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A meta-analysis of the effects of treatments used to break dormancy in seeds of the megagenus *Astragalus* (Fabaceae)

Elias Soltani[1], Jerry M. Baskin[2], Carol C. Baskin[2,3] and Fatemeh Benakashani[1]

[1]Department of Agronomy and Plant Breeding Sciences, College of Aburaihan, University of Tehran, Tehran, Iran; [2]Department of Biology, University of Kentucky, Lexington, KY 40506-0225, USA and [3]Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546-0312, USA

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Author for Correspondence:
Elias Soltani,
E-mail: elias.soltani@ut.ac.ir

Abstract

Astragalus is the largest genus of seed plants with 3000 or more species that occurs naturally on several continents. The genus has some use as a forage and medicine and in industry, and many of the species are rare endemics threatened with extinction. The seeds are reported to be dormant at maturity, and various treatments have been used in an attempt to germinate them. Our primary aim was to determine *via* a meta-analysis the most effective way(s) to break dormancy in seeds of this species-rich genus. Mechanical and chemical (conc. sulphuric acid) scarification were by far the best of 12 treatments for breaking seed dormancy of the 40 species included in our meta-analysis, whereas prechilling, gibberellin and smoke were ineffective. These results along with those of imbibition tests confirm that seeds of the examined *Astragalus* species have physical dormancy (PY). Further, PY in these 40 species and (its documented occurrence) in 118 species that could not be included in our meta-analysis transcends climatic and geographic boundaries, edaphic conditions, life cycle/life form types and infrageneric phylogeny. Thus, it seems likely that most species of *Astragalus* have PY. However, in addition to PY, physiological dormancy (PD), that is, combinational dormancy (PY + PD), has been reported in a few species of *Astragalus*. This study should be useful to both basic and applied scientists who want to germinate seeds of *Astragalus*.

Introduction

Astragalus L. (Fabaceae, subfam. Papilionoideae, tribe Galegeae) is the largest genus of seed plants with 3000 or more species (Frodin, 2004; Chaudhary et al., 2008) that occurs naturally on several continents (Chaudhary et al., 2008; Mabberley, 2008). *Astragalus* has some economic value for use as forage for livestock (Butkutė et al., 2018; Tahmasebi et al., 2020), as a medicine (Li et al., 2013, 2014; Liu et al., 2017) and in the industry (Lôpez-Franco and Higuera-Ciapara, 2009; Ferhi et al., 2014). However, some species of *Astragalus* are poisonous to livestock (Cook et al., 2009, 2017), thus causing economic loss to the livestock grazing industry.

Many *Astragalus* species are rare and threatened with extinction. For example, NatureServe (2019) lists 81 species of *Astragalus* in the USA and Canada as imperilled or critically imperilled. The red list report of the International Union for the Conservation of Nature (IUCN) (2019) reported that one species is extinct and 32 endangered or critically endangered. Factors implicated in the rarity and threatened status of *Astragalus* species include low seed germination percentage and slow seedling growth (Kunz et al., 2016; Statwick, 2016), pollinator (pollen) limitation (Becker et al., 2011; Baer and Maron, 2018; Schurr et al., 2019), urban and land development (Decker, 2005, 2006), herbivory (Lesica 1995; Dianati Tilaki et al., 2010; Baer and Maron, 2018) and small population size (Decker, 2006; Wells, 2006).

For *Astragalus*, sowing seeds (usually scarified) or planting *ex situ*-produced seedlings/juveniles (usually from scarified seeds but also from stem cutting in the case of *A. tennesseensis*; Bowles et al., 1988) in the field can be used for reintroduction of extirpated populations (Bowles, 1988; Bowles et al., 1993; Erisen et al., 2010; Naseri and Adibi, 2016), introduction for the establishment of new populations (Baskin and Baskin, 1981; Bowles et al., 1993; Kondo and Takeuchi, 2004; Albrecht and McCue, 2010; Albrecht and Penagos, 2012; Albrecht and Long, 2019) or for augmentation of existing populations (Becker, 2010). Also, plants can be produced by tissue and cell culture methods. Plant tissue culture and somatic embryogenesis are used for propagation of *Astragalus* species (Hou and Jia, 2004; Erisen et al., 2010). *Ex situ* propagation *via* tissue culture has been reported in *A. adsurgens* (Luo and Jia, 1998), *A. sinicus* (Cho and Widholm, 2002), *A. cicer* (Uranbey et al., 2003), *A. melilotoides* (Hou and Jia, 2004), *A. chrysochlorus* (Turgut-Kara and Ari, 2008), *A. canadensis*, *A. racemosus* (Hung and Xie, 2008) and *A. schizopterus* (Yorgancilar and Erisen, 2011). The explants produced have been successfully grown in *ex vitro* conditions such as those in a

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greenhouse (Yorgancilar and Erisen, 2011). Using seeds to propagate *Astragalus* plants is more reliable, costs less and is simpler than doing so by tissue and cell culture (Statwick, 2016; Albrecht and Long, 2019). Germination and seedling survival/establishment are the most critical stages of the life cycle in the conservation of endangered species. Many seedlings will die during the reintroduction of a plant species; thus, it is necessary to produce seedlings in consecutive years in a successful reintroduction program (Maunder, 1992).

Seeds of *Astragalus* are reported to be dormant at maturity, and various treatments have been used in an attempt to germinate them (e.g. see Baskin and Baskin, 2014; Rosbakh et al., 2020). Based on the fact that many (but not all, see Rubio de Cases et al., 2017) seeds of Fabaceae have physical dormancy (PY), we hypothesized that scarification is the most effective treatment for breaking dormancy in seeds of *Astragalus*. Thus, our objectives were (1) to identify the kind(s) of seed dormancy in *Astragalus* species, in order to confirm (or not) that PY is the most represented class of seed dormancy in this megagenus and (2) to determine *via* a meta-analysis the most effective treatment(s) among those reported to break dormancy in seeds of *Astragalus*.

Methods

We did a meta-analysis on the effect of various treatments that have been used in attempting to germinate seeds of *Astragalus* species. Data were collected from indexed papers in the ISI-Web of Science (WOS) database published before February 2019. The search terms were '*Astragalus* dormancy' (11 publications), '*Astragalus* germination' (59 publications), '*Astragalus* seed coat' (17 publications) and '*Astragalus* scarification' (22 publications). Additional publications (75) were found in papers cited in the Persian indexed database (www.sid.ir) and in references cited in the WOS publications. Publications were included in the meta-analysis if they compared seed germination after a dormancy-breaking treatment with control (not treated) and if the information was provided on mean values and number of replications. Fifty-two publications met these requirements, from which data were extracted for 40 species of *Astragalus* (Table 1). Dormancy-breaking treatments were immersion in sulphuric acid (Acid), dry freeze shock (DryFreeze), wet freeze shock (WetFreeze), dry storage (DryStorage), heat plus cold shock (HeatCold), dry heat shock (DryHeat), wet heat shock (WetHeat), prechilling (Prechill), mechanical methods (Mechanical), dry heat shock plus wet heat shock (Dry + WetHeat), gibberellic acid (GA) and smoke (Smoke). For each species, germination improvement (%) was calculated as germination of treatment minus germination of control seeds.

The effect of dormancy-breaking treatments on seed germination in *Astragalus* was investigated by calculating effect size (Hedges et al., 1999; Soltani and Soltani, 2015; Soltani et al., 2018). The ratio of germination of treated seeds ($\bar{X}_T$) to control seeds ($\bar{X}_C$, not treated) is calculated to obtain a response ratio, and the natural logarithm of the response ratio (ln *R*) is applied to determine effect size:

$$\ln R = \ln\left(\frac{\bar{X}_T}{\bar{X}_C}\right) \quad (1)$$

When germination percentage of control seeds approaches zero, the response ratio and the natural logarithm of the response ratio approach infinity. Thus, we added/subtracted 0.5% to it, when germination percentage of control seeds was zero (Robertson et al., 2006). After calculation of ln *R* values, the mean effect size ($\overline{\ln R}$)is calculated by weighting to each study (Gurevitch and Hedges, 1999; Hedges et al., 1999; Soltani and Soltani, 2015; Soltani et al., 2018):

$$\overline{\ln R} = \frac{\sum(\ln R_i \times w_i)}{\sum(w_i)} \quad (2)$$

where w_i is the weight for the *n* observation, that is the number of replications per dormancy-breaking treatment. Significant changes are evaluated by confidence intervals (CIs) as shown in the following equation (Neyeloff et al., 2012):

$$\text{CI} = \overline{\ln R} \pm 1.96 \times \text{SE} \quad (3)$$

where standard error (SE) was estimated for n observations from standard deviation (SD) as shown below:

$$\text{SD} = \sqrt{\frac{\sum(\ln R_i - \overline{\ln R})^2}{n-1}} \quad (4)$$

The Chi-square (*Q*) test was used to evaluate total heterogeneity (Q_T), between-study heterogeneity (Q_B), within-study heterogeneity (Q_W) and heterogeneity within subgroups (Q_{Wj}) (Gurevitch and Hedges, 1999; Traveset and Verdu, 2002; Rosenberg et al., 2004). When Q_W is significant, further heterogeneity remains unexplained within that group. A portion of the heterogeneity can be explained by subgrouping the studies into categories if Q_B is significant. The Chi-square tests were performed by fixed effects (Traveset and Verdu, 2002). All calculations of heterogeneities and effect sizes were conducted in Microsoft Excel (Neyeloff et al., 2012).

Results and discussion

Overall, dormancy-breaking treatments increased germination of *Astragalus* compared with the control (mean effect size $(\overline{\ln R})$ = 1.40; Fig. 1). The Chi-square test indicated that Q_T, Q_W and Q_B were significant in the studies, showing that some other variable(s) can explain the variations among the studies. Thus, we categorized the dormancy-breaking treatments into 12 categories. Dormancy-breaking treatments differed significantly ($Q_B = 4799.14$, $P < 0.0001$), implying significant changes among the treatments. The fact that scarification with sulphuric acid (effect size = 1.99) and mechanical methods (effect size = 2.05) had the most positive effects on germination among the dormancy-breaking treatments coupled with the zero or negative effects of prechilling, GA and smoke strongly indicates that seeds of most species of *Astragalus* have PY (Fig. 1). Furthermore, the few studies in which imbibition was compared in scarified *versus* intact seeds of *Astragalus* (e.g. Kim et al., 2008; Long et al., 2012; Han et al., 2018) have shown that the seed coat is water impermeable, thus conclusively demonstrating that the seeds of the investigated species have a water-impermeable coat, indicating PY.

There are few reports indicating combinational dormancy (PY + PD) in seeds of a few species of *Astragalus* (Pickart et al., 1992; Kaye, 1997; Eisvand et al., 2006; Meinke et al., 2013; Bushman et al., 2015, 2019; Jones et al., 2016; Kildisheva et al.,

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Table 1. Germination improvement (germination of treatment – germination of control, %) after treatment of seeds of 40 *Astragalus* species included in the meta-analysis

Astragalus species	Treatments	Germination improvement (%)	Number of observations	Reference
A. adscendens	Prechill, Acid, WetHeat	13	3	Tavili et al. (2014)
A. adsurgens	Acid, Dry Storage	65	11	Kondo and Takeuchi (2004)
A. agnicidus	Prechill, Acid	45	7	Pickart et al. (1992)
A. amblytropis	Mechanical, Acid, Prechill	53	4	Rittenhouse and Rosentreter (1994)
A. armatus	Acid, WetHeat	51	7	Kheloufi et al. (2018)
A. arpilobus[a]	Mechanical, WetHeat, Acid, HeatCold, DryStorage	49	74	Long et al. (2012)
A. australis	Mechanical	78	1	Kaye (1999)
A. bibullatus	Mechanical, DryHeat	57	8	Albrecht and Penagos (2012)
A. bisulcatus	Mechanical/Acid*, Prechill, WetHeat	13	4	Smreciu et al. (1988)
A. cicer	WetHeat, Acid, Mechanical, DryStorage, HeatCold	44	60	Statwick (2016), Sahbaz et al. (2012), Townsend and McGinnies (1972), Acharya et al. (1993), Acharya et al. (2006) and Miklas et al. (1987)
A. contortuplicatus	Mechanical, WetHeat, DryHeat, DryFreeze, WetFreeze	28	6	Molnár et al. (2015)
A. crassicarpus	Acid, Mechanical, Smoke, DryHeat, Smoke, WetHeat	28	21	Cox et al., (2017), Wells (2006) and Smreciu et al. (1988)
A. cyclophyllon	Mechanical, Acid, GA	15	7	Keshtkar et al. (2008) and Moshtaghyan et al. (2009)
A. drummondii	Mechanical/Acid*, Prechill	29	3	Smreciu et al. (1988)
A. filipes	Mechanical, Acid, GA, Prechill	23	25	Kildisheva et al. (2018), Kildisheva et al., (2019), Jones et al. (2016), Bushman et al. (2015) and Fund et al. (2019)
A. fridae	Mechanical, WetHeat, WetFreez, Prechill	32	7	Arbabian et al. (2009)
A. gilviflorus[b]	Mechanical/Acid*, Prechill, WetHeat	60	1	Smreciu et al. (1988)
A. gines-lopezii	Mechanical, DryFreeze, HeatCold, WetHeat, Acid	25	24	Schnadelbach et al. (2016) and Martínez-Fernández et al. (2014)
A. gossypinus[c]	Acid, Mechanical, WetHeat	63	4	Tavili et al. (2012) and Moammeri et al. (2012)
A. hamosus	Acid, WetFreeze, Mechanical, WetHeat, DryFreeze	4	60	Siles et al. (2016), Siles et al. (2017) and Patanè and Gresta (2006)
A. lehmannianus	Mechanical, Acid, WetHeat, Dry + WetHeat,	40	33	Abudureheman et al. (2014) and Han et al. (2018)
A. looseri	Prechill	9	1	Cavieres and Sierra-Almeida (2018)
A. maritimus	Mechanical, Acid, DryHeat	50	17	Bacchetta et al. (2011)
A. michauxii	DryHeat, DryHeat + Smoke, Acid	24	4	Weeks (2004)
A. mongholicus	WetFreeze	65	7	Shibata and Hatakeyama (1995)
A. nanjiangianus	Mechanical	92	2	Abudureheman et al. (2014)
A. nitidiflorus	Mechanical	53	8	Segura et al. (2015)
A. parrowianus[d]	Mechanical	71	7	Khayat Moghadam and Sadrabadi Haghighi (2015)
A. peckii	Mechanical	46	6	Pearson (2015)
A. pectinatus	Mechanical/Acid*, Prechill, WetHeat	38	4	Smreciu et al. (1988)
A. penduliflorus	Mechanical, Acid	72	5	Dziurka et al. (2019)
A. podolobus	Prechill, Acid, DryFreeze, WetFreeze, WetHeat,	4	15	Agh et al. (2017) and Tavili et al. (2014)

(*Continued*)

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Table 1. (*Continued.*)

Astragalus species	Treatments	Germination improvement (%)	Number of observations	Reference
A. siliquosus	Mechanical, Acid, DryFreeze, WetHeat, WetFreeze	29	12	Eisvand et al. (2005) and Eisvand et al. (2005)
A. sinicus	DryStorage, Prechill, WetHeat, Mechanical, Acid, DryHeat	40	83	Kim et al. (2008) and Lee et al. (2006)
A. spp.[e]	GA	–9	6	Ikram et al. (2014)
A. striatus[f]	Mechanical/Acid, Prechill, WetHeat	38	4	Smreciu et al. (1988)
A. tennesseensis	Mechanical, Acid	32	5	Baskin and Quarterman (1969)
A. tribuloides	Mechanical, WetHeat, WetFreeze, Prechill	35	7	Fateh et al. (2006)
A. verrucosus	Acid, Mechanical	56	15	Bacchetta et al. (2011)
A. vulnerariae	Acid	77	4	Dilaver et al. (2017)

These species were extracted from Web of Science database and included searching for references and papers cited in the Persian indexed database (www.sid.ir) published before 24 February 2019. Germination improvement (%) is an average for pre-treatment(s) listed for the species.
[a]syn. *Astragalus harpilobus*.
[b]syn. *Orophaca caespitosa*.
[c]syn. *Astracantha gossypina*.
[d]syn. *Astracantha parrowiana*.
[e]The authors said '*Astragalus* spp.' but did not give any specific epithets. Thus, we counted this entry as one species.
[f]syn. *Astragalus adsurgens*.
*The authors say that they used acid or mechanical scarification to break dormancy, but they did not distinguish which one was used for each species and gave the term 'scarification' for both of them. These data were used to calculate the overall value (Fig. 1) but not for the categorization of treatments.

2018). Seeds of *A. amphioxys* required scarification of the seed coat plus 'a period of refrigeration' (cold stratification?) before they would germination (Spellenberg, 1976). Presumably then, seeds of this species have PY + PD. Scarified and non-stratified seeds of *A. agnicidus* gave 'very poor germination', whereas those scarified and then cold stratified at 4°C for 20 d germinated to 89% (Pickart et al., 1992). In another study on this species, scarified–non-stratified and scarified–stratified seeds germinated to 38–43 and 98%, respectively (Meinke et al., 2013). Seeds of *A. cottonii* have PY + PD. Although 100% of scarified seeds eventually germinated, the t_{50} of seeds stored for 1 and 9 months was 73 and 7 d, respectively (Kaye, 1997). In other words, the seeds afterripened, that is, germination rate ($1/t_{50}$) increased, during dry storage. Eisvand et al. (2006) indicated that dormancy in 95% of the seeds of *A. siliquosus* was caused by PY and only 5% by PD. Seeds of *A. filipes* have been reported to have PD in addition to PY, that is, PY + PD (Jones et al., 2016; Kildisheva et al., 2018). Germination of scarified seeds of *A. filipes* that had been stored dry at 4 months was 19 and 34% in water and GA_3, respectively (Kildisheva et al., 2018). Jones et al. (2016) tested the effect of different dormancy-breaking treatments on germination of *A. filipes* seeds that had been stored for at least 8 months. Non-treated (intact) seeds germinated to 12%, seeds mechanically scarified with sandpaper to 20%, seeds prechilled for 3 weeks to 20% and seeds scarified plus prechilled to 35%. Physiological dormancy (PD) of the embryo may be the reason why Bushman et al. (2015) obtained very low germination and seedling emergence (from soil) percentages for mechanically and acid-scarified seeds of *A. filipes*, although seed viability was very high. In another study, Bushman et al. (2019) indicated that seedling emergence of *A. filipes* was higher when scarified seeds were planted in autumn than in spring and concluded that they need cold stratification for germination. Treatments to overcome PD or PY + PD did not increase germination to a high percentage, and also scarification alone was not beneficial in some cases. Thus, combinational dormancy appears to be present in at least a portion of the seeds in a seed cohort of a few species of *Astragalus*.

Seeds of *A. tennesseensis* have a water-impermeable outer seed coat and a tough membranous water-permeable inner seed coat (Baskin and Quarterman, 1969). Thus, seeds will imbibe water upon scarification of the outer seed coat. However, the seeds will not germinate unless the inner seed coat is removed from the seed. Although these authors concluded that the seeds had PY, it seems likely that the embryo has some PD. However, the effect of neither cold stratification nor any other PD-breaking treatment on germination of imbibed seeds was tested. We now speculate that the seeds of *A. tennesseensis* have PY + PD and require both scarification and cold stratification to germinate. In other words, without PD-breaking treatment, the embryo in imbibed seeds cannot generate enough growth potential to overcome the resistance of the inner seed coat and germinate.

Seeds of *A. michauxii* are reported to have an inner and outer seed coat like *A. tennesseensis* (Weeks, 2004; Kunz et al., 2016). Also like *A. tennessensis*, scarification of the outer seed coat only will allow the seed to imbibe water but not to germinate. The inner seed coat also must be scarified in several places for a high percentage of the seeds of *A. michauxii* to germinate (Kunz et al., 2016). Whereas Kunz et al. stated that the seeds have PY only, this may not be the case, as in *A. tennessensis*. Therefore, we suggest that seeds of *A. michauxii* may have PY + PD.

In our database, germination of control seeds was higher than 30% in five species, including *A. adscendens*, *A. cyclophyllon*, *A. gilviflorus*, *A. gines-lopezii* and *A. peckii* (Table 1; Fig. 2). Keshtkar et al. (2008) found that germination of control seeds of *A. cyclophyllon* was 55% and germination of treated seeds was 70% (an average of treatments), which is equal to 15%

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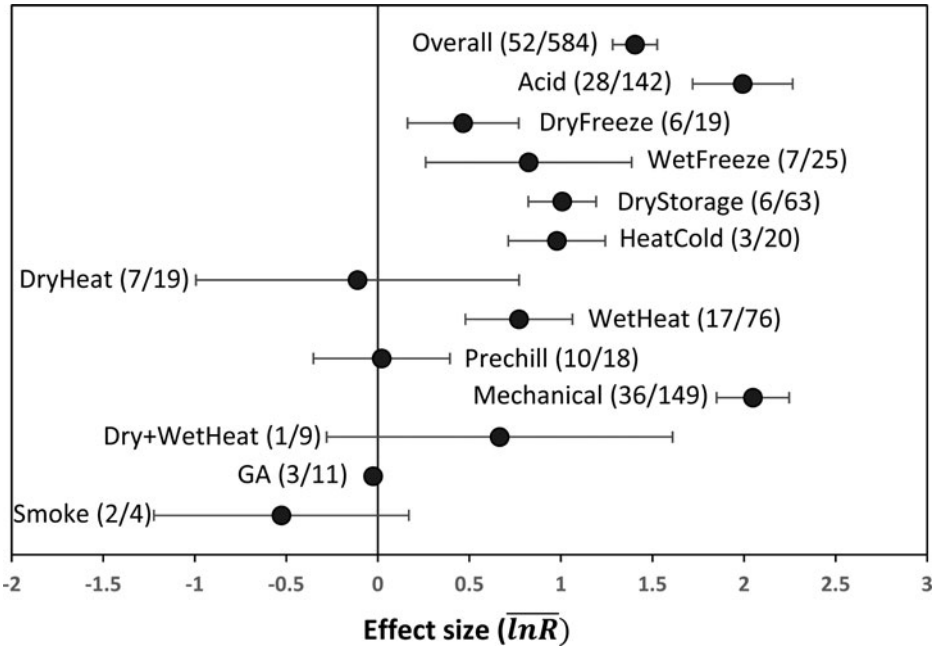

Fig. 1. Effect size of various pre-treatments and of all treatments combined (Overall) on germination percentages of treated seeds of *Astragalus*. Treatments were sulphuric acid (Acid), dry freeze shock (DryFreeze), wet freeze shock (WetFreeze), dry storage (DryStorage), heat plus cold shock (HeatCold), dry heat shock (DryHeat), wet heat shock (WetHeat), prechilling (Prechill), mechanical scarification (Mechanical), dry plus wet heat shock (Dry + WetHeat), Gibberellin (GA) and smoke (Smoke). Number of studies (first number) and number of observations (second number) are shown in parenthesis. Error bars show 95% confidence intervals (CIs). No overlap of error bars with zero indicates that treatment significantly affected germination percentage.

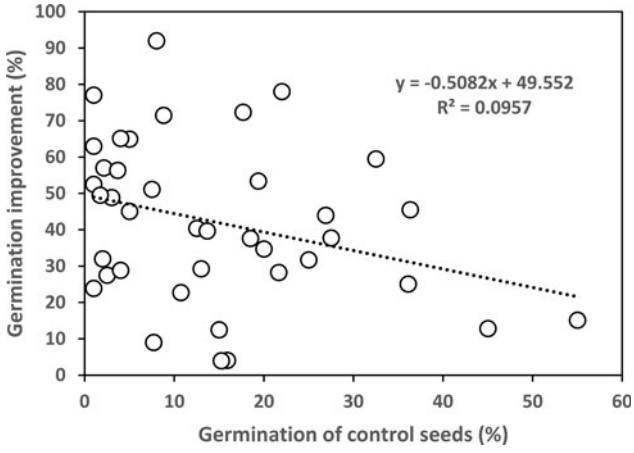

Fig. 2. Relationship between germination improvement (%) and initial germination of control seeds (no treatment). The values of germination improvement (%) are given in Table 1. Data for *Astragalus* spp. treated with GA had a negative value, and it is not included in this figure.

germination improvement (Table 1). Ramos et al. (2010) concluded that seeds of the Spanish endemic *A. gines-lopezii* were non-dormant. However, since the seeds were stored for 1 year before they were tested for germination it is not known if fresh seeds were dormant or non-dormant. The results of Ramos et al. (2010) do not agree with those of Martínez-Fernández et al. (2014), who compared dormancy/germination of the only two known populations of *A. gines-lopezii*. They found that mechanical scarification increased mean germination from 27 to 95% in the Andres population and from 30 to 91% in the Calera population. Dormancy and germination of seeds from these two populations were investigated by Schnadelbach et al. (2016), who found that mechanical scarification significantly improved germination over that of the non-scarified control.

Seeds of *A. peckii*, with PY, seem to have a portion of water-permeable seeds (36%), which varies among years and study sites (Pearson, 2015), implying that only part of the seeds of this species have PY. Moreover, there are five species (*A. clerceanus*, *A. karelinianus*, *A. laguroides*, *A. sulcatus* and *A. tibetanus*) included in Supplementary Table S1 for which in addition to PY non-dormancy (ND) is recorded (Rosbakh et al., 2020). In *A. clerceanus* and *A. karelinianus*, the proportion of freshly collected seeds with a water-permeable seed coat was 30–35% and in dry-stored seeds (1 month) 85–93%. Information regarding dormancy in fresh seeds in the other three species is unclear, but 20–40% of the seeds had a water-impermeable seed coat. Although the meta-analysis showed that PY was present among the studied species, in some species, there was an appreciable proportion of non-dormant seeds. Thus, the seed lot would have 'partial PY'.

The heterogeneity test indicated that Q_{Wj} decreased for each kind or category of dormancy-breaking treatment. The lowest Q_{Wj} values were observed for smoke (3.26) and GA (7.44) treatments, both of which were non-significant. The Q_{Wj} was 46.8 for prechilling treatment, in which variation was low. Thus, GA, smoke and prechilling that break PD in seeds of various species are ineffective in breaking dormancy in seeds of *Astragalus*. The highest values for Q_{Wj} were obtained for sulphuric acid (Q_{Wj} = 12398.5, $P < 0.0001$) and mechanical treatments ($Q_{Wj} = 8908.9$, $P < 0.0001$) treatments, indicating high variation among species and/or studies. For the other treatments that were significant, Q_{Wj} ranged from 21.95 (Dry + WetHeat) to 1124.38 (WetHeat).

Dry and wet freeze shocks (freeze-thaw), heat plus cold shock and wet heat shock significantly increased germination of *Astragalus* seeds by effect sizes of 0.47, 0.82, 0.98 and 0.77, respectively (Fig. 1). There have been various studies on the effect of wet and dry heat on dormancy break in seeds of *Astragalus* species. Fotheringham and Keeley (1998) reported that germination of seeds of *A. brauntonii* was increased from 13 to 85% by dry heat (95 or 105°C for 5 min); however, 100% of the seeds germinated after mechanical scarification. Bacchetta et al. (2011) compared the effects of boiling water (100°C; time not given), acid scarification and mechanical scarification on PY-break in seeds of *A. maritimus* and *A. verrucosus* and found that all treatments improved germination in both species. A low percentage (≤5)

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of the seeds of *A. arpilobus* exposed to different temperatures and durations of dry heat (60, 70 and 80°C for 0, 3, 6, 12, 24 and 48 h) became water-permeable (Long et al., 2012). However, the seeds that did not imbibe were still viable, and 99% of them germinated after mechanical scarification. Treating seeds of *A. arpilobus* with wet heat also was not as effective as mechanical scarification. The highest germination in wet heat-treated seeds (31%) was for those submerged in boiling water (100°C) for 10 min; longer/shorter times and lower temperatures were less effective in promoting germination (Long et al., 2012). Exposure to hot water (100°C) for 5 min significantly improved germination of seeds of *A. podolobus* but not those of *A. adscendens* (Tavili et al., 2014). Neither wet heat (100°C for 10 min) nor dry heat (80°C for 10 min) had an effect on germination of *A. contortuplicatus* seeds (Molnár et al., 2015). Kheloufi et al. (2018) tested the effect of hot water (100°C) for 10 min on dormancy break in seeds of *A. armatus*, but this treatment did not improve germination. Thus, our synthesis of results reported in the literature strongly indicate that neither dry nor wet heat is as effective in breaking dormancy in seeds of *Astragalus* as are acid and mechanical scarification. However, a possible reason for the general ineffectiveness of wet or dry heat on dormancy break in seeds of *Astragalus* may be that in some of the studies seeds were killed by the high heat level (°C × time). For example, 90% of the seeds of *A. bibullatus* lost viability during treatment with dry heat (125°C) for 30 min (simulation of fire) (Albrecht and Penagos, 2012).

A positive change in germination percentage was obtained for all *Astragalus* species included in the meta-analysis, except for those treated with GA_3, in which case the change was negative (Table 1). Dormancy-breaking treatments for a species were not the same in all studies, and this may be a reason for different results. We found a relationship between response to dormancy-breaking treatment (based on % germination improvement) and initial germination (control without any dormancy-breaking treatment) in which seeds with low initial germination percentages had higher response to dormancy-breaking treatment than seeds with high initial germination percentages (Fig. 2). Thus, the magnitude of germination improvement depended on the initial germination percentage of seeds.

The germination improvement of *A. gines-lopezii* seeds was 25% (average for two studies) (Table 1), which was obtained from studies by Martínez-Fernández et al. (2014) and Schnadelbach et al. (2016). In the study by Schnadelbach et al. (2016), the seeds of *A. gines-lopezii* were stored in a dry condition for 7 months, which obviously did not have much of an influence on increasing water-permeability of the seed coat. In contrast, in our study, dry storage improved germination of *Astragalus* seeds by an effect size of 1.01 (Fig. 1). Positive effects of dry storage previously have been reported for *A. cicer* (Acharya et al., 1993, 2006) and *A. sinicus* (Lee et al., 2006; Kim et al., 2008). However, Long et al. (2012) found no significant changes in germination of *A. arpilobus* (*A. harpilobus*) seeds dry stored for 1 year. Kondo and Takeuchi (2004) observed only a 9% increase in germination of *A. adsurgens* seeds after 9 months of dry storage. Dormancy break in seeds with PY during dry storage is due to the seeds becoming water-permeable and not to afterripening *per se* such as occurs in seeds with PD. Seeds of *A. tennesseensis* did not become permeable after 54 years of dry storage at room temperatures (Baskin and Baskin, unpublished).

Sulphuric acid, DryFreeze, WetFreeze, DryStorage, HeatCold, WetHeat and Mechanical treatments had positive effects on germination of *Astragalus* seeds (Fig. 1). Among these treatments, scarification with sulphuric acid and by mechanical methods were the best treatments to break dormancy. Scarification by sulphuric acid is not a good choice for commercial usage in breaking PY since it is dangerous and less practical than mechanical scarification. For commercial use of *Astragalus*, mechanical scarifier machines can be used to scarify large seed lots (Townsend and McGinnies, 1972; Acharya et al., 2006; Patanè and Gresta, 2006; Kimura and Islam, 2012; Kildisheva et al., 2018, 2019). Townsend and McGinnies (1972) used a scarifier equipped with a small drum with the inside covered by abrasive paper, and seeds of *A. cicer* were air-compressed into the drum to scarify them. The authors determined how to effectively scarify the seeds with the machine, but the percentage of seeds damaged increased with the number of scarifications at 80 psi (551.6 kilopascals). However, Patanè and Gresta (2006) observed that germination of *A. hamosus* seeds passed through the same scarifying machine ten times was only about 7%, which was not a significant change from the control. Small seeds may pass through the scarifier without the seed coat being scarified. Thus, the machine must be adjusted according to the size of the seeds Mechanical scarification can be done manually with a blade or sandpaper for small seed lots (Acharya et al., 2006) or small seeds (Patanè and Gresta, 2006) of *Astragalus*.

Phylogenetic history can restrict variation in seed dormancy and germination traits, and thus, related species often have the same traits. Nevertheless, adaptation to different environmental conditions may affect these traits and lead to significant variation between related species (Willis et al., 2014; Arène et al., 2017; Seglias et al., 2018). The 40 species in our meta-analysis belong to 32 sections of *Astragalus* that are phylogenetically and geographically (including climate and edaphic factors) widely distributed (Table 2). These 40 species include annuals and perennials and shrubs/subshrubs and herbs. In addition to the 52 papers used in the meta-analysis (Table 1), there are various studies on *Astragalus* in which the germination data did not meet the requirements for inclusion in our meta-analysis (Supplementary Table S1). The 118 species listed in this table belong to an additional 58 sections of the genus *Astragalus*, making a total of 90 sections of *Astragalus* represented in our study, which is about 36% of the sections in the genus. Thus, it seems likely that seeds of most species of *Astragalus* have PY, while (as described above) a few have PY + PD, at least for a proportion of the seeds in a seed lot. In the latter case, a seed cohort might consist of seeds with PY and of those with PY + PD.

Conclusions and recommendations

The 158 species of *Astragalus* for which we evaluated the kind of seed dormancy represent different types of life cycles and life forms that are widely distributed geographically, climatically, edaphically and phylogenetically in the genus, and most species of *Astragalus* have PY, that is, have a water-impermeable seed coat. However, we did find some evidence that seeds of a few species have PD in addition to PY, that is, PY + PD. Thus, we conclude that while most likely the great majority of *Astragalus* species produce seeds with PY only, some of them produce seeds with PY + PD or a mixture of PY and PY + PD.

The best ways to break PY in seeds of *Astragalus* species are by mechanical or chemical (conc. sulphuric acid) scarification. Compared with the control and other treatments, mechanical and chemical scarification improved seed germination significantly. Where high numbers of plants are required, such as

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Table 2. *Astragalus* species (and botanical section) included in the meta-analysis and their geographical distribution, life cycle and life form (Barneby, 1964; Langran, 2010; Masumi, 2018; refer to citations in Table 1)

Astragalus species	Section	Distribution	Life cycle	Life form
A. adscendens	*Tragacantha* DC.	W Asia, Turkey	Perennial	Shrub
A. adsurgens	*Onobrychoidei* DC.	North America, Siberia, Mongolia, China	Perennial	Herb
A. agnicidus	*Miselli* Barneby	Northern California	Perennial	Herb
A. amblytropis	*Platytropides* Barneby	Central Idaho	Perennial	Herb
A. armatus	*Poterium* Bunge	Northern Africa	Perennial	Shrub
A. arpilobus[a]	*Haematodes* Bunge	Central Asia, SW Asia, Eastern Europe, China	Annual	Herb
A. australis	*Hemiphragmium* (Koch) Bunge	Olympic Mountains, Washington	Perennial	Herb
A. bibullatus	*Sarcocarpi* Gray	Central Basin of Tennessee	Perennial	Herb
A. bisulcatus	*Bisulcati* Gray	North America	Perennial	Herb
A. cicer	*Hypoglottidei* DC.	Continental Europe	Perennial	Herb
A. contortuplicatus	*Cycloglottis* Bunge	Continental Eurasia, from China to central Europe	Annual	Herb
A. crassicarpus	*Sarcocarpi* Gray	North America	Perennial	Herb
A. cyclophyllon	*Incani* DC.	Endemic to Iran	Perennial	Herb
A. drummondii	*Drummondiani* Barneby	North America	Perennial	Herb
A. filipes	*Cusickiani* Torr & Gray	North America	Perennial	Herb
A. fridae	*Incani* DC.	Endemic to Iran	Perennial	Shrub
A. gilviflorus[b]	*Orophaca* (T. & G.) Barneby	North America	Perennial	Herb
A. gines-lopezii	*Platyglottis* Bunge	Endemic to southern part of the Iberian Peninsula, Spain	Perennial	Herb
A. gossypinus[c]	*Platonychium* Bunge	SW Asia	Perennial	Shrub
A. hamosus	*Bucerates* DC.	Throughout Europe and part of Asia	Annual	Herb
A. lehmannianus	*Aegacantha* Bunge	Russia, Turkmenistan, Kazakhstan, Uzbekistan, China	Annual	Herb
A. looseri[d]	Undetermined	Chile	Perennial	Herb
A. maritimus	*Thlaspidium* Lipsky	Sulcis-Iglesiente area (SW Sardinia)	Annual	Herb
A. michauxii	*Michauxiani* Barneby	Southeastern USA	Perennial	Herb
A. mongholicus	*Cenantrum* Koch	Russian Federation, Mongolia, Kazakhstan, China	Perennial	Herb
A. nanjiangianus	*Poliothrix* Bunge	China	Perennial	Herb
A. nitidiflorus	*Platyglottis* Bunge	Spain	Perennial	Herb
A. parrowianus[e]	*Rhacoohorus* Bunge	Iran	Perennial	Shrub
A. peckii	*Neonix* Barneby	Oregon	Perennial	Herb
A. pectinatus	*Pectinati* Gray	North America	Perennial	Herb
A. penduliflorus	*Cenantrum* Koch	Europe	Perennial	Herb
A. podolobus	*Ammodendron* Bunge	Iran	Perennial	Sub-shrub
A. siliquosus	*Theiochrus* Bunge	Iran	Perennial	Herb
A. sinicus	*Lotidium* Bunge	E Asia, China	Annual	Herb
A. striatus[f]	*Onobrychoidei* DC.	North America	Perennial	Herb
A. tennesseensis	*Tennesseensis* Barneby	Tennessee, northern Alabama, central Illinois	Perennial	Herb
A. tribuloides	*Oxyglottis* Bunge	Central Asia, SW Asia (Caucasus), N Africa	Annual	Herb
A. verrucosus	*Platyglottis* Bunge	Sulcis-Iglesiente area (SW Sardinia)	Perennial	Herb
A. vulneraria	*Vulneraria* DC.	Turkey	Perennial	Herb

[a]syn. *Astragalus harpilobus*.
[b]syn. *Orophaca caespitosa*.
[c]syn. *Astracantha gossypina*.
[d]South American species not assigned to sections.
[e]syn. *Astracantha parrowiana*.
[f]syn. *Astragalus adsurgens*.

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growth of an *Astragalus* species for forage or large-scale revegetation (conservation) projects, we suggest the use of a scarifier/huller machine to break dormancy. Mechanical scarification by hand (with sandpaper or a blade) is suggested for situations in which a small or relatively small number of plants are required, for example, testing the effect of dormancy break on germination in the laboratory.

Supplementary material. To view supplementary material for this article, please visit: https://doi.org/10.1017/S0960258520000318.

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