are unable to release mucilage upon seed hydration. Similar to other epidermal cells, MSCs observed with TEM have a cuticle-like osmophilic layer coating their outer cell walls; alterations to this cuticular layer or compensatory changes to the subtending cell wall could prevent mucilage extrusion in *dcr* seeds (Panikashvili *et al.*, 2009).

Distorted external MSC morphology is also seen in several mutants affected in the production of and response to gibberellins (GAs). These include mutants for the biosynthetic genes *GA1* (*ent*-copalyl diphosphate synthetase 1) and *GIBBERELLIN-3-OXIDASE4* (*AtGA3OX4*), and a double mutant for the GA receptors *GIBBERELLIN-INSENSITIVE DWARF 1A* (*GID1A*) and *GID1B* (Kim *et al.*, 2005; Iuchi *et al.*, 2007). A study of the regulation of α-amylase genes by GA synthesis in Arabidopsis seeds identified a role for *AtGA3OX4* in the regulation of starch metabolism in seed coats (Kim *et al.*, 2005). *ga3ox4* knockout seeds had delayed starch degradation in the seed coat coupled with reduced mucilage release upon seed hydration. Both phenotypes could be rescued by treatment of developing seeds with GA. Thus GA may regulate substrate availability for both mucilage and columella secondary cell wall production through α-amylase-mediated starch degradation (Fig. 2F) (Kim *et al.*, 2005).

Ecological significance of seed coat mucilages – germination and dispersal

Seed coat mucilage production requires a significant carbon allocation during early seed development, suggesting that there must be selection for myxodiaspory. A number of hypotheses have been suggested for the presence of seed coat mucilages, including: (1) aid for seed hydration, especially under conditions of water or salt stress; (2) water reservoir for germination and/or early seedling development; (3) inhibition of germination by preventing oxygen flow to the embryo; (4) aid to germination by promoting embryo DNA repair (seed priming); (5) seed adhesion to soil to prevent removal by water or predators; (6) seed adhesion to animal vectors for dispersal (epizoochory); and (7) aid to seed dispersal by animal ingestion (endozoochory) (see Table 1).

Role of seed coat mucilages in seed hydration and germination

Due to the significant hydrophilicity of mucilages and their impressive swelling and water retention abilities, a role for mucilages in seed hydration and/or as a water reservoir for germination seems obvious (Grubert, 1974; Fahn, 1982; Kreitschitz, 2009). Such a role is also suggested by the high frequency of myxospermic plants in arid habitats (Grubert, 1974; Ellner and Shmida, 1981; Ryding, 2001; Kreitschitz and Vàlles, 2007; Kreitschitz, 2009). Ryding (2001) performed a thorough study of myxocarpy in 400 species across the Nepetoideae (Lamiaceae), where he used both experimentation and the literature to analyse the correlations between the amount of mucilage produced, presence of nutlet trichomes, plant habitat and plant duration (annual versus perennial). He found that species from dry habitats had a greater frequency of myxospermy. Interestingly, this held for both arid and more humid regions, with tropical Africa having the highest relative proportion of myxodiaspory of the regions where Nepetoideae are found (Ryding, 2001).

Direct experiments for a role of mucilages in seed hydration have been performed on a number of species. Harper and Benton (1966) compared germination of seeds with copious mucilage, less-copious mucilage, bumpy (tuberculate) surface and smooth surface on filter paper versus sintered glass plates with 0–200 cm water tension. Seeds with copious mucilage were able to germinate in all treatments, with some slowing of germination at higher water tensions. Those

https://doi.org/10.1017/S0960258511000249 Published online by Cambridge University Press

with less-copious mucilage had poorer germination at higher water tensions, while those with tuberculate or smooth surfaces generally had very poor germination. Upon wetting, the swollen mucilage increased contact between the seed and the pores of the plate, presumably aiding water flow to the seed. Further investigations between a species with copious mucilage (*L. sativum*) and a tuberculate seed of similar size (*Agrostemma githago,* Caryphyllaceae) revealed that seeds with mucilage could also germinate well at high water tensions on simulated soil particles of different grades. Further, germination of *A. githago* under high water tension could be increased by the addition of exogenous *Plantago psyllium* mucilage, burying of the seed and covering the plates with plastic wrap to increase humidity. From these experiments, they concluded that germination is a balance between water uptake from the substrate and water loss to the atmosphere, and that mucilage aids the former through increased surface contact and may also reduce the latter (Harper and Benton, 1966). Mott (1974) compared germination between three species from an arid region of Western Australia: *Helipterum craspedioides* (Asteraceae; mucilaginous pericarp), *Helichrysum cassinianum* (Asteraceae; pericarp with long hairs) and *Aristida contorta* (Poaceae; no surface features). Tests were performed comparing intact seeds with those with mucilage or hairs removed. While both the hairs and mucilage spread out when wet and promoted seed–substrate contact, there was no benefit from either when imbibed or dehydrated under non-limiting water conditions. When tested on soil with different moisture contents, however, both mucilage and hairs aided seed germination if seeds were first sprayed with water to promote mucilage release/hair spreading. This suggests that mucilage aids seed hydration and germination in the presence of some surface water (Mott, 1974). Achenes of *Artemisia sphaerocephala* (Asteraceae) were tested for germination of intact achenes versus those with mucilage removed (Yang *et al.*, 2010). While no difference was found under non-limiting conditions, 'demucilaged' achenes showed greater sensitivity to both increasing osmotic potential [polyethylene glycol (PEG) concentration] and salinity (NaCl) stress. Further, mucilaginous achenes whose germination was inhibited by high concentrations of NaCl were more likely to germinate when moved to non-limiting conditions. Thus, the presence of mucilage can aid germination in desert habitats with both drought and salt-stress (Yang *et al.*, 2010).

Recent work in Arabidopsis has also suggested a role for mucilage in seed hydration during germination. Mutant *ttg1, gl2* and *myb61* reduced-mucilage seeds are more sensitive to low water potential than wild-type seeds, as determined by germination on increasing concentrations of PEG. *myb61* seeds, which have more mucilage than those of *ttg1* and *gl2*, had germination intermediate between wild type and the other two mutants (Penfield *et al.*, 2001). Arsovski *et al.* (2009b) demonstrated that seeds of the reduced mucilage mutant *mum4* had slow germination under non-water-limiting conditions, which was exacerbated in double mutants with those *men* genes that further decrease the amount of mucilage. Preliminary studies of imbibition of *mum4* and *men4 mum4* double mutants suggest that imbibition is slowed and/or reduced with decreasing mucilage production (A.A. Arsovski and T.L. Western, unpublished data). Mutants affected in mucilage release also have defective germination: *sbt1.7* and *dcr* mutants have reduced germination under low water potential conditions (PEG solutions), while *bxl1* and *mum1/luh* mutants have slow germination under non-water-limiting conditions (Rautengarten *et al.*, 2008; Sitaraman *et al.*, 2008; Arsovski *et al.*, 2009a; Panikashvili *et al.*, 2009). A slowing of *mum4* germination occurs also in double mutants for *men* genes proposed to be affected in mucilage release (Arsovski *et al.*, 2009b). Together these results suggest that both the amount of mucilage present and the ability of mucilage to expand and extrude from epidermal cells to surround the seed are important for seed hydration and subsequent germination.

Grubert, in his comprehensive survey of angiosperm myxospermy (1974), argues against mucilages as a water reservoir, citing his own and other experiments that demonstrate rapid water loss from mucilages. While there are cases, e.g. some desert plants (Kreitschitz, 2009), where germination may be rapid enough to occur before mucilage dehydration, it seems more likely from the above experiments that mucilage speeds initial seed hydration and/or hydration under mild water stress. Another germination-promoting role of seed mucilages was suggested by Huang *et al.* (2008). They propose that mucilages maintain sub-germination seed hydration from overnight dew to allow seed priming in desert species. DNA repair of embryos of two species of *Artemisia* occurs in intact achenes but not in those where mucilage was removed. Further, DNA repair was faster in *Artemisia sphaerocephala* (copious pericarp mucilage) than in *A. ordosica* (moderate pericarp mucilage) (Huang *et al.*, 2008). Garwood (1985), found no effect of mucilage on cuipo (*Cavanillesia plantanifolia*, Bombaceae) germination. Rather, the water retention of the seed mucilage appeared to increase the speed of seedling development and to prevent wilting. Removal of mucilage from seeds of *Dillenia indica* (Dilleniaceae) also has no effect on germination under non-water-limiting conditions. The authors suggest that the seed mucilage acts to clump seeds together to retain them in the fruit to escape excessive drying and predation prior to germination (Thapliyal *et al.*, 2008).

In contrast to a role in the promotion of germination, several studies suggest that mucilages can inhibit

germination. Seeds of *Diptychocarpus strictus*, *Lesquerella perforata* and *Lesquerella stonensis* (Brassicaceae) had increased germination when mucilage was removed (Fitch *et al.*, 2007; Lu *et al.*, 2010). Incubation of demucilaged *D. strictus* seeds with the isolated mucilage demonstrated that there was not an inhibitor in the mucilage, suggesting that the layer of gel around the seed was responsible for inhibition of germination (Lu *et al.*, 2010). Detailed studies of the mechanism of mucilage inhibition of germination have been performed for spinach (*Spinacia oleracea*, Amaranthaceae) and *B. persica* (Acanthaceae) seeds (Heydecker and Orphanos, 1968; Witztum *et al.*, 1969). Similar to *D. strictus*, tests for both species showed no soluble chemical inhibitor in the mucilage. Spinach seeds are surrounded by a mucilaginous fruit coat. Germination decreases with increasing amounts of water and can be rescued by removal of part of the fruit coat, removal of mucilage, low temperature (increased oxygen solubility in water) and treatment with oxidizing chemicals such as hydrogen peroxide (Heydecker and Orphanos, 1968). A similar effect was seen with seeds of *B. persica*, which have long mucilaginous hairs. In small amounts of water, the hairs expand separately, while under excess water the mucilage swells to fill the space between the hairs (Witztum *et al.*, 1969) and germination is prevented. Germination can be promoted by removing the seed coat, stripping mucilage from the seed, treatment with $CaCl_2$ (reducing mucilage swelling), low temperature and increasing the percentage of oxygen in the germination chamber (Witztum *et al.*, 1969). Together these suggest that mucilage inhibits germination under excessively moist conditions by preventing the diffusion of oxygen to the embryo.

Role of seed coat mucilages in seed dispersal

Hydrated pectinaceous mucilages are very sticky, leading to hypotheses of their role as adhesives for seeds to adhere to soil or to animal vectors (Grubert, 1974; Fahn, 1982; Kreitschitz, 2009). Grubert (1974) used binding of seeds to glass plates to assess the adhesive properties of almost 500 species across 20 families that varied in the amount of mucilage released, type of mucilage cells and mucilage structure. He found strong adhesion across all types of mucilage cells and hypothesized that the presence of cellulosic fibres and/or hair cells stabilized substrate binding. For seven species across six families, he determined precise forces for seed removal from the substrate and demonstrated substantial seed weight increase through adhesion of soil particles, especially to those seeds with mucilaginous hairs or highly soluble (mobile) mucilage (Grubert, 1974). Finally, he performed a time course on mucilage dehydration and found that all seven species returned to their dry weight within 2–7 h. Based on the high frequency of myxodiaspory in arid regions and his experimental results on seed adhesion and mucilage dehydration, Grubert proposed that the primary role of seed coat mucilage is fixation to the soil to prevent dispersal to less favourable habitats (antitelechory *sensu* Ellner and Shmida, 1981) (Grubert, 1974). He also noted that in more humid climates, myxodiaspory could be acting in dispersal through sticking to animals (exozoochory) (Grubert, 1974). Increased seed weight through adhesion of sand and soil to mucilage has been demonstrated for other species, including *D. strictus* and *Salvia columbariae* (Lamiaceae) (Fuller and Hay, 1983; Lu *et al.*, 2010). García-Fayos *et al.* (2010) investigated seed characteristics affecting seed removal from slopes by rainfall runoff. Using simulated rainfall on a sandpaper-covered incline, they tested 141 species from dry regions of Spain, including 29 species with mucilaginous diaspores. Overall they demonstrated that seeds with mucilage were generally small and had a lower susceptibility to removal by runoff compared with non-myxospermous species of similar mass. Adaptations to immobilize seeds to prevent dispersal (antitelechory) are found widely among desert species, and have been proposed to keep progeny with the mother plant where conditions are appropriate for growth, a scarce commodity in arid regions. Based on a long-term survey of desert flora in Israel, Ellner and Shmida (1981) proposed that desert conditions of open ground instead favour atelechory (lack of any dispersal mechanism). Thus, they conclude any antitelechoric characteristics of dispersal units of desert species are a side-effect of mechanisms with other adaptive significance. Further, they suggested that myxodiaspory is not a mechanism of antitelechory *per se*, since it acts on the seed after its initial dispersal from the mother plant.

In addition to the prevention of seed removal by abiotic forces, adhesion of the seed to soil, or soil to the seed, has been suggested to prevent predation – either by increasing the mass of the seed to prevent its removal by insects such as ants, or by camouflaging the seed from various predators (Young and Evans, 1973; Fuller and Hay, 1983; Gutterman and Shem-Tov, 1997). Fuller and Hay (1983) tested the response of various desert granivores to naked *S. columbariae* seeds versus those that had been wetted and coated in sand. Diurnal predators that hunt by sight, such as birds and ground squirrels, had a significant preference for uncoated seeds, while nocturnal rodents, which use scent to track food, had less difference in their choice. Ants seemed less likely to notice the covered seeds and, when they did, they had trouble moving the seeds that were approximately 11 times their uncoated weight, requiring several ants to move the seeds (Fuller and Hay, 1983).

https://doi.org/10.1017/S0960258511000249 Published online by Cambridge University Press

As mentioned above, the adhesive power of mucilage has also been suggested to aid seed dispersal by sticking to animal fur or bird feathers (Young and Evans, 1973; Grubert, 1974). The conclusion of Ryding's survey of Nepetoideae (2001) mentioned above was that the primary role of myxocarpy in the Nepetiodeae was epizoochory – or at least that there was no evidence in this case to support antitelechory. Lobova *et al*. (2003) described a potential role for mucilage in endozoochory in *Cecropia* species in French Guiana. They proposed that the slimy mucilage lubricates seeds to aid their passage through the gut of bats.

Conclusions and future perspectives

Myxodiaspory is a common adaptation in at least 100 families of angiosperms. A rich literature over the past 150 years exists on the presence and distribution of seed coat/pericarp mucilages (see Table 1 and the extensive reviews and literature lists in Grubert, 1974, 1981), though less attention has been paid to it in recent decades. A resurgence in interest, however, has occurred recently due to its study in the model genetic plant Arabidopsis, leading to a literature of over 46 papers since 2000. At least 44 genes have been identified with roles in mucilage production or MSC differentiation in Arabidopsis (Fig. 2F and Table 2), with many more under investigation. The MSCs of Arabidopsis are of particular interest in the field of cell wall biology, where they have become a model system for the identification and investigation of genes involved in pectin synthesis and post-deposition modification (reviewed in Arsovski *et al*., 2010). Genes involved in secondary cell wall production and mucilage secretion have also been identified recently (Kulich *et al*., 2010; Stork *et al*., 2010), suggesting further utility in other aspects of cell wall production. Despite considerable progress, many aspects of mucilage synthesis, secretion and MSC differentiation are still poorly understood, thus continued gene identification and investigation of their roles is required.

Comparisons between the recent detailed studies of mucilage composition and production in Arabidopsis and those performed in the past in other species have highlighted a number of commonalities. These include the primarily pectinaceous nature of seed coat mucilages, the frequent presence of dispersed cellulose microfibrils and cellulosic fibres or inclusions, and the hypersecretory activity of the Golgi apparatus during mucilage synthesis. While the chemical and/or physical properties have been determined in detail for a number of mucilages of economically important species, e.g. mustard (*S. alba*), linseed flax (*L. usitatissimum*) and psyllium (*Plantago* spp.), Arabidopsis studies have linked chemical composition with structural domains within extruded mucilage (e.g. Macquet *et al*., 2007a; Harpaz-Saad *et al*., 2011; Sullivan *et al*., 2011). A layer of diffuse mucilage primarily comprised of unbranched RG I is dispersed beyond a dense layer of complex pectic and cellulosic composition that appears to be linked to the seed. The increased solubility and swelling of the outer mucilage layer may be the driver of mucilage expansion for cell wall breakage, while the inner layer acts in seed hydration (or adhesion, see below). The differential extractability of these layers means that most quantitative information on the chemical composition of Arabidopsis mucilage only reflects the outer soluble mucilage, which may also be true for other species where compositional studies were performed with mild extraction procedures. Work in Arabidopsis has also revealed the complex modifications of apoplastic mucilage and/or the outer primary cell wall that are required for mucilage to be released upon seed hydration. These include both trimming of pectin side-chains and the regulation of pectin gel status through the degree of methylesterification of HG (Dean *et al*., 2007; Macquet *et al*., 2007b; Rautengarten *et al*., 2008; Arsovski *et al*., 2009a; Bui *et al*., 2011; Huang *et al*., 2011; Walker *et al*., 2011).

Despite the obvious selection for myxodiaspory in many species, its precise ecological role is still unclear. As suggested by the studies described in this review, it may have a number of roles in germination and dispersal, depending on the species and its environmental context. However, as described here and in the recent review by Kreitschitz (2009), the number of well-controlled studies performed on the role of mucilages is limited and, generally, they test only one aspect of mucilage function. While some Arabidopsis mutants affected in mucilage production and its release to surround the seed have been tested for their effects on germination, more detailed studies comparing a range of mucilage defects directly with each other under more conditions are needed. Further studies in other species, both in controlled conditions and in their ecological contexts, are also necessary.

Acknowledgements

My apologies to those researchers whose studies I was unable to include in this review due to space considerations. I would like to thank Dr Derek Bewley, Dr Andrej Arsovski, Dr Uday Divi, Bronwen Forward and Cheng-En Tsai for helpful comments on the manuscript, Dr Thomas Bureau and Dr George Haughn for seeds of *Aethionema arabicum* and *Salvia hispanica*, respectively, and the Natural Sciences and Engineering Research Council of Canada for Discovery Grant funding.

References

Abeysekera, R.M. and Willison, J.H.M. (1988) Development of helicoidal texture in the prerelease mucilage of quince (*Cydonia oblonga*) seed epidermis. *Canadian Journal of Botany* **66**, 460–467.

Anderson, E. and Fireman, M. (1935) The mucilage from psyllium seed, *Plantago psyllium* L. *Journal of Biological Chemistry* **109**, 437–442.

Anderson, E. and Lowe, H.J. (1947) The composition of flaxseed mucilage. *Journal of Biological Chemistry* **168**, 289–297.

Anjaneyalu, Y., Khan, M.-R. and Tharanathan, R.N. (1983) An acidic xylan from the capsular polysaccharide complex of *Ocimum gratissimum* seeds. *Carbohydrate Research* **116**, 83–88.

Anjaneyalu, Y., Khan, M.-R. and Tharanathan, R.N. (1984) An acidic polysaccharide from the seeds of *Ocimum adscendens*. *Phytochemistry* **23**, 2243–2245.

Arsovski, A.A., Popma, T.M., Haughn, G.W., Carpita, N.C., McCann, M.C. and Western, T.L. (2009a) *AtBXL1* encodes a bifunctional β-D-xylosidase/ α-L-arabinofuranosidase required for pectic arabinan modification in Arabidopsis mucilage secretory cells. *Plant Physiology* **150**, 1219–1234.

Arsovski, A.A., Villota, M.M., Rowland, O., Subramaniam, R. and Western, T.L. (2009b) *MUM ENHANCERS* are important for seed coat mucilage production and mucilage secretory cell differentiation in *Arabidopsis thaliana*. *Journal of Experimental Botany* **60**, 2601–2612.

Arsovski, A.A., Haughn, G.W. and Western, T.L. (2010) Seed coat mucilage cells of *Arabidopsis thaliana* as a model for plant cell wall research. *Plant Signaling and Behavior* **5**, 1–6.

Beeckman, T., De Rycke, R., Viane, R. and Inzé, D. (2000) Histological study of seed coat development in *Arabidopsis thaliana*. *Journal of Plant Research* **113**, 139–148.

Boesewinkel, F.D. (1980) Development of ovule and testa of *Linum usitatissimum* L. *Acta Botanica Neerlandia* **29**, 17–32.

Boesewinkel, F.D. and Bouman, F. (1984) The seed: structure. pp. 567–610 *in* Johri, B.M. (Ed.) *Embryology of angiosperms*. New York, NY, Springer-Verlag.

Bouman, F. (1975) Integument initiation and testa development in some Cruciferae. *Botanical Journal of the Linnean Society* **70**, 213–229.

Bowman, J.L. and Koornneef, M. (1994) Seed morphology. pp. 398–401 *in* Bowman, J.L. (Ed.) *Arabidopsis: an atlas of morphology and development*. New York, NY, Springer-Verlag.

Bui, M., Lim, N., Sijacic, P. and Liu, Z. (2011) LEUNIG_HOMOLOG and LEUNIG regulate seed mucilage extrusion in Arabidopsis. *Journal of Integrative Plant Biology* **53**, 399–408.

Burn, J.E., Hurley, U.A., Birch, R.J., Arioli, T., Cork, A. and Williamson, R.E. (2002) The cellulose-deficient *Arabidopsis* mutant *rsw3* is defective in a gene encoding a putative glucosidase II, an enzyme processing N-glycans during ER quality control. *Plant Journal* **32**, 949–960.

Bushway, A., Belyea, P. and Bushway, R. (1981) Chia seed as a source of oil, polysaccharide, and protein. *Journal of Food Science* **46**, 1349–1356.

Buth, G.M. and Ara, R. (1981) Seed coat anatomy of some cultivated Brassicas. *Phytomorphology* **31**, 69–78.

Caffall, K.H. and Mohnen, D. (2009) The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. *Carbohydrate Research* **344**, 1879–1900.

Caffall, K.H., Pattathil, S., Phillips, S.E., Hahn, M.G. and Mohnen, D. (2009) *Arabidopsis thaliana* T-DNA mutants implicate *GAUT* genes in the biosynthesis of pectin and xylan in cell walls and seed testa. *Molecular Plant* **2**, 1000–1014.

Campanoni, P. and Blatt, M.R. (2007) Membrane trafficking and polar growth in root hairs and pollen tubes. *Journal of Experimental Botany* **58**, 65–74.

Cole, R. and Fowler, J. (2006) Polarized growth: maintaining focus on the tip. *Current Opinion in Plant Biology* **9**, 579–588.

Cui, W., Eskin, N.A.M. and Biliaderis, C.G. (1993) Chemical and physical properties of yellow mustard (*Sinapis alba* L.) mucilage. *Food Chemistry* **46**, 169–176.

Cui, W., Eskin, N.A.M. and Biliaderis, C.G. (1994a) Fractionation, structural analysis, and rheological properties of water-soluble yellow mustard (*Sinapis alba* L.) polysaccharides. *Journal of Agricultural and Food Chemistry* **42**, 657–664.

Cui, W., Mazza, G. and Biliaderis, C.G. (1994b) Chemical structure, molecular size distributions, and rheological properties of flaxseed gum. *Journal of Agricultural and Food Chemistry* **42**, 1891–1895.

Dean, G.H., Zheng, H., Tewari, J., Huang, J., Young, D.S., Hwang, Y.T., Western, T.L., Carpita, N.C., McCann, M.C., Mansfield, S.D. and Haughn, G.W. (2007) The Arabidopsis *MUM2* gene encodes a β-galactosidase required for the production of seed coat mucilage with correct hydration properties. *Plant Cell* **19**, 4007–4021.

Debeaujon, I., Léon-Kloosterziel, K.M. and Koornneef, M. (2000) Influence of the testa on seed dormancy, germination, and longevity in Arabidopsis. *Plant Physiology* **122**, 403–413.

Diederichsen, A., Raney, J.P. and Duguid, S.D. (2006) Variation of mucilage in flax seed and its relationship with other seed characters. *Crop Science* **46**, 365.

Duletiae-Lauševiae, S. and Marin, P.D. (1998) Pericarp structure and myxocarpy in selected genera of Nepetoideae (Lamiaceae). *Nordic Journal of Botany* **19**, 435–446.

Edwards, M.M. (1968) Dormancy in seeds of charlock I. Developmental anatomy of the seed. *Journal of Experimental Botany* **19**, 575–582.

Ellner, S. and Shmida, A. (1981) Why are adaptations for long-range seed dispersal rare in desert annual plants? *Oecologia* **51**, 133–144.

Esau, K. (1977) *Anatomy of seed plants* (2nd edition). Toronto, Wiley.

Eskin, N.A.M. (1992) Effect of variety and geographical location on the incidence of mucilage in canola seeds. *Canadian Journal of Plant Science* **72**, 1223–1225.

Fahn, A. (1979) *Secretory tissues in plants*. New York, NY, Academic Press.

Fahn, A. (1982) *Plant anatomy* (3rd edition). New York, NY, Pergamon Press.

Fedeniuk, R.W. and Biliaderis, C.G. (1994) Composition and physicochemical properties of linseed (*Linum usitatissimum* L.) mucilage. *Journal of Agricultural and Food Chemistry* **42**, 240–247.

Fitch, E.A., Walck, J.L. and Hidayati, S.N. (2007) Germinating seeds of *Lesquerella perforata* and *stonensis*: substrate

effects and mucilage production. *Native Plants Journal* **8**, 4–10.

Frey-Wyssling, A. (1976) *The plant cell wall* (3rd edition). Berlin, Gebr. Borntraeger.

Fuller, P.J. and Hay, M.E. (1983) Is glue production by seeds of *Salvia columbariae* a deterrent to desert granivores? *Ecology* **64**, 960–963.

García-Fayos, P., Bochet, E. and Cerdà, A. (2010) Seed removal susceptibility through soil erosion shapes vegetation composition. *Plant and Soil* **334**, 289–297.

Garwood, N.C. (1985) The role of mucilage in the germination of cuipo, *Cavanillesia platanifolia* (H. & B.) H.B.K. (Bombacaceae), a tropical tree. *American Journal of Botany* **72**, 1095–1105.

Gonzalez, A., Mendenhall, J., Huo, Y. and Lloyd, A. (2009) TTG1 complex MYBs, MYB5 and TT2, control outer seed coat differentiation. *Developmental Biology* **325**, 412–421.

Goto, N. (1985) A mucilage polysaccharide secreted from testa of *Arabidopsis thaliana*. *Arabidopsis Information Service* **22**, 143–145.

Gowda, D.C. (1984) Polysaccharide components of the seed-coat mucilage from *Hyptis suaveolens*. *Phytochemistry* **23**, 337–338.

Grant, G.T., McNab, C., Rees, D.A. and Skerrett, R.J. (1969) Seed mucilages as examples of polysaccharide denaturation. *Journal of the Chemical Society D: Chemical Communications*, 805–806.

Grubert, M. (1974) Studies on the distribution of myxospermy among seeds and fruits of Angiospermae and its ecological importance. *Acta Biologica Venezuelica* **8**, 315–551.

Grubert, M. (1981) *Mucilage or gum in seeds and fruits of angiosperms: a review.* Munich, Minerva Press.

Guo, Q., Cui, S.W., Wang, Q., Goff, H.D. and Smith, A. (2009) Microstructure and rheological properties of psyllium polysaccharide gel. *Food Hydrocolloids* **23**, 1542–1547.

Gutterman, Y. and Shem-Tov, S. (1997) Mucilaginous seed coat structure of *Carrichtera annua* and *Anastatica heirochuntica* from the Negev Desert highlands of Israel, and its adhesion to the soil crust. *Journal of Arid Environment* **35**, 695–705.

Gutterman, Y., Witztum, A. and Heydecker, W. (1973) Studies of the surfaces of desert plant seeds. II. Ecological adaptations of the seeds of *Blepharis persica*. *Annals of Botany* **37**, 1051–1055.

Hamilton, K.N., Ashmore, S.E., Drew, R.A. and Pritchard, H.W. (2007) Seed morphology and ultrastructure in *Citrus garrawayi* (Rutaceae) in relation to germinability. *Australian Journal of Botany* **55**, 618–627.

Harpaz-Saad, S., McFarlane, H.E., Xu, S., Divi, U.K., Forward, B., Western, T.L. and Kieber, J.J. (2011) Cellulose synthesis via the FEI2 RLK/SOS5 pathway and CELLULOSE SYNTHASE 5 is required for the structure of seed coat mucilage in Arabidopsis. *Plant Journal* DOI: 10.1111/j.1365-313X.2011.04760.x.

Harper, J. and Benton, R. (1966) The behaviour of seeds in soil. II. The germination of seeds on the suface of a water supplying substrate. *Journal of Ecology* **54**, 151–166.

Heydecker, W. and Orphanos, P. (1968) The effect of excess moisture on the germination of *Spinacia oleracea* L. *Planta* **83**, 237–247.

Hirst, E.L., Rees, D.A. and Richardson, N.G. (1965) Seed polysaccharides and their role in germination. *Biochemical Journal* **95**, 453–458.

Hoth, S., Morgante, M., Sanchez, J.-P., Hanafey, M.K., Tingey, S.V. and Chua, N.-H. (2002) Genome-wide gene expression profiling in *Arabidopsis thaliana* reveals new targets of abscisic acid and largely impaired gene regulation in the *abi1-1* mutant. *Journal of Cell Science* **115**, 4891–4900.

Hsiao, Y.-C. and Chuang, T. (1981) Seed-coat morphology and anatomy in *Collomia* (Polemoniaceae). *American Journal of Botany* **68**, 1155–1164.

Huang, J., Bowles, D., Esfandiari, E., Dean, G., Carpita, N.C. and Haughn, G.W. (2011) The Arabidopsis transcription factor LUH/MUM1 is required for extrusion of seed coat mucilage. *Plant Physiology* **156**, 491–502.

Huang, Z.Y. and Gutterman, Y. (1999) Water absorption by mucilaginous achenes of *Artemisia monosperma*: floating and germination as affected by salt concentrations. *Israel Journal of Plant Sciences* **47**, 27–34.

Huang, Z., Boubriak, I., Osborne, D.J., Dong, M. and Gutterman, Y. (2008) Possible role of pectin-containing mucilage and dew in repairing embryo DNA of seeds adapted to desert conditions. *Annals of Botany* **101**, 277–283.

Hyde, B.B. (1970) Mucilage-producing cells in the seed coat of *Plantago ovata*: developmental fine structure. *American Journal of Botany* **57**, 1197–1206.

Ibrahim, N., El-Eraky, W., El-Gengaihi, S. and Shalaby, A. (1997) Chemical and biological evaluation of proteins and mucilages from roots and seeds of *Glossostemon bruguieri* Desf. (Moghat). *Plant Foods for Human Nutrition* **50**, 55–61.

Iglesias-Fernández, R., Matilla, A.J., Pulgar, I. and de la Torre, F. (2007) Ripe fruits of *Sisymbrium officinale* L. contain heterogeneous endospermic seeds with different germination rates. *Seed Science and Biotechnology* **1**, 18–24.

Inceer, H. (2011) Achene slime content in some taxa of *Matricaria* L. (Asteraceae). *Acta Botanica Croatica* **70**, 109–114.

Iuchi, S., Suzuki, H., Kim, Y.-C., Iuchi, A., Kuromori, T., Ueguchi-Tanaka, M., Asami, T., Yamaguchi, I., Matsuoka, M., Kobayashi, M. and Nakajima, M. (2007) Multiple loss-of-function of Arabidopsis gibberellin receptor AtGID1s completely shuts down a gibberellin signal. *Plant Journal* **50**, 958–966.

Jofuku, K.D., den Boer, B.G.W., Montagu, M.V. and Okamuro, J.K. (1994) Control of Arabidopsis flower and seed development by the homeotic gene *APETALA2*. *Plant Cell* **6**, 1211–1225.

Johnson, C.S., Kolevski, B. and Smyth, D.R. (2002) *TRANSPARENT TESTA GLABRA2*, a trichome and seed coat development gene of Arabidopsis, encodes a WRKY transcription factor. *Plant Cell* **14**, 1359–1375.

Jordaan, A. (2008) Ultrastructure and histochemistry of seeds of *Colophospermum mopane* during imbibition. *South African Journal of Botany* **74**, 591–597.

Jurgens, G. (2005) Cytokinesis in higher plants. *Annual Review Plant Biology* **56**, 281–299.

Karssen, C.M., Brinkhorst-van der Swan, D.L.C., Breekland, A.E. and Koornneef, M. (1983) Induction of dormancy during seed development by endogenous

abscisic acid: studies on abscisic acid deficient genotypes of *Arabidopsis thaliana* (L.) Heynh. *Planta* **157**, 158–165.

Khan, M.-R., Salimath, P., Anjaneyalu, Y. and Tharanathan, R.N. (1987) Structural features of an arabinogalactan from the seeds of *Becium filamentosum*. *Phytochemistry* **26**, 1197–1198.

Kim, Y.-C., Nakajima, M., Nakayama, A. and Yamaguchi, I. (2005) Contribution of gibberellins to the formation of *Arabidopsis* seed coat through starch degradation. *Plant and Cell Physiology* **46**, 1317–1325.

Kong, Y., Zhou, G., Yin, Y., Xu, Y., Pattathil, S. and Hahn, M.G. (2011) Molecular analysis of a family of Arabidopsis genes related to galacturonosyltransferases. *Plant Physiology* **155**, 1791–1805.

Koornneef, M. (1981) The complex syndrome of *ttg* mutants. *Arabidopsis Information Service* **18**, 45–51.

Kraemer, H. (1898) Origin and detection of mucilage in plants. *American Journal of Pharmacy* **70**.

Kreitschitz, A. (2009) Biological properties of fruit and seed slime envelope: how to live, fly, and not die. pp. 11–30 *in* Gorb, S.N. (Ed.) *Functional surfaces in biology*. New York, NY, Springer Science.

Kreitschitz, A. and Vàlles, J. (2007) Achene morphology and slime structure in some taxa of *Artemisia* L. and *Neopallasia* L. (Asteraceae). *Flora* **202**, 570–580.

Kreitschitz, A., Tadele, Z. and Gola, E.M. (2009) Slime cells on the surface of *Eragrostis* seeds maintain a level of moisture around the grain to enhance germination. *Seed Science Research* **19**, 27–35.

Kulich, I., Cole, R., Drdová, E., Cvrčková, F., Soukup, A., Fowler, J. and Žárský, V. (2010) Arabidopsis exocyst subunits *SEC8* and *EXO70A1* and exocyst interactor *ROH1* are involved in the localized deposition of seed coat pectin. *New Phytologist* **188**, 615–625.

Kunieda, T., Mitsuda, N., Ohme-Takagi, M., Takeda, S., Aida, M., Tasaka, M., Kondo, M., Nishimura, M. and Hara-Nishimura, I. (2008) NAC Family proteins NARS1/NAC2 and NARS2/NAM in the outer integument regulate embryogenesis in *Arabidopsis*. *The Plant Cell* **20**, 2631–2642.

Léon-Kloosterziel, K.M., Keijzer, C.J. and Koornneef, M. (1994) A seed shape mutant of Arabidopsis that is affected in integument development. *Plant Cell* **6**, 685–692.

Lerouxel, O., Cavalier, D., Liepman, A. and Keegstra, K. (2006) Biosynthesis of plant cell wall polysaccharides – a complex process. *Current Opinion in Plant Biology* **9**, 621–630.

Li, S.F., Milliken, O.N., Pham, H., Seyit, R., Napoli, R., Preston, J., Koltunow, A.M. and Parish, R.W. (2009) The *Arabidopsis* MYB5 transcription factor regulates mucilage synthesis, seed coat development, and trichome morphogenesis. *Plant Cell* **21**, 72–89.

Lobova, T.A., Mori, S.A., Blanchard, F., Peckham, H. and Charles-Dominique, P. (2003) *Cecropia* as a food resource for bats in French Guiana and the significance of fruit structure in seed dispersal and longevity. *American Journal of Botany* **90**, 388–403.

Lu, J., Tan, D., Baskin, J.M. and Baskin, C.C. (2010) Fruit and seed heteromorphism in the cold desert annual ephemeral *Diptychocarpus strictus* (Brassicaceae) and possible adaptive significance. *Annals of Botany* **105**, 999–1014.

Macquet, A., Ralet, M.C., Kronenberger, J., Marion-Poll, A. and North, H.M. (2007a) In situ, chemical and macromolecular study of the composition of *Arabidopsis thaliana* seed coat mucilage. *Plant and Cell Physiology* **48**, 984–999.

Macquet, A., Ralet, M.C., Loudet, O., Kronenberger, J., Mouille, G., Marion-Poll, A. and North, H.M. (2007b) A naturally occurring mutation in an Arabidopsis accession affects a β-D-galactosidase that increases the hydrophilic potential of rhamnogalacturonan I in seed mucilage. *Plant Cell* **19**, 3990–4006.

McAbee, J.M., Hill, T.A., Skinner, D.J., Izhaki, A., Hauser, B.A., Meister, R.J., Venugopala Reddy, G., Meyerowitz, E.M., Bowman, J.L. and Gasser, C.S. (2006) *ABERRANT TESTA SHAPE* encodes a KANADI family member, linking polarity determination to separation and growth of Arabidopsis ovule integuments. *Plant Journal* **46**, 522–531.

McFarlane, H.E., Young, R.E., Wasteneys, G.O. and Samuels, A.L. (2008) Cortical microtubules mark the mucilage secretion domain of the plasma membrane in Arabidopsis seed coat cells. *Planta* **227**, 1363–1375.

Mendu, V., Griffiths, J., Persson, S., Stork, J., Downie, B., Voiniciuc, C., Haughn, G. and DeBolt, S. (2011) Subfunctionalization of cellulose synthases in seed coat epidermal cells mediate secondary radial wall synthesis and mucilage attachment. *Plant Physiology* **157**, 441–453.

Moffatt, B.A., Stevens, Y.Y., Allen, M.S., Snider, J.D., Pereira, L.A., Todorova, M.I., Summers, P.S., Weretilnyk, E.A., Martin-McCaffrey, L. and Wagner, C. (2002) Adenosine kinase deficiency is associated with developmental abnormalities and reduced transmethylation. *Plant Physiology* **128**, 812–821.

Molina, I., Ohlrogge, J.B. and Pollard, M. (2008) Deposition and localization of lipid polyester in developing seeds of *Brassica napus* and *Arabidopsis thaliana*. *Plant Journal* **53**, 437–449.

Mott, J. (1974) Factors affecting seed germination in three annual species from an arid region of Western Australia. *Journal of Ecology* **62**, 699–709.

Mühlethaler, K. (1950) The structure of plant slimes. *Experimental Cell Research* **1**, 341–350.

Müller, K., Tintelnot, S. and Leubner-Metzger, G. (2006) Endosperm-limited Brassicaceae seed germination: abscisic acid inhibits embryo-induced endosperm weakening of *Lepidium stativum* (cress) and endosperm rupture of cress and *Arabidopsis thaliana*. *Plant & Cell Physiology* **47**, 864–877.

Muralikrishna, G., Salimath, P. and Tharanathan, R.N. (1987) Structural features of an arabinoxylan and a rhamnogalacturonan derived from linseed mucilage. *Carbohydrate Research* **161**, 265–271.

Naran, R., Chen, G. and Carpita, N.C. (2008) Novel rhamnogalacturonan I and arabinoxylan polysaccharides of flax seed mucilage. *Plant Physiology* **148**, 132–141.

Oka, T., Nemoto, T. and Jigami, Y. (2007) Functional analysis of *Arabidopsis thaliana* RHM2/MUM4, a multidomain protein involved in UDP-D-glucose to UDP-L-rhamnose conversion. *Journal of Biological Chemistry* **282**, 5389–5403.

Oomah, D.B., Kenaschuk, E.O., Cui, W. and Mazza, G. (1995) Variation in the composition of water-soluble polysaccharides in flaxseed. *Journal of Agricultural and Food Chemistry* **43**, 1484–1488.

Panigrahi, S.G. (1986) Seed morphology of *Rotala* L., *Ammannia* L., *Nesaea* Kunth and *Hionanthera* Fernandes & Diniz (Lythraceae). *Botanical Journal of the Linnean Society* **93**, 389–403.

Panikashvili, D., Shi, J.X., Schreiber, L. and Aharoni, A. (2009) The Arabidopsis *DCR* encoding a soluble BAHD acyltransferase is required for cutin polyester formation and seed hydration properties. *Plant Physiology* **151**, 1773–1789.

Penfield, S., Meissner, R.C., Shoue, D.A., Carpita, N.C. and Bevan, M.W. (2001) *MYB61* is required for mucilage deposition and extrusion in the Arabidopsis seed coat. *Plant Cell* **13**, 2777–2791.

Rautengarten, C., Usadel, B., Neumetzler, L., Hartmann, J., Büssis, D. and Altmann, T. (2008) A subtilisin-like serine protease essential for mucilage release from Arabidopsis seed coats. *Plant Journal* **54**, 466–480.

Razavi, S.M.A., Mortazavi, S.A., Matia-Merino, L., Hosseini-Parvar, S.H., Motamedzadegan, A. and Khanipour, E. (2009) Optimisation study of gum extraction from basil seeds (*Ocimum basilicum* L.). *International Journal of Food Science and Technology* **44**, 1755–1762.

Rerie, W.G., Feldmann, K.A. and Marks, D.M. (1994) The *GLABRA2* gene encodes a homeo domain protein required for normal trichome development in *Arabidopsis*. *Genes and Development* **8**, 1388–1399.

Ryding, O. (2001) Myxocarpy in the Nepetoideae (Lamiaceae) with notes on mxyodiaspory in general. *Systematics and Geography of Plants* **71**, 502–514.

Schnepf, E. and Deichgräber, G. (1983a) Structure and formation of fibrillar mucilage in seed epidermis cells. I. *Collomia grandiflora* (*Polemoniaceae*). *Protoplasma* **114**, 210–221.

Schnepf, E. and Deichgräber, G. (1983b) Structure and formation of fibrillar mucilage in seed epidermis cells. II. *Ruellia* (*Acanthaceae*). *Protoplasma* **114**, 222–234.

Sharma, P. and Koul, A. (1986) Mucilage in seeds of *Plantago ovata* and its wild allies. *Journal of Ethnopharmacology* **17**, 289–295.

Siddiqui, I.R., Yiu, S.H., Jones, J.D. and Kalab, M. (1986) Mucilage in yellow mustard (*Brassica hirta*) seeds. *Food Microstructure* **5**, 157–162.

Sitaraman, J., Bui, M. and Liu, Z. (2008) *LEUNIG_HOMOLOG* and *LEUNIG* perform partially redundant functions during Arabidopsis embryo and floral development. *Plant Physiology* **147**, 672–681.

Srinivas, M., Joshi, S. and Krishnan, R. (1998) Ontogeny of solasodine-containing mucilage layer in *Solanum viarum* Dunal, ploidy types. *Journal of Biosciences* **23**, 155–162.

Stork, J., Harris, D., Griffiths, J., Williams, B., Beisson, F., Li-Beisson, Y., Mendu, V., Haughn, G. and DeBolt, S. (2010) CELLULOSE SYNTHASE9 serves a nonredundant role in secondary cell wall synthesis in Arabidopsis epidermal testa cells. *Plant Physiology* **153**, 580–589.

Sullivan, S., Ralet, M.C., Berger, A., Diatloff, E., Bischoff, V., Gonneau, M., Marion-Poll, A. and North, H.M. (2011) CESA5 is required for the synthesis of cellulose with a role in structuring the adherent mucilage of Arabidopsis seeds. *Plant Physiology* **156**, 1725–1739.

Swarbrick, J. (1971) External mucilage production by seeds of British plants. *Botanical Journal of the Linnean Society* **64**, 157–162.

Thapliyal, R., Phartyal, S., Baskin, J.M. and Baskin, C.C. (2008) Role of mucilage in germination of *Dillenia indica* (Dilleniaceae) seeds. *Australian Journal of Botany* **56**, 583–589.

Tomoda, M., Yokoi, M. and Ishikawa, K. (1981) Plant mucilages XXIX. Isolation and characterization of a mucous polysaccharide, 'Plantago-mucilage A' from the seeds of *Plantago major* var. *asiatica*. *Chemical and Pharmaceutical Bulletin* **29**, 2877–2884.

Usadel, B., Kuschinsky, A.M., Rosso, M.G., Eckermann, N. and Pauly, M. (2004) RHM2 is involved in mucilage pectin synthesis and is required for the development of the seed coat in Arabidopsis. *Plant Physiology* **134**, 286–295.

Van Caeseele, L., Mills, J.T., Sumner, M. and Gillespie, R. (1981) Cytology of mucilage production in the seed coat of Candle canola (*Brassica campestris*). *Canadian Journal of Botany* **59**, 292–300.

Van Caeseele, L., Kovacs, M.I.P. and Gillespie, R. (1987) Netural sugar analysis of polysaccharides from the seed epidermis of *Brassical campestris*. *Journal of the American Oil Chemists' Society* **64**, 761–762.

Vaughan, J.G. and Whitehouse, J.M. (1971) Seed structure and the taxonomy of the Cruciferae. *Botanical Journal of the Linnean Society* **64**, 383–409.

Vose, J.R. (1974) Chemical and physical studies of mustard and rapeseed Coats. *Cereal Chemistry* **51**, 658–665.

Walker, A.R., Davison, P.A., Bolognesi-Winfield, A.C., James, C.M., Srinivasan, N., Blundell, T.L., Esch, J.J., Marks, D.M. and Gray, J.C. (1999) The *TRANSPARENT TESTA GLABRA1* locus, which regulates trichome differentiation and anthocyanin biosynthesis in Arabidopsis, encodes a WD40 repeat protein. *Plant Cell* **11**, 1337–1349.

Walker, M., Tehseen, M., Doblin, M.S., Pettolino, F.A., Wilson, S.M., Bacic, A. and Golz, J.F. (2011) The transcriptional regulator LEUNIG_HOMOLOG regulates mucilage release from the Arabidopsis testa. *Plant Physiology* **156**, 46–60.

Warrand, J., Michaud, P., Picton, L., Muller, G., Courtois, B., Ralainirina, R. and Courtois, J. (2003) Large-scale purification of water-soluble polysaccharides from flaxseed mucilage, and isolation of a new anionic polymer. *Chromatographia* **58**, 331–335.

Western, T.L. (2006) Changing spaces: the *Arabidopsis* mucilage secretory cells as a novel system to dissect cell wall production in differentiating cells. *Canadian Journal of Botany* **84**, 622–630.

Western, T.L., Skinner, D.J. and Haughn, G.W. (2000) Differentiation of mucilage secretory cells of the Arabidopsis seed coat. *Plant Physiology* **122**, 345–355.

Western, T.L., Burn, J., Tan, W.L., Skinner, D.J., Martin-McCaffrey, L., Moffatt, B.A. and Haughn, G.W. (2001) Isolation and characterization of mutants defective in seed coat mucilage secretory cell development in Arabidopsis. *Plant Physiology* **127**, 998–1011.

Western, T.L., Young, D.S., Dean, G.H., Tan, W.L., Samuels, A.L. and Haughn, G.W. (2004) *MUCILAGE-MODIFIED4* encodes a putative pectin biosynthetic enzyme developmentally regulated by *APETALA2, TRANSPARENT TESTA GLABRA1,* and *GLABRA2* in the Arabidopsis seed coat. *Plant Physiology* **134**, 296–306.

Willats, W.G.T., McCartney, L. and Knox, J.P. (2001) In-situ analysis of pectic polysaccharides in seed mucilage and at the root surface of *Arabidopsis thaliana*. *Planta* **213**, 37–44.

Windsor, J.B., Symonds, V.V., Mendenhall, J. and Lloyd, A.L. (2000) *Arabidopsis* seed coat development: morphological differentiation of the outer integument. *Plant Journal* **22**, 483–493.

Witztum, A., Gutterman, Y. and Evenari, M. (1969) Integumentary mucilage as an oxygen barrier during germination of *Blepharis persica* (Burm.) Kuntze. *Botanical Gazette* **130**, 238–241.

Woods, D. and Downey, R. (1980) Mucilage from yellow mustard. *Canadian Journal of Plant Science* **60**, 1031–1033.

Wu, Y., Cui, W., Eskin, N.A.M. and Goff, H.D. (2009) Fractionation and partial characterization of non-pectic polysaccharides from yellow mustard mucilage. *Food Hydrocolloids* **23**, 1535–1541.

Xu, S.L., Rahman, A., Baskin, T.I. and Kieber, J.J. (2008) Two leucine-rich repeat receptor kinases mediate signaling, linking cell wall biosynthesis and ACC synthase in Arabidopsis. *Plant Cell* **20**, 3065–3079.

Yang, X., Dong, M. and Huang, Z. (2010) Role of mucilage in the germination of *Artemisia sphaerocephala* (Asteraceae) achenes exposed to osmotic stress and salinity. *Plant Physiology and Biochemistry* **48**, 131–135.

Young, J.A. and Evans, R.A. (1973) Mucilaginous seed coats. *Weed Science* **21**, 52–54.

Young, J.A., Evans, R.A., Gifford, R.O. and Eckert, R.E.J. Jr (1970) Germination characteristics of three species of *Cruciferae*. *Weed Science* **18**, 41–48.

Young, R.E., McFarlane, H.E., Hahn, M.G., Western, T.L., Haughn, G.W. and Samuels, A.L. (2008) Analysis of the Golgi apparatus in Arabidopsis seed coat cells during polarized secretion of pectin-rich mucilage. *Plant Cell* **20**, 1623–1638.

Zeng, C.-L., Wang, J.-B., Liu, A.-H. and Wu, X.-M. (2004) Seed coat microsculpturing changes during seed development in diploid and amphidiploid *Brassica* species. *Annals of Botany* **93**, 555–566.

Zhang, F., Gonzalez, A., Zhao, M., Payne, C.T. and Lloyd, A. (2003) A network of redundant bHLH proteins functions in all TTG1-dependent pathways of Arabidopsis. *Development* **130**, 4859–4869.

Zhang, Y., Liu, C.-M., Emons, A.-M.C. and Ketelaar, T. (2010) The plant exocyst. *Journal of Integrative Plant Biology* **52**, 138–146.

Zohuriaan-Mehr, M.J. and Kabiri, K. (2008) Superabsorbent polymer materials: a review. *Iranian Polymer Journal* **17**, 415–477.

Zwieniecki, M.A., Melcher, P.J. and Holbrook, N.M. (2001) Hydrogel control of xylem hydraulic resistance in plants. *Science* **291**, 1059–1062.

https://doi.org/10.1017/S0960258511000249 Published online by Cambridge University Press