Canella, Rossi, Mondoni and Guzzon (2020).
Seed Science and Technology, **48**, 2, 189-199.
https://doi.org/10.15258/sst.2020.48.2.06

Promoting seed germination of *Bunias erucago*, a Mediterranean leafy vegetable

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(Submitted January 2020, Accepted March 2020; Published online April 2020)

Abstract

Knowledge of germination and propagation requirements are crucial for the use of plant genetic resources. Here, we compare different treatments to improve the germination of *Bunias erucago* (Cruciferae), a Mediterranean vegetable herb of growing commercial interest. Its cultivation is limited by the poor germination shown by the seeds which are enclosed in indehiscent silicles. To improve the germination, we investigated four treatments: (1) pre-soaking silicles in water for 48 hours; (2) scarification of silicles with sandpaper; (3) seed extraction; and (4) seed exposure to gibberellic acid (GA_3). The same treatments (except the GA_3 treatment) were also used to investigate seedling emergence under glasshouse conditions. In the absence of treatments and/or soaking in water, there was very low final germination (< 10%), while scarification and seed extraction increased the germination to ~ 40% and > 90%, respectively. Scarification was the most effective treatment in a horticultural context, since the brittleness of seeds makes their extraction too delicate and time-consuming. The seedling emergence results confirmed those of seed germination in the laboratory, underlining the effectiveness of the treatments for plant cultivation. Seed germination performance varied among wild populations, underlining the importance of provenance when using wild plants as new crops.

Keywords: dispersal unit, dormancy, neglected crops, seed germination, useful plants

Introduction

Neglected crops and wild food plants represent genetic resources of great importance for food security, for future agriculture as new or re-discovered crops, and in supporting people's livelihoods in rural communities (Hammer *et al*., 2001; Sõukand, 2016; Ulian *et al*., 2019). Not only are they often adapted to thrive under marginal conditions, but they also have interesting organoleptic characteristics (Ceccanti *et al.*, 2018). The utilisation of additional edible plants is important to diversify our diets that are currently based on an extremely small number of crop species (Li and Siddique, 2018). Most neglected crops and wild food plants have an additional importance for local cultural identities because they are associated with traditional food products and local recipes (Padulosi and

Hoeschle-Zeledon, 2004; Savo *et al*., 2019). One of the crucial steps for the use of wild useful plants and neglected crops is to make them accessible and attractive for growers (Khoury *et al*., 2019). Poor knowledge of the germination requirements often limits their cultivation and use (Benvenuti and Parossi, 2016).

Corn rocket (*Bunias erucago* L.) is an annual or biennial herb in the Brassicaceae family, which includes several other Mediterranean crops and edible wild plants (for example, the genera *Eruca*, *Brassica*, *Sinapis*, *Raphanus* and *Capsella*). *B. erucago* is considered relatively widespread in the south Euro-Mediterranean sub-region (Wilson, 1852; Saatkamp *et al.*, 2014), where it naturally grows in nutrient poor soils such as ruderal environments and dry meadows. In some European areas, *B. erucago* is considered a weed. In the USA, it is considered a potential minor invader and grain contaminant (Bojňanský and Fargašová, 2007; USDA, 2016). *B. erucago* is characterised by branched, erect stems, growing 200-600 mm-tall. It has flowers that change colour from cream to yellow during the day in accordance to light strength and glucosinolate concentration (Bennett *et al.*, 2006; Ishida *et al.*, 2014). *B. erucago* is considered a potential nutraceutical crop (Benvenuti and Parossi, 2016), showing high levels of β-carotene, vitamin E and polyphenols (Ranfa *et al*., 2013).

Several ethnobotanical surveys underlined the use of this species as a wild collected leafy vegetable and as an ingredient in many traditional dishes: it is mainly consumed raw in salads (Salisbury, 1961; Di Novella *et al*., 2013; Romano *et al*., 2013) or as a cooked vegetable especially in Italy (Guarrera and Savo, 2005) and the Balkans (Dolina and Łuczaj, 2014; Łuczaj and Dolina, 2015). In addition, a recent ethnobotanical field survey (Ardenghi *et al*., 2017) documented that *B. erucago* is not only harvested in the wild but also cultivated, as a neglected crop, in backyards in the province of Pavia (Lombardy region, northern Italy) where it is locally known as "*landar*", "*landra*" or "*barlánd*" and used to enrich a typical rice dish called "*ris e landar*". The cultivation of *B. erucago*, once much more widespread in northern Italy, is currently very limited and mostly carried out by elderly farmers. In addition, the wild populations have shown a marked decline in the last decades due to the effects of intensive agriculture (e.g. wider use of herbicides), which limits its use as a wild food plant. Nevertheless, in recent years, a growing interest of local communities of the Province of Pavia to cultivate and use *B. erucago* can be noticed (Guzzon *et al*., 2019). In the Province of Pavia, propagation material, in the form of silicles, is sold by at least two small companies. The standard growing procedure for *B. erucago* is to sow its peculiar dispersal units. These dispersal units are four-chambered, indehiscent silicles, with ovoid shapes, presenting four cristate wings and a subconical beak (Bojňanský and Fargašová, 2007; figure 1). In some other cases, local growers leave the plant to self-sow the silicles, often hundreds per plant. Local growers use this self-sowing method to overcome the poor germination of this species. But even if this practice might be effective for very small production, it presents some obvious weaknesses such as the impossibility of crop rotation and tillage. The poor germination of the seeds, which remain enclosed in the silicles, is recognised as the most limiting factor in the cultivation of *B. erucago* (Benvenuti and Parossi, 2016), a fact that one of the small seed traders who produce and commercialise this species in northern Italy could confirm (Battista Carrara, *personal communication*).

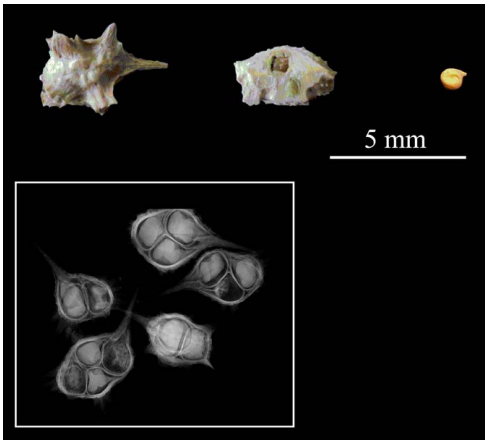

Figure 1. Silicles of *Bunias erucago*. Top, left to right: silicle, scarified silicle and extracted seed. Bottom: X-ray scan of silicles.

The aim of this study was to identify treatments that could improve the germination performance of *B. erucago* and therefore enhance its cultivation as a leafy vegetable, considering the importance of this species in the traditional food knowledge of several Mediterranean areas in Europe.

Material and methods

Seed lots and treatments

Silicles were collected at the time of natural dispersal (June-July 2019) from two wild populations growing in the province of Pavia (Becca and Moranda localities; table 1) and from a home garden cultivation carried out for four consecutive years by of one of the authors (G. Rossi), using seeds of a mixed seed lot of wild and commercial seeds (Fratelli Carrara, San Genesio ed Uniti, Italy); silicles purchased from this seed company, produced in 2019, were also tested. Silicle weight, seed weight and number of seeds per silicle were determined by weighing 20 individual silicles or seeds from each seed lot using an ALJ 220-4 nm microbalance (Kern & Sohn GmbH, Balingen, Germany).

Germination tests in the laboratory

Immediately after harvest, silicles were brought to the germination laboratory of the University of Pavia. In order to increase the germination performance of *B. erucago*, we tested different treatments for all the seed lots: (1) soaking silicles for 48 hours in distilled water (as recommended by some Italian growers); (2) scarifying silicles with commercial sandpaper (FEPA P180, 3M, USA); (3) extracting seeds from silicles; and (4) exposing manually-extracted seeds from silicles to gibberellic acid (GA_3, 0.25 g l^{-1} in agar medium). Finally, as a control, silicles were sown without any treatment. Germination

Table 1. Information on the four *Bunias erucago* seed lots used in the experiments.

Seed lot	Collection site	Average silicle weight (mg) ± standard deviation	Average seed weight (mg) ± standard deviation	Average number of seeds per silicle ± standard deviation
Carrara	Purchased at Fratelli Carrara (San Genesio ed Uniti, Italy)	24.6 ± 7.2	2.5 ± 1.3	1.9 ± 0.6
Home garden	Carbonara al Ticino (Pavia, Italy)	21.9 ± 2.5	2.5 ± 0.1	2.9 ± 0.4
Wild (Moranda)	Loc. la Moranda, Albaredo Arnaboldi (Pavia, Italy) 45°06'53.6"N, 9°14'10.8"E	24.1 ± 5.6	2.6 ± 0.7	2.0 ± 0.3
Wild (Becca)	Loc. Ponte della Becca (Linarolo, Italy) 45°08'58.3"N, 9°13'43.0"E	27.5 ± 4.2	2.7 ± 0.1	2.9 ± 0.8

tests were performed in light and temperature-controlled incubators (LMS 250A, LMS Ltd., Sevenoaks, UK), sowing three replicates of 20 silicles or seeds each per treatment on 1% agar in 90 mm-diameter Petri dishes. In the tests with silicles as sowing units, the total number of seeds sown and germinated was calculated for each seed lot (table 1). The Petri dishes were put in transparent plastic bags to avoid evaporation and randomly positioned in the incubators. Each treatment was tested at two alternating temperatures (25/15°C and 10/15°C) using a 12-hour daily photoperiod (light was provided during the warm phase; photosynthetically active radiation 40–50 μmol m^{-2} s^{-1}; LMS 250A). The two temperature and light conditions were chosen based on the results of preliminary trials (F. Guzzon, unpublished). Seed germination was defined as visible radicle protrusion and elongation > 2 mm and it was checked daily for four weeks. For the tests in which silicles were the sowing units, we considered both the percentage of germinated silicles (at least one seed germinated per each silicle) and the percentage of germinated seeds (calculated by multiplying the number of silicles sown and the number of germinated silicles by the average number of seeds per silicle; table 1). As an additional parameter, we calculated the percentage of germinated silicles, since silicles are currently the propagation material sown by growers and in a horticultural context one germinated seed per silicle is enough to allow cultivation. Upon completion of each germination test, ungerminated silicles and seeds were cut-tested with empty seeds or silicles disregarded in the dataset.

Seedling emergence in the glasshouse

The same treatments used in the laboratory (except the GA_3) were repeated in nursery pots in a non-heated glasshouse at the Botanic Garden of the University of Pavia, in order to understand the effects of the germination treatments on seedling emergence as well as to obtain data from conditions closer to a natural/cultivation environment. For this

glasshouse test, we used the seed lot with the highest final germination (home garden). Pots were filled with sifted sand collected at one of the wild population sites (Becca) and watered every other day during the whole duration of the experiment (six weeks). Maximum, minimum and average temperatures were recorded at one-hour intervals using a Tinytag View2 data logger (Gemini, Chichester, UK) located at the surface of the central pot. Seedlings emergence was checked daily for six weeks. As for the laboratory tests, for each treatment we considered both the percentage of germinated silicles and the percentage of germinated seeds.

Data analysis

Final seed germination and seedling emergence were analysed by means of Generalized Linear Models (GLMs), using a binomial probability distribution and a logit link function. Pairwise comparisons were utilised to find differences among treatments. Furthermore, we calculated the mean germination time (MGT) of seeds extracted from the dispersal unit, expressed in days, following Ellis and Roberts (1980):

$$MGT = \Sigma (nt) / N$$

where n = number of seeds that germinated at time T; T = days between the beginning of the test and the measurement; N = total number of germinated seeds. Other treatments were not considered in terms of MGT due to the low final germination percentage. An additional GLM was used to analyse MGT values, using a gamma distribution and an identity link function. All analyses were computed in SPSS 21.

Results

Germination tests in the laboratory

Final seed germination differed significantly among seed lots (Wald $\chi^2 = 19.21$, d.f. = 3, $P < 0.001$), incubation temperatures (Wald $\chi^2 = 20.04$, d.f. = 1, $P < 0.001$) and treatments (Wald $\chi^2 = 917.45$, d.f. = 4, $P < 0.001$). Only data from the temperature treatment 25/15°C are shown (figure 2), since this treatment achieved the best germination results. The interactions between seed lots and treatments (Wald $\chi^2 = 64.73$, d.f. = 12, $P < 0.001$) and seed lot and temperature (Wald $\chi^2 = 27.57$, d.f. = 3, $P < 0.001$) were also significant, while no significant differences were found among replicates (Wald $\chi^2 = 1.16$, d.f. = 2, $P = 0.56$). There were no silicles with more than one seed that germinated. In all seed lots, seed germination from intact silicles was very low (< 5% germinated seeds or silicles). Soaking in water did not improve the germination, except for the Becca seed lot at 25/15°C. Nonetheless, the final germination of the soaked silicles was always lower than 10%. Mechanical scarification of silicles with sandpaper significantly improved the germination compared with untreated silicles (Wald $\chi^2 = 0.01$, d.f. = 1, $P < 0.001$), which ranged from 8.2 to 18% (seeds) and from 20 to 46.6% (silicles). Seeds extracted from the silicles and exposed to 25/15°C showed the highest final germination, though with significant differences between the wild seed lots (78 to 98%) and the commercial seed lot Carrara (36.7%). Adding GA_3 to the germination medium significantly improved the germination

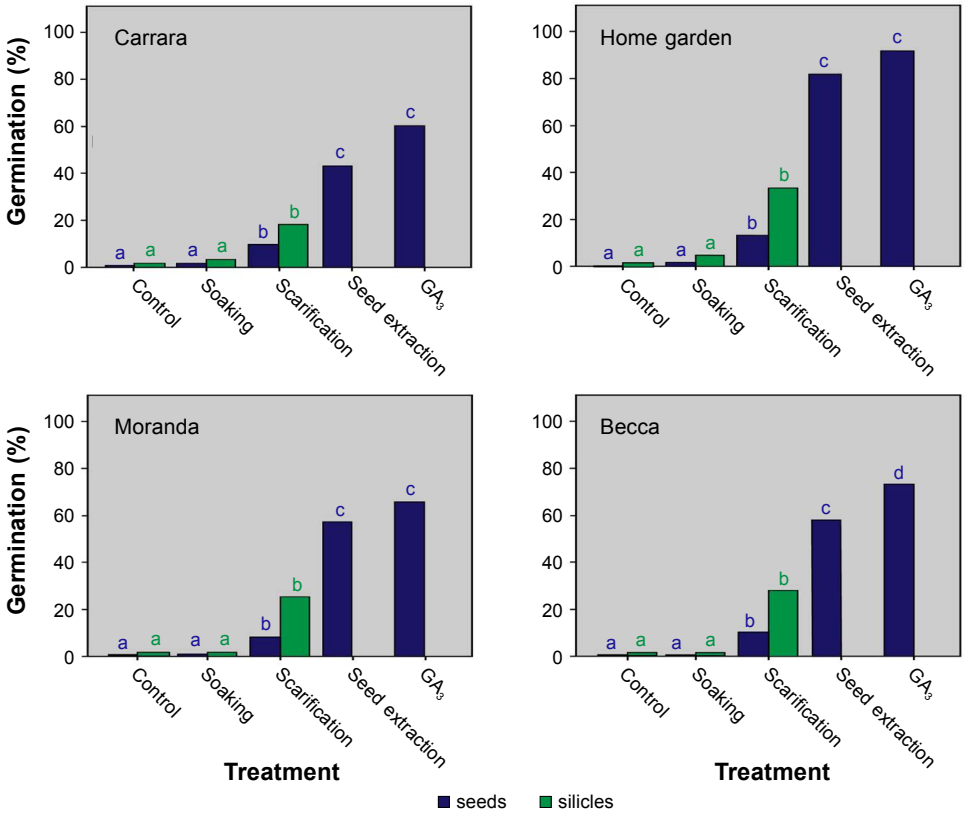

Figure 2. Final germination at 25/15°C of each *Bunias erucago* seed lot after different treatments: silicle soaking, silicle scarification, seed extraction, application of GA_3 and control (no treatment). Blue columns represent mean final germination of seeds, green columns show the final mean germination of silicles. Different letters above the columns indicate statistically significant differences ($P < 0.05$). Comparisons were made separately for seeds and silicles.

of extracted seeds in just one seed lot (Becca at 15/5°C Wald $\chi^2 = 0.07$, d.f. = 1, $P = 0.007$; and at 25/15°C Wald $\chi^2 = 0.05$, d.f. = 1, $P = 0.029$). Significant differences in MGT were observed among populations (Wald $\chi^2 = 31.66$, d.f. = 3, $P < 0.001$), incubation temperature (Wald $\chi^2 = 33.17$, d.f. = 1, $P < 0.001$) and their interaction. MGT was faster at 25/15°C, showing the highest value for the Carrara seed lot (5.6 days) and the lowest value for the home garden seed lot (2.0 days) (figure 3).

Seedling emergence tests in the glasshouse

The average air temperature during the glasshouse experiment was 16.0°C (mean minimum 11.6°C, mean maximum 24.4°C; figure 4). Seedling emergence varied significantly among treatments (Wald $\chi^2 = 49.13$, d.f. = 3, $P < 0.001$). No significative differences were found among replicates (Wald $\chi^2 = 0.02$, d.f. = 2, $P = 0.99$). Seedling emergence was 86.7% for seeds extracted from silicles, while it was significantly lower in seeds enclosed in untreated silicles

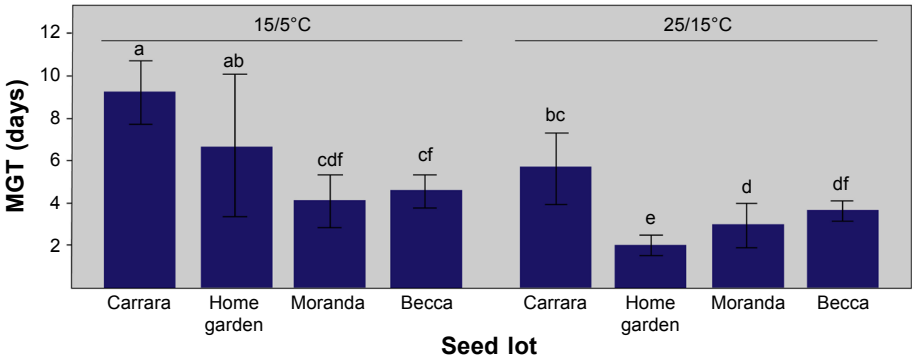

Figure 3. Mean germination time (MGT) of extracted *Bunias erucago* seeds at 15/5°C and 25/15°C. Lengths of error bars are double the standard error. Different letters above bars indicate statistically significant differences ($P < 0.05$). Comparisons were made considering the whole dataset.

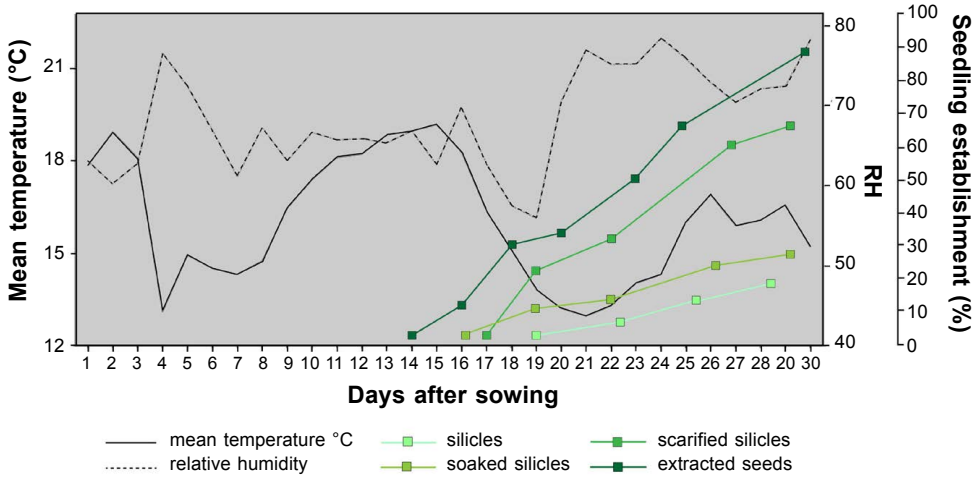

Figure 4. Cumulative seedling emergence of *Bunias erucago* (home garden seed lot) sown in the glasshouse after different treatments: silicle soaking, silicle scarification, seed extraction and control (no treatment). Percentages are based on the number of sown units (silicles or seeds) that showed germination. Lines show the mean daily air temperature (solid line) and relative humidity (dashed line) during the cultivation.

(18.3% for germinated silicles, 7.2% for germinated seeds). Soaking resulted in a seedling emergence of 11.5% (26.7% for germinated silicles), which was not significantly different from that of untreated silicles (Wald $\chi^2 = 0.03$, d.f = 1, $P = 0.28$). As for the experiments in the laboratory, scarification significantly improved the seedling emergence compared with untreated (Wald $\chi^2 = 0.04$, d.f. = 1, $P < 0.001$) and soaked silicles (Wald $\chi^2 = 0.04$, d.f. = 1, $P < 0.001$), showing a final value of 25.9% (66.7% for germinated silicles). Unlike in the laboratory tests, we found that multiple seedlings per silicle emerged in the glasshouse experiment, although this was infrequent: 6.7% for the untreated silicles, 8.3% for soaked silicles and 23.3% for scarified silicles.

Discussion

The germination experiments, both in the laboratory and the glasshouse confirmed the observation of farmers that sowing untreated silicles results in extremely low germination. Similarly, soaking in water does not lead to a significant increase in germination. The results of this study (i.e. the very high germination of extracted seeds compared with the very poor germination of seeds enclosed in the silicles) suggest that the indehiscent silicles inhibit germination in *B. erucago*. The low germination performance is likely to be due to a physical constraint imposed by the dispersal unit, something which is documented for the congeneric *B. orientalis* (Gomez-Campo, 1990; Royal Botanic Gardens Kew, 2019). Seed dormancy and the ability to form a persistent soil seed bank are typical strategies of plants which grow in variable environments with unpredictable resource availability (Guzzon *et al*., 2018; Peng *et al*., 2018). Such strategies are well documented for several species belonging to the Brassicaceae family (Lu *et al*., 2010; Eskandari *et al*., 2017). As showed by Cousens *et al*. (2010) for *Raphanus raphanistrum* L., the persistency of fruit wall delays germination and is involved in water regulation, both limiting imbibition of seeds and slowing seed desiccation. Although we did not study the soil seed bank in the current paper, a physical restriction of germination imposed by the dispersal units may contribute to its formation. This possibility is supported by the fact that this species grows in ruderal environments characterised by a dry, sandy substrate, low nutrient input and recurring soil manipulations (Dölle and Schmidt, 2009).

In our experiments, the scarification of the silicles with sandpaper significantly improved the germination in all seed lots. Though seed germination in scarified silicles never exceeded 20% (Moranda at 25/15°C), the percentage of germinated silicles was significantly higher than in the control (reaching 47% for Moranda). Seeds extracted from the silicles had the highest germination, a result which is consistent with the findings of Benvenuti and Parossi (2016). Even if the manual extraction of seeds from the silicles leads to high germination of *B. erucago*, this treatment is time-consuming and certainly not feasible for large quantities of material in a commercial context. In addition, it could lead to loss of material since, if not properly performed, the fragile seeds can be damaged, especially at their thin, pointed end. On the other hand, scarification of silicles with sandpaper significantly improved the silicle germination and is more likely to be practicable at a commercial scale. The germination performance should therefore be evaluated on a sowing-unit basis. For example, for *Beta vulgaris* L., the official minimum germination which is required to reach 50-70% refers to germination of units (here referred as “clusters”), not to seed germination (European Union, 2002). In this context, the germination performance of scarified silicles would fit well to the EU minimum requirements for some Brassicaceae crops. Therefore, silicle scarification could be an effective method for growers to improve the currently poor germination of *B. erucago*. Moreover, scarified silicles sown on sand germinated better (approximately 66% of silicles) than the same treatment in the laboratory (approximately 45% of silicles), which suggests that sand is a better germination medium than agar for *B. erucago* silicles. This observation is in line with the growing conditions at its natural sites (dry, sandy soils). Silicle scarification could even lead to better results in the field than the ones

obtained in laboratory conditions. Further research is needed to reduce the time required for (or even automate) the scarification of the dispersal units, in order to make high performing propagation material available. Considering the use of plant hormones, the application of GA_3 improved the germination in only one seed lot (Becca, one of the two wild populations), suggesting a certain level of physiological dormancy in seeds of this population. In the commercial seed lot (Carrara) we observed the lowest germination, coupled with the longest MGT (5.58 ± 1.5 days at 25/15°C), suggesting either low vigour (perhaps due to a premature seed collecting or bad handling of the seeds) or higher dormancy in seeds of this seed lot. Further viability tests (e.g. tetrazolium test) should be applied to understand the poor gemination performance of this seed lot. The significant differences among wild populations and cultivated seed lots in the final germination and germination timing highlight how important it is to select the right source populations for *B. erucago* as a crop, in particular sampling new wild populations that show a high final germination. Finally, further treatments (dry after-ripening, cold stratification and the use of nitrates) that proved to be efficient in dormancy-breaking in Brassicaceae (for example, Hilhorst *et al*., 1986; Hilhorst and Karssen, 1988; Müller *et al*., 2006; Lu *et al*., 2015) should be tested in *B. erucago* in order to gain a better understanding of the seed dormancy mechanisms of this useful species.

Conclusion

Bunias erucago is a wild edible plant well represented in the folk plant knowledge of several Mediterranean areas and of great value as a leafy vegetable. The first barrier for its cultivation is represented by the physical constraint imposed by the dispersal unit. The extraction of seeds significantly enhanced germination while the practice of soaking in water, as currently used by growers, was not effective. However, since seed extraction is a time-consuming practice and not feasible on a large, commercial scale, thinning of silicles through scarification with sandpaper could be the most promising treatment from an agricultural perspective. The variability in final germination among the seed lots highlight the importance of collecting and testing several wild populations in order to identify high performing material with high germination and suitable for cultivation.

Acknowledgements

Fondazione Cariplo is gratefully acknowledged for funding this study in the context of the project “La Sostenibilità che Include”. The authors thank Silvia Bodino, Paolo Cauzzi and Silvano Lodetti (University of Pavia) for their help during seed curation and the setting up of the glasshouse experiment. We thank Valeria Cavalloro (University of Pavia) for fruitful considerations concerning glucosinolates in *B. erucago*. We are also indebted to Maraeva Gianella (University of Pavia) for her valuable suggestions on data analysis. Finally, we thank Jonas V. Müller for language editing of the text and valuable suggestions to improve the clarity of the manuscript.

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