Khajeh-Hosseini, M. and Mostashar-Shahidi, T. (2016), *Seed Sci. & Technol.,* **44**, 435-440.
http://doi.org/10.15258/sst.2016.44.2.17

Research Note

Role of mucilage in germination of fourteen species of medicinal plants

M. KHAJEH-HOSSEINI AND T. MOSTASHAR-SHAHIDI

Department of Crop Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
(E-mail: saleh@ferdowsi.um.ac.ir)

(Accepted April 2016)

Abstract

The role of mucilage in the germination of 14 species of medicinal plants from six families was investigated. Germination tests were conducted using four replications of 25 seeds of each species in Petri dishes lined with filter paper and premoistened with either distilled water or experimental solutions. For seven species that showed germination below 80% in water, scarification, prechilling, KNO_3 and GA_3 were applied on both intact and demucilaged seeds to investigate the possible role of mucilage in controlling seed dormancy. Removing the mucilage increased both the speed and final germination. All dormancy-breaking treatments improved the germination of both intact and demucilaged seeds, though GA_3 was the most effective, indicating the possibility of physiological dormancy.

Experimental and discussion

Seeds of some medicinal plant species produce mucilage which have specific chemical characteristics that are used in pharmaceutical industries such as construction of adhesive granules and laxatives (Mirmasoomi, 1992). They are also used in the food industry as thickening and gelling agents (Koocheki *et al.*, 2009).

The mucilage is thought to play an important role in maintaining seed viability and ensuring seed germination by either protecting the seed against drying during germination (Garwood, 1985) and increasing the seed-soil contact area (Grubert, 1974), or preventing further dispersal of the seed by rain and wind (Huang *et al*., 2004). In addition, mucilage formed by wetting with dew at night or a small amount of rain enables the embryo to repair DNA and thus help maintain its viability under harsh desert conditions. Therefore, development of mucilage seems to be one of the evolutionary adaptations of plants to desert environments (Huang *et al*., 2008). This is by either regulating seed water relations and aiding germination in osmotically stressful habitats of the cold desert environment (Yang *et al*., 2010) or by controlling seed dormancy to regulate the germination and

emergence when sufficient rain is available to complete the plant life cycle. The objective of this study was to assess the role of mucilage in seed dormancy in 14 species of medicinal plants (table 1).

Table 1. One thousand seed weight (g), mucilage thickness (mm), ratio of mucilage thickness to 1000 seed weight, and germination percentages of 14 species of medicinal plants in H_2O.

Latin name	Family	1000 seed weight (g)	Mucilage thickness (mm)	Mucilage thickness/ 1000 seed weight	Germination (%)
Alyssum homalocarpum Fish & C. A. Mey Boiss.	Brassicaceae	0.92	0.73	0.79	83
Descurainia sophia L.	Brassicaceae	0.16	1.40	8.75	72
Lallemantia iberica M. B.	Lamiaceae	1.66	1.30	0.78	14
Linum usitatissimum L.	Linaceae	4.77	0.40	0.08	95
Melissa officinalis L.	Lamiaceae	1.80	1.50	0.83	97
Ocimum basilicum (green) L.	Lamiaceae	1.75	1.90	1.09	48
Ocimum basilicum (violet) L.	Lamiaceae	1.34	2.00	1.49	52
Plantago lanceolata L.	Plantaginaceae	0.94	2.20	2.34	31
Plantago major L.	Plantaginaceae	0.18	0.30	1.67	43
Plantago ovata Forssk.	Plantaginaceae	1.73	1.90	1.10	83
Plantago psyllium L.	Plantaginaceae	1.22	2.10	1.72	92
Salvia officinalis L.	Lamiaceae	1.10	1.20	1.09	82
Salvia sclarea L.	Lamiaceae	3.70	1.50	0.41	68
Trigonella foenumgraecum L.	Fabaceae	8.90	0.75	0.08	97

Seeds were collected in June 2012 from the Medicinal Plants Garden, Ferdowsi University of Mashhad, Iran. To study the shape and amount of the mucilage, intact seeds were submerged in water for 10 minutes and observed using a binocular (model OB) and photographed. The thickness of the mucilage produced in each species was measured with JMicroVision Software (Version 1.2.7) using scaled photos. To remove the mucilage, intact seeds were submerged in water for 10 minutes and then rubbed gently on filter paper several times until all the mucilage was released. The seeds with removed mucilage are termed "demucilaged seeds".

Germination tests were conducted using four replicates of 25 seeds each on filter paper premoistened with 2.5 ml distilled water in 90 mm-diameter Petri dishes and placed at 25°C. Germinated seeds (2 mm radicle emergence) were recorded daily for 14 days. Dormancy-breaking treatments of prechilling (4°C for seven days), scarification (with P100 sandpaper), KNO_3 (2 g l^{-1}) and GA_3 (250 mg l^{-1}) were applied on intact and demucilaged seeds. Mean germination time (MGT) was calculated using the formula:

$$MGT = \Sigma\, nt / \Sigma\, n$$

where, n is the number of seeds newly germinated at time t; t = days from when set

to germinate. Data were analysed by one-way ANOVA using the statistical package MSTST-C followed by the calculation of LSD. Percentage data were arcsine-transformed prior to analysis.

Range in seed size (0.16 to 8.90 g) and mucilage thickness (0.3 to 2.2 mm) was observed across the different species (table 1). There was no significant correlation between seed size and the mucilage thickness ($r = 0.28$). The relative thickness of mucilage was reflected in the ratio of mucilage thickness to 1000 seed weight, where *Trigonella foenumgraecum* (ratio 0.08) showed a relatively thin layer of mucilage while there was a thick layer of mucilage around the *Descurainia sophia* seeds (ratio 8.75) after imbibition. A wide range of germination from 14% in *D. sophia* to 97% in *T. foenumgraecum* was observed in water. Hence, there was no significant relation between germination percentage with 1000 seed weight ($r = 0.38$) and the thickness of the mucilage of the species ($r = 0.27$). However, seven species had germination below 80% possibly due to seed dormancy. Therefore, the mucilage of the seeds was removed to investigate its possible involvement in inducing the seed dormancy.

There was generally an increase in germination following removal of the mucilage (table 2). In the demucilaged seeds of *Plantago major*, germination increased from 43 to 56% while that of *L. iberica* increased from 14 to 73%.

Scarification of the intact seeds was not effective in increasing germination of seeds of *Salvia sclarea*, *Ocimum basilicum* (green) or *P. major* while in *P. lanceolata* and *Lallemantia iberica*, germination increased from 29 to 90% and from 14 to 98%, respectively. Scarification of the demucilaged seeds improved the germination of three species (e.g. *Salvia sclarea*) but decreased the germination of *D. sophia* and *L. iberica* in comparison with scarified intact seeds.

Prechilling generally increased the germination of the species in the intact seeds (table 2). In particular, a high germination was observed for seeds of *L. iberica* and *D. sophia*, 94 and 99% respectively, compared with germination of the intact seeds in water. Interestingly, prechilling did not improve the germination of demucilaged seeds compared with the prechilled intact seeds except in *O. basilicum* (green). However, the germination of the demucilaged seeds after prechilling compared with demucilaged seeds in water showed contrasting results in different species: germination decreased in *P. major* and *O. basilicum* (green) while it increased in *L. iberica* and *P. lanceolata*.

KNO_3 treatment on the intact seeds enhanced the germination of five species, particularly those of *P. lanceolata* for which 96% germination was observed. It improved the germination of demucilaged seeds for only three species and to a lesser extent. GA_3 treatment improved the germination of five species in both intact and demucilaged seeds, particularly those of *L. iberica* and *P. lanceolata* for which >96% germination was achieved.

Removing the mucilage increased the speed of the germination reflected in a shorter MGT in all species, particularly *Ocimum basilicum* (violet) (1.55 days) and *L. iberica* (1.45 days) compared with the intact seeds in water. However, removing the mucilage increased the average MGT of the seven species given dormancy-breaking treatments. In addition, both KNO_3 and GA_3 treatments increased the MGT of the demucilaged seeds of *D. sophia* in comparison with the intact seeds. Prechilling and KNO_3 treatments also delayed the germination of the demucilaged seeds of *L. iberica* compared with the intact seeds.

Table 2. Germination (G) and mean germination time (MGT) of seven species with mucilage and demucilaged seeds in water (control) and with dormancy-breaking treatments.

Species	GA_3		KNO_3		Prechilling		Scarification		Water	
	Without mucilate	With mucilage	Without mucilate	With mucilage	Without mucilate	With mucilage	Without mucilate	With mucilage	Without mucilate	With mucilage
	G (%)									
Plantago major	56	61	47	69	29	37	45	48	56	43
Ocimum basilicum (violet)	69	42	67	42	52	53	60	51	58	44
Descurainia sophia	99	93	80	90	86	94	70	90	92	67
Lallemantia ibrica	100	99	50	52	98	99	53	98	73	14
Plantago lanceolata	96	98	79	96	87	88	86	90	53	29
Salvia sclarea	84	86	76	86	65	86	85	73	64	73
Ocimum basilicum (green)	72	46	67	46	53	42	55	45	67	48
	MGT (days)									
Plantago major	7.02	4.38	6.57	5.80	6.70	3.82	7.37	5.62	3.65	4.85
Ocimum basilicum (violet)	2.17	2.52	2.35	2.15	3.92	4.47	2.30	3.00	1.05	2.60
Descurainia sophia	3.40	2.10	4.57	2.22	3.35	3.80	2.92	3.95	2.60	2.72
Lallemantia iberica	1.60	1.90	3.66	2.35	2.52	1.27	2.62	2.97	1.90	3.35
Plantago lanceolata	1.60	2.27	2.42	3.05	2.57	2.65	2.62	2.20	3.32	4.41
Salvia sclarea	3.28	2.00	5.73	2.00	6.18	3.37	5.55	2.42	1.90	2.25
Ocimum basilicum (green)	1.75	2.62	2.05	2.05	3.81	4.88	2.92	2.67	1.15	2.10

LSD (germination) = 7.2; LSD (MGT) = 0.47

In general, removing the mucilage improved both the speed of germination (shorter MGT) and final germination (table 2) indicating its possible involvement in seed dormancy. The structure and chemical composition of the mucilage varies between species (Geneve *et al*., 2013) and hence its mechanism could differ to control the germination process either as an osmotica surrounding the seeds after imbibition or as a chemical inhibitor delaying or preventing germination. Toncer and Tansi (2000) reported that mucilage forms a layer around the seed capable of preventing gas exchange and prevents uniform germination of seeds of some species of *Capparis*. Cirak *et al*. (2007) also reported that rinsing the mucilaginous seeds of *Hypericum* leads to rapid seed germination. These studies and ours have been carried out in controlled, laboratory conditions and with sufficient water for the seeds during germination. However, seeds could benefits from the mucilage as a water retainer in water stress conditions (Garwood, 1985). Yang *et al.* (2010) suggested the higher germination of intact achenes of *Artemisia sphaerocephala* Krasch. benefited from the hydrophilic property of mucilage, which can be vital for germination in the harsh desert environment.

Although removing the mucilage generally increased the germination of the species, complete germination was not achieved possibly due to the involvement of the seed coat itself or germination inhibitors of the embryo to induce dormancy. A large increase in the germination of the intact seeds of *P. lanceolata* and *L. iberica* after scarification revealed the involvement of both mucilage and seed coat itself (not mucilage) in controlling germination. Since scarification of the intact seeds removed a part of the mucilage as well as scratching the seed coat, it indicates the mechanical resistance of the seed coat against imbibition.

Prechilling improved the germination of the intact seeds in five species but only in two species in the case of demucilaged seeds (table 2). Tavili *et al*. (2010) also reported the positive effect of prechilling on seed germination in a number of medicinal plant species. However, in the current research, mucilage structure may change at low temperatures and subsequently its function may change after a cold period in some species, depending on its chemical compositions. In nature, this seems to be in order to protect the seeds in cold conditions to allow them to germinate (e.g. *L. iberica*), while in other species (e.g. *P. major*) these changes could prevent germination to some degree until the cold period is passed.

Chemical treatments improved germination of the majority of species as seen before in *D. sophia* and *P. ovata* (Tavili *et al*., 2010) and *O. basilicum* (Aghilian *et al.*, 2014) indicating the possibility of physiological dormancy in some species with mucilaginous seeds. However, in *O. basilicum* (green and violet), GA_3 and KNO_3 treatments had no effect on intact seeds but improved the germination of demucilages seeds, although complete germination was not achieved. It seems removing the mucilage facilitated oxygen transfer to the embryos hence increasing the respiration rate of the seeds (Witztum *et al*., 1969). It is also possible that GA_3 and KNO_3 bond with the components of the mucilage and are trapped in the mucilage, hence they are not able to reach the embryos to be effective.

In conclusion, removing the mucilage increased both the speed and final germination of the seeds of all species in water. All dormancy-breaking treatments improved the

germination of both intact and demucilaged seeds, although GA_3 was the most effective treatment with an average germination of 82% in demucilaged seeds indicating the possibility of physiological dormancy (Baskin and Baskin, 2004). However, germination below 70% was observed in some species (e.g. *P. major*) hence other dormancy-breaking treatments such as longer prechilling is needed. Germination above 80% of intact seeds of the other seven species (e.g. *M. officinalis*) in water indicated no dormancy in the seeds, hence mucilage may play a role in seed water regulation during germination.

Acknowledgements

We would like to thank Dr. Sara Sanjani for help to analyse the data.

References

Aghilian, S., Khajeh-Hosseini, M. and Anvarkhah, S. (2014). Evaluation of seed dormancy in forty medicinal plant species, *International Journal of Agriculture and Crop Sciences*, **10**, 760-768.

Baskin, J.M. and Baskin, C.C. (2004). A classification system for seed dormancy, *Seed Science Research*, **14**, 1-16.

Cirak, C., Kevseroglu, K. and Ayan, A.K. (2007). Breaking of seed dormancy in a Turkish endemic *Hypericum* species: *Hypericum aviculariifolium* subsp. *depilatum* var. *depilatum* by light and some pre-soaking treatments. *Journal of Arid Environments*, **68**, 159-164.

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.

Geneve, R., Hildebrand, D., Philips, T. and Kirk, J. (2013). A comparison of seed mucilage production in several members of the Lamiaceae. *Proccedings of 30th ISTA Congress*, Antalya, Turkey.

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.

Huang, Z., Gutterman, Y. and Osborne, D.J. (2004). Value of the mucilaginous pellicle to seeds of the sand-stabilizing desert woody shrub *Artemisia sphaerocephala* (Asteraceae). *Trees*, **18**, 669-676.

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.

Koocheki, A., Mortazavi, S.A., Shahidi, F., Razavi, S.M.A., and Taherian, A.R. (2009). Rheological properties of mucilage extracted from *Alyssum homolocarpum* seed as a new source of thickening agent. *Journal of Food Engineering*, **91**, 490-496.

Mirmasoomi, M. (1992). *Evaluation of Mucilage in Plantago Family by Tissue Culture and Cultivation in the Field*, M.Sc. Thesis, Tehran University.

Tavili, A., Pouzesh, H., Farajolahi, A., Zare, S. and Zare-Chahooki, M.A. (2010). The effect of different treatments on improving seed germination characteristics in medicinal species of *Descurainia sophia* and *Plantago ovata*. *African Journal of Biotechnology*, **9**, 6588-6593.

Toncer, O.G. and Tansi, S. (2000). The caper (*Capparis ovata*) culture in Turkey. *Pakistanian Journal of Biological Science*, **3**, 568-570.

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

Yang, X., Dong, M. and Huang, Z. (2010). Role of mucilage in the germination of *Artemizia spharocephala* (Astraceae) achenes exposed to osmotic stress and salinity. *Plant Physiology and Biochemistry*, **48**, 131-135.