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Research Note

Seed dormancy and seedling growth changes in response to scarification treatments and population origin in *Kosteletzkya pentacarpos* (Malvaceae)

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Abstract

Kosteletzkya pentacarpos is important for the restoration of coastal habitats in Asia and is vulnerable in Europe. Physical dormancy (PY) prevents ready seed germination and seedling production for restoration and conservation purposes. In this study, seed germination and seedling growth of *K. pentacarpos* were investigated in response to scarification treatments (manual scarification, percussion) and population origin (i.e., wild, cultivated *ex situ* and reintroduced). Manual scarification best promoted seed germination (98-100%), but resulted in damages to the cotyledons (on 64% of seedlings, on average) and lower seedling growth. Percussion did not significantly increase seed germination, but best promoted seedling growth. There was a significant reduction of PY in seeds produced by plants cultivated *ex situ* and/or reintroduced. Our results indicate that scarification treatments and *ex situ* cultivation may have important implications in translocation and other uses of *K. pentacarpos*.

Keywords: physical dormancy, reintroduction, scarification treatments, seashore mallow, seedling growth, translocation

Experimental and discussion

Seashore mallow, *Kosteletzkya pentacarpos* (L.) Ledeb. (Malvaceae), is a perennial halophytic plant found in North America and along the coasts of the Caspian and Mediterranean Seas. The species is listed as Vulnerable (VU) in the European Union (EU) red list (Bilz *et al.*, 2011) and considered of high conservation relevance, being listed in Annex II of the EU Directive 92/43/EEC and in Annex I of the Bern Convention (Rossi *et al.*, 2016). Where *K. pentacarpos* is abundant (e.g. North America), it is cultivated and used as a source of textile fibres or biomass to produce biofuel (Markevicius *et al*., 2010; Fan *et al*., 2011). Moreover, the species is recommended for the restoration of degraded coastal soils for its salt tolerance. In China, where it was introduced more than 20 years ago, *K. pentacarpos* has been used to restore degraded saline lands with positive effects on local soil conditions, growth of native species and plant diversity (Qin *et al*., 2015).

The propagation of *K. pentacarpos* via seed for restoration purposes is made difficult primarily due to physical dormancy (i.e. hardseededness), that prevents movement of water to the embryo (Poljakoff-Mayber *et al*., 1992). Various treatments have been proposed for reducing hardseededness in plants, including for example, mechanical scarification with scalpel or sandpaper (Harijati and Widoretno, 2019), acid scarification (Kimura and Islam, 2012) and thermal scarification with hot water (McNair, 1917). However, some scarification methods may excessively degrade the seed-coat causing embryo damage (e.g. acid treatments; Kelly and Van Staden, 1987) or are time-consuming (e.g. mechanical scarification by hand). In this regard, percussion (i.e. seeds repeatedly propelled against a hard surface), has been found to be an effective and user-friendly method of scarification for large quantities of legume seeds (Mondoni *et al*., 2013). In this study, we investigated the effects of percussion and manual scarification on seed germination, cotyledon damage and seedling growth in *K. pentacarpos*, from wild, *ex situ* cultivated and reintroduced populations, in order to identify the most effective method to remove dormancy and produce healthy seedlings.

Seeds of *K. pentacarpos* were collected at the time of seed dispersal in September 2013 from two wild populations in Albufera, Spain (45°27'/9°11') and Volano, Italy (44°48'/12°16'), hereafter referred to as wES and wIT, respectively; one reintroduced population in Algemesi, Spain (45° 27'/9°11'), hereafter referred to as rES; and one *ex situ* population cultivated at the Botanical Garden of the University of Ferrara, Italy, for one generation, hereafter referred to as cIT. Plants of rES and cIT were derived from seeds collected at wES and wIT, respectively. In both cases, the distance between wild and cultivated/reintroduced populations was about 10 km. After collection seeds were held under laboratory ambient conditions (approx. 23°C, 30% RH) for two weeks, before the start of the test.

The following treatments were applied: (1) seed-coats were gently chipped by hand using a scalpel (manual scarification); (2) 5 and (3) 10 minutes percussion treatments using a pneumatic paint shaker (CycloneTM, BroncorpMfg. Co., Denver, CO, USA) as described in Mondoni *et al*. (2013); (4) control treatment (no scarification). After scarification, three replicates of 20 seeds each per population and treatment were sown in 90 mm-diameter Petri dishes with 1% agar and placed in temperature and light controlled incubators (LMS Ltd., Sevenoaks, UK) at conditions previously found to elicit high germination (Poljakoff-Mayber *et al*., 1992), i.e. 12-hour daily photoperiod (photosynthetically active radiation of 40–55 µmol m^{-2} s^{-1}, 400–700 nm) and 30/25°C (with light provided during the warmest temperature). As soon as radicles emerged, seedlings were transferred to 57 mm-diameter pot trays containing a commercial soil mixture (perlite and peat; Terflor s.r.l., Capriolo, Brescia, Italy) and were cultivated for three weeks in a greenhouse (20 ± 4°C, natural light). Plants from cIT were used for plant translocation (Abeli *et al*., 2017; Brancaleoni *et al*., 2018).

Cotyledon damage was assessed as the presence/absence of cuts or holes due to the scarification treatment, two weeks after radicle emergence, when all the cotyledons were fully developed. Plant height and leaf length were measured on 35 randomly selected individuals three weeks after radicle emergence, when plants have developed 3-4 mature leaves (except for wES under control and percussion treatments where, due to poor germination, only 8 and 15 seedlings could be used, respectively).

Germination data were analysed with a binary logistic Generalised Linear Model (GLM) with logit link function, with germination as response variable, scarification treatment as fixed factor, and population and replicate as random factors. Similarly, damage data were analysed with the same binary logistic GLM with damage as response variable. Plant height and leaf length were analysed by fitting a linear GLM.

Seed germination was significantly affected by scarification treatments (table 1), with manual scarification always showing higher germination compared with the control and percussion treatments, except in cIT (figure 1A-D). Seeds produced by cIT were not dormant, germinating to almost 100% in absence of scarification. Consistently, seeds from rES germinated more under the control (and the percussion treatments), than those of the related wild population (wES). Significant differences were detected among populations, with wES and cIT populations having the lowest and the highest germination percentage, respectively.

Scarification treatment significantly affected the presence/absence of cotyledon damage (table 1), which was greater in seedlings resulting from manually-scarified seeds (64%, on average), than in seedlings from percussed or untreated seeds (both 0%).

After three weeks, plant height and leaf length were significantly affected by scarification treatment (table 1). In particular, manual scarification and control treatments resulted in significantly fewer plants and smaller leaves compared with both percussion treatments (figure 1B, C). Seedlings belonging to wIT were significantly bigger and with larger leaves, compared with those of the other populations.

Table 1. Results of fitting binary logistic generalised linear models to different performance variables of *Kosteletzkya pentacarpos* seeds following different scarificiation treatments.

	Germination			Damage		
	Wald χ^2	Degrees of freedom	*P*	Wald χ^2	Degrees of freedom	*P*
Treatment	24.799	3	**<0.001**	103.635	3	**<0.001**
Population	44.506	3	**<0.001**	2.874	3	0.411
Population × Treatment	14.504	9	0.106	4.162	9	0.900
	Plant height			**Leaf length**		
Treatment	50.730	3	**<0.001**	57.917	3	**<0.001**
Population	162.974	3	**<0.001**	510.230	3	**<0.001**
Population × Treatment	46.995	9	**<0.001**	44.312	9	**<0.001**

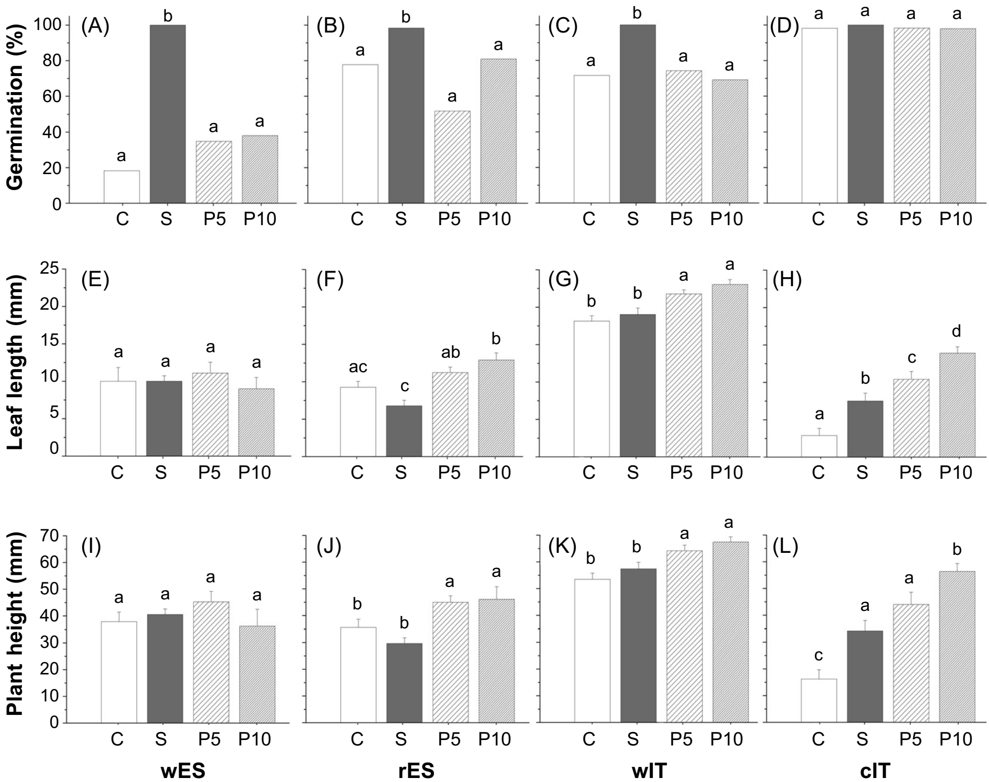

Figure 1. Mean germination percentage (**A**-**D**), leaf length (± s.e. **E**-**H**) and plant height (± s.e. **I**-**L**) in two wild populations (wES, wIT), one reintroduced population (rES) and one cultivated populated (cIT) of *Kosteletzkya pentacarpos* subjected to four scarification treatments [control (C), manual scarification (S), 5 and 10 minutes of percussion (P5, P10)]. Different letters indicate significant differences on measured variables at $P < 0.05$ level.

Hand-scarification of seeds of *K. pentacarpos* of wild origin resulted in more successful germination compared with the control and other treatments (→ 100%), confirming the presence of physical dormancy in this species (Poljakoff-Mayber *et al*., 1992). However, while Poljakoff-Mayber *et al*. (1994) reported that the permeability of the seed coat to water in *K. pentacarpos* differs in seeds harvested in different years, our study highlight significant variation of dormancy traits also across wild populations and in response to cultivation and reintroduction, showing a significant decrease in rES (compared with the related wES) and disappearance in cIT. Changes in seed characteristics (e.g. dormancy or germination) have often been observed in plants cultivated *ex situ* (Brancaleoni *et al*., 2018), where the loss of seed dormancy may be due to unconscious selection of plants resulting from non-dormant seeds (Ensslin *et al*., 2011). Given the high degree of non-dormant seeds found in wIT and used for cultivation in the Botanic Garden (i.e. 71%), this latter possibility cannot be ruled out.

Knowledge of the variation in degree of dormancy has allowed selection for either dormancy or non-dormancy (see e.g. Burson *et al*., 2009). In the case of *K. pentacarpos*, lack of dormancy may be advantageous for the cultivation of the species, in particular for the production of biofuel or textiles. However, a reduction of dormancy may limit the translocation (e.g. reintroduction) of the species, as it may lead to an increase in recruitment and consequent reduction of the soil seed bank, which is an important way of plant population persistence (Stocklin *et al*., 1999).

Manual scarification resulted in visible damage in the form of cuts or missing parts in the cotyledons, but did not affect the first true leaves. Damage of cotyledons due to scarification treatments have been rarely investigated, though they have been found to negatively affect subsequent plant growth performance (see e.g. Giertych and Suszka, 2011; Hossain *et al*., 2018;). Consistently, in our study, plant height and leaf length were shorter in seedlings from hand-scarified seeds for three of the four populations tested (i.e. rES, wIT, cIT; figure 1F-H, C). In contrast, 10 minutes-percussed seeds developed into the tallest seedlings with longest leaves, indicating that this treatment may enhance growth, at least in the early phases. Further research is needed to explain this response; for example, other mechanical seed treatments, such as ultrasonic waves have shown to induce micro-cracks on cell walls, improving water permeability and oxygen entry, and thereby germination and other growth traits (Sharififar *et al.*, 2015; Nazari and Eteghadipour, 2017). Another possibility is that percussion could weaken the seed coat, resulting in lower energy required for radicle protrusion and, consequently, availability of more storage reserves for subsequent growth.

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