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Seed ecology and ex situ conservation of *Aegilops* and *Citrullus* in the context of climate change

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Contents

Abstract

In the next decades extreme climate conditions will pose new challenges to agricultural production and therefore to global food security. To cope with this challenge, the selection of new crop cultivars with favourable traits, such as increased drought tolerance, will be of key-importance. Considering the eroded genepool of modern crops, the selection of resilient cultivars is achievable only by conserving and characterizing the widest possible genepool for each crop, mainly in terms of landraces and crop wild relatives (CWR). In this thesis, I studied the germination ecology of landraces and CWR in relation to drought stress adaptations. In addition, my work aimed at finding and bridging gaps in the seed conservation of landraces and CWR, by improving seed conservation standards and passport data of seed accessions. Two genera were chosen as case study: *Aegilops* (the secondary genepool of wheat, *Triticum*) and *Citrullus* (the primary genepool of watermelon). Both genera hold a great importance for food security and breeding and grow in semi-arid and arid environments, showing interesting adaptations towards abiotic stresses.

I investigated seed germination under different drought stress of different populations of *Ae. geniculata* collected from a rainfall gradient. Different accessions of *Triticum aestivum* and *T. durum* were used as comparison. Despite *Ae. geniculata* was proved to be a drought tolerant species at germination level, significant differences in germination were detected among populations. Wheat and the southern population of *Ae. geniculata* were not significantly affected by drought stress. However, seed germination of the northern populations of *Ae. geniculata* was significantly reduced under high water stress. Differences between populations of the same species could be explained by rainfall experienced during seed development and maturation.

The response of seed germination and seedling growth to different water availabilities was also evaluated on the genus *Citrullus*, focusing on landraces of *Citrullus amarus* and in a commercial cultivar of *C. lanatus* (‘Sugar Baby’). ‘Sugar Baby’ germinated better to lower water potential than all *C. amarus* landraces, while seedling establishment was much higher in *C. amarus* than in *C. lanatus*. Finally, seedling growth of *C. amarus* landraces and the *C. lanatus* cultivar followed different patterns depending on water availability and showing enhanced biomass growth under wet conditions, while no changes between dry and wet growing treatments, respectively. The different water use strategies found in the two crops highlight drought avoidance strategies linked to the species growing environment in *C. amarus*, not present in the *C. lanatus* cultivar.

As a genus with only annual species, regeneration from seeds in *Aegilops* is crucial. In several species in *Aegilops*, spikes produce different seed morphs, both in size and germination patterns. I investigated the germination phenology of *Ae. neglecta* under laboratory and field conditions and assessed longevity of different seed morphs of five additional *Aegilops* species using controlled ageing tests. My results indicate that different longevities in seed morphs of *Aegilops* may reflect a different soil seed bank persistence, with small seeds being longer lived than big seeds, since they have to persist longer before germination. Differences of seed germination and longevities between seed morphs detected here may have important implications for the *ex situ* seed conservation and for plant regeneration in variable environments.

The conservation, accessibility and use of seed accessions of landraces and CWR stored ex situ, relies on their correct taxonomic labelling and on the building of a searchable database that links collections together. I analysed the impact of taxonomic misnaming in the global databases of plant genetic resources (PGR) using, as a case study, the genus *Citrullus*. Taxonomic misnaming greatly limits PGR conservation with only 3% of the accessions listed in the databases correctly named; moreover, 28% were affected by taxonomic errors that prevent the establishment of the accessions' taxonomic identity, with consequences on their conservation and use. I have proposed a series of actions that could solve the extant misnaming issues in the databases and prevent their reoccurrence, allowing the correct conservation and the usability by the stakeholders of all accessions.

The results of these studies provide novel insights about the complicated regeneration ecology of the genus *Aegilops*. Moreover, drought resistance and drought avoidance adaptive traits, respectively, were detected, in *Aegilops* and *Citrullus,* linked with the plants growth environments. Finally, I have suggested practical measures to improve the ex situ seed conservation of PGR of crucial importance for food security, including how to properly conserve seed morphs with different longevities and how to avoid taxonomic misnaming in the passport data.

General statement

This thesis is based on the following four scientific articles published in index journals (ISI). I have personally planned, performed and written a significant portion of all of these papers.

I- Orsenigo S, **Guzzon F**, Abeli T, Rossi G, Vagge I, Balestrazzi A, Mondoni A, Müller JV (2017) Comparative germination responses to water potential across different populations of *Aegilops geniculata* and cultivar varieties of *Triticum durum* and *T. aestivum*. Plant Biology, 19, 165-171.

II- **Guzzon F**, Müller JV, do Nascimiento Araujo M, Cauzzi P, Orsenigo S, Mondoni A, Abeli T (2017) Drought avoidance adaptive traits in seed germination and seedling growth of *Citrullus amarus* landraces. South African Journal of Botany, 113, 382-388.

III- **Guzzon F**, Orsenigo S, Gianella M, Müller JV, Vagge I, Rossi G, Mondoni A (2018) Seed heteromorphy influences seed longevity in *Aegilops*. Seed Science Research, 28, 277-285.

IV- **Guzzon F**, Ardenghi NMG (2018) Could taxonomic misnaming threaten the ex situ conservation and the usage of plant genetic resources? Biodiversity and Conservation, 27, 1157-1172. Selected for topical collection: Ex-situ conservation.

With the exception of the Chapter 1 (Introduction) and Chapter 6 (Discussion and conclusions), each chapter is a published manuscript. Because of this, there is some overlap between chapters, particularly in relation to the introduction and references paragraphs.

1. Introduction

1.1 Seed traits in landraces and crop wild relatives (CWR) to face the challenges of climate change

According to several predictions, climate changes will not only increase the global temperatures, but also the frequency and intensity of extreme climatic events such as droughts and irregular rainfall (Meehl and Tebaldi 2004; Orlowsky and Seneviratne 2013). This will greatly affect plant life and consequently natural ecosystems and agricultural production (Orsenigo et al. 2014; Lesk et al. 2016). Climate-change driven drought events are already causing significant yield losses for many food crops worldwide (Lesk et al. 2016; Zhang et al. 2011). Therefore, in the next decades, extreme climate conditions, a growing human population, lower availability of natural resources and the need to minimize impacts on ecosystems, will pose new challenges to global food security (Godfray et al. 2010; Asseng et al. 2015).

To cope with the aforementioned challenges, both agricultural production and sustainability need to increase (Tilman et al. 2002). The selection of new crop cultivars with favourable traits such as increased drought tolerance will be key to this process (Esquinas-Alcazar 2005; McCouch et al. 2013). To meet this goal, the eroded genepool of modern crop plants needs to be broadened and the widest genetic diversity needs to be available and exploitable (Ford-Llyod et al. 2011; Guarino and Lobell 2011; Vincent et al. 2013; Warschefsky et al. 2014). This is achievable only by conserving and characterising the widest possible genepool for each crop (in terms of the genetic diversity that can be tapped into by scientists for plant breeding and crop improvement; McCouch et al. 2013). The bulk of genetic diversity in crops is found in landraces and crop wild relatives (CWR) (Camacho Villa et al. 2006). Landraces are defined as: “plant materials consisting of cultivated varieties that have evolved and may continue evolving, using conventional or modern breeding techniques, in traditional or new agricultural environments within a defined eco-geographical area and under the influence of local human culture" (Casañas et al. 2017). Conversely, CWR “include the progenitors of crops, as well as species more or less closely related to them” (Heywood et al. 2007).

CWR and crop landraces are known to be adapted to marginal environments and to tolerate high levels of abiotic stress (Zeven 1998; Camacho Villa et al. 2006; Mabhaudhi et al. 2016; Warschefsky et al. 2014). There are several examples of useful traits of CWR and landraces to improve crop performance in relation with abiotic and biotic stress resistance (Dodig et al. 2012; Hajjar and Hodgking 2017). As example, CWR and landraces has been used to provide crop resistance to several pest and diseases, increase tolereance to salinity, acidic-sulfate soil content and drought as well as improve yield and being selected as rooststock lines (Cohen et al. 2007; Hajjar and Hodgking 2017). The utilization of those plant genetic resources (PGR) requires an in-depth knowledge of their ecological characteristics, especially in relation to climate change adaptation (Dempewolf et al. 2014).

As part of the pre-breeding activities, an ecological characterisation of seed germination traits of CWR and landraces is of key importance. Seed germination is a key-event in plant life. Therefore, understanding the germination potential of seeds under drought conditions is an important parameter, because germination usually occurs at or near to the soil surface, where drought conditions may compromise radicle emergence and thus seedling establishment. This is particularly true for annual species in which regeneration from seeds is crucial (Albuquerque and de Carvalho 2003). Wild plant species show different adaptations to drought at germination stage. The two main ecological strategies plants use to withstand drought at seed stage are drought tolerance and avoidance, in which seeds are able to germinate or not under low water potentials (wp), respectively. This latter strategy prevents seedlings emergence during dry conditions, which are unfavourable for establishment (Evans and Etherington 1990; Gutterman 2000; Bader et al. 2009). Moreover, other adaptive strategies are involved in the germination ecology of annual species growing in marginal environments. In particular, seed heteromorphy, defined as the production of different types of seeds by a single plant, is a common strategy to cope with unpredictable climatic conditions (Bhatt and Santo 2016) and it is diffused in seveal crop genepools, especially among cereals (Venable 1985). Seed heteromorphy influences several other traits such as: seed dispersal ability, germination, seed dormancy (the incapacity of viable seeds to germinate under favorable conditions) and persistence in the soil (Duràn and Retamal 1989). The different germination strategies and patterns in the loss of viability detected in different seed morphs, suggest that different seed longevities could be found in different morphs (Dyer 2017). Seed longevity is an important plant trait, allowing not only seed persistence in the soil but also long-term ex situ plant conservation in germplasm banks (Walters et al. 2005; Probert et al. 2009).

1.2 Ex situ seed conservation of plant genetic resources (PGR)

In order to exploit potential useful traits embedded in CWR and landraces, those plants need to be long-term conserved as well as readily available for research and breeding programmes (McCouch et al. 2013). This is particularly important considering that in the last few decades, genetic erosion (the loss of genetic diversity in the form of alleles and genotypes as well as domestic crop accessions) as higher than the 70% has been observed in several areas of the world (Hammer et al. 1996; Veteläinen et al. 2009; van de Wouw et al. 2010). Therefore, several crop landraces went extinct or were subjected to unidirectional introgression or substitution from high-yielding modern cultivars (Ardenghi et al. 2018). Additionally, several CWR species are currently endangered (Bilz et al. 2011). In this scenario, it is of key-importance to long-term conserve agrobiodiversity defined as: “the diversity of crop species used in different agro-ecosystems, as well as the genetic diversity within and among crop and CWR accessions” (Last et al. 2014).

One of the most effective strategies to ensure the conservation and availability of CWR and landraces is through ex situ conservation in genebanks, biorepositories that preserve genetic material (McCouch et al. 2013; Davies and Allender 2017). In particular, seed banking shows several advantages as a long-term ex situ conservation strategy for plant species and populations. Seed material is relatively easy to collect, can be stored in small spaces, can provide a representative sample of the genetic variability within the species, remains viable for long periods and does not require a high level of maintenance (Li and Pritchard 2009; Riviere and Muller 2017). Worldwide there are more than 1750 genebanks containing over 7.4 million of seed accessions (FAO 2010; Davies and Allender 2017). Seed conservation of CWR and landraces accessions needs to be particularly efficient since it should aim at capturing all the possible adaptive traits in the seed samples (Warschefsky et al. 2014). Seed banking is a multistep step activity and therefore several prerequisites need to be met for a successful seed conservation scheme. The following steps were considered in the current work:

- understanding the germination requirements of the accessions, essential to perform viability testing and propagation activities (Guzzon et al. 2015);

- understanding the seed longevity of the accessions, to plan their viability testing, regeneration or recollection intervals (Hay and Probert 2013);

- implementing efficient PGR databases, linking together ex situ collections conserved worldwide, to make the seed material accessible and usable by end users; the conservation and use of PGR accessions relies on their correct taxonomic naming in the databases (Khoury et al. 2010).

The latter point is of particular relevance considering that all countries are nowadays highly dependent for research and breeding upon PGR conserved beyond their borders. This global interdependence, and therefore global flows of PGR, are likely to increase in order to cope with climatic changes (Galluzzi et al. 2016).

1.3 The study genera: *Aegilops* and *Citrullus*

Among thousands of crop genepools, the current thesis focussed on two plant genera: *Aegilops* L. and *Citrullus* Schrad..

The genus *Aegilops* includes 23 species of annual grasses (family Poaceae), occurring in southern Europe, North Africa, the Near East and western and central Asia. *Aegilops* species occur in various dry, thermophile and low nutrient, disturbed habitats (e.g. pastures, roadsides, field margins, wastelands; van Slageren, 1994). *Aegilops* represents the largest part of the secondary gene pool of wheat (*Triticum* L.), one of the most important and widely cultivated cereal crops, with over 700 million tonnes harvested annually (FAOSTAT 2017). *Aegilops* played a key role in wheat evolution and domestication and has been used extensively in wheat breeding programmes (Kilian et al. 2011; Vincent et al. 2013). Some species of the genus display seed heteromophy (the production of different types of seeds by a single plant; Bhatt and Santo 2016); it was hypothesized that the production of different seed morphs is linked to the germination phenology of the genus to cope with environments with unpredictable resources availability (Datta et al. 1970; Dyer 2004). However this latter aspect has not been fully clarified and despite the importance for breeding and food security of *Aegilops*, little is known the ecology of seed germination in this genus.

Citrullus is a xerophytic genus including seven herbaceous species diffused in desert and semi-arid areas of Africa and southwest Asia. Four species of the genus are annuals with the other three being perennials (Chomicki and Renner 2014). Three species of the genus are widely cultivated (*C. amarus* Schrad., *C. lanatus* (Thunb.) Matsum. & Nakai, *C. mucosospermus* (Fursa) Fursa) and one only locally (*C. colocynthis* (L.) Schrad.) (Paris 2015). Watermelons have the greatest world production of any cucurbit (family Cucurbitaceae), with 3.5 million hectares of agricultural land used to cultivate watermelons in 2014 when annual production reached 111 million tons, which was 9.5% of global vegetable production, grown on 6% of the area used globally for the cultivation of vegetables (FAOSTAT 2017). The genus is of great importance in terms of food security in desert areas (Mujaju et al. 2011; Modi and Zulu 2012). Genetic resources of the genus *Citrullus* that grow in arid areas, either as CWR or as landraces, are considered potential donors of useful traits to improve abiotic and biotic stress resistances of modern watermelon cultivars (*C. lanatus*; Hwang et al. 2011; Jarret 2014). As in many desert plant species, in several wild populations of *Citrullus*, seed dormancy was detected as well as specific temperature and photoperiod requirements (Ramirez et al. 2014; El-Keblawy et al. 2017).

The two genera, comprising mostly annual species, were chosen as study taxa because both hold a great importance for food security and breeding. Moreover, they show important, albeit very different, adaptations towards dry environments where they thrive.

1.4 Aims and structure of the work

The aim of this study was to clarify the germination ecology of landraces and CWR, in relation to drought stress adaptations, using the genera *Aegilops* and *Citrullus* as case study. Moreover, I investigated potential gaps in the ex situ seed conservation of those PGR, proposing practical solutions to fill those gaps. More in detail:

1) In **article I,** I studied the germination of *Aegilops* under osmotic stress (imitating the effects of drought). I used as study taxa *Aegilops geniculata* one of the most diffused species in the genus (van Slageren 1994). I selected different population along a rainfall gradient to understand if the precipitation experienced by the mother plant can influence the germination performance. I compared the germination of the wild populations with domesticated cultivars of wheat.

2) In **article II**, I studied the germination under osmotic stress of several landraces of citron melon (*Citrullus amarus*) compared with a modern dessert melon (*C. lanatus*) variety. Citron melon is a neglected crop of southern African origin cultivated worldwide that shows interesting adaptations towards arid environments especially at seedling stage (Kawasaki et al. 2000; Laghetti and Hammer

2007). To test the latter observation I performed a growth experiment applying two different water treatments to the study varieties.

3) In **article III,** I investigated the seed dimorphism of *Aegilops* and its influence on seed longevity and phenology. More in detail, I studied the seed longevity of different seed morphs of five *Aegilops* species using controlled ageing tests (CAT). I integrated the CAT with a field experiment to clarify the germination phenology of *Ae. neglecta* Bertol..

4) In **article IV**, I analysed the passport data of *Citrullus* seed accessions listed in the major databases that gather information about PGR conserved worldwide. The aims were to understand which are the most frequently nomenclature-related issues in databases of PGR accessions and to clarify if these issues could prevent or corrupt the usage of these accessions. Eventually, three accessions, that appeared to be misnamed in the databases, were propagated are re-determined.

References

Albuquerque FM., de Carvalho NM (2003) Effect of type of environmental stress on the emergence of sunflower (*Helianthus annuus* L.), soyabean (*Glycine max* (L.) Merril) and maize (*Zea mays* L.) seeds with different levels of vigor. Seed Science and Technology, 31, 465–467.

Ardenghi N, Rossi G, Guzzon F (2018) Back to beaked: *Zea mays* subsp. *mays* Rostrata Group in Northern Italy, refugia and revival of open pollinated maize landraces in an intensive cropping system. PeerJ, DOI 10.7717/peerj.5123.

Asseng S, Ewert F, Martre P, Rötter RP, Lobell DB, Cammarano D, Kimball BA, Ottman MJ, Wall GW, White JW, Reynolds MP, Alderman PD, Prasad PVV, Aggarwal PK, Anothai J, Basso B, Biernath C, Challinor AJ, De Sanctis G, Doltra J, Fereres E, Garcia-Vila M, Gayler S, Hoogenboom G, Hunt LA, Izaurralde RC, Jabloun M, Jones CD, Kersebaum KC, Koehler A-K, Müller C, Naresh Kumar S, Nendel C, O'Leary G, Olesen JE, Palosuo T, Priesack E, Eyshi Rezaei E, Ruane AC, Semenov MA, Shcherbak I, Stöckle C, Stratonovitch P, Streck T, Supit I, Tao F, Thorburn PJ, Waha K, Wang E, Wallach D, Wolf J, Zhao Z, Zhu Y (2015) Rising temperatures reduce global wheat production. Nature Climate Change, 5, 143–147.

Bader MY, Menke G, Zotz G (2009) Pronounced drought tolerance characterizes the early life stages of the epiphytic bromeliad *Tillandsia flexuosa* (2009) Functional Ecology, 23, 472–479.

Bhatt A., Santo A (2016) Germination and recovery of heteromorphic seeds of *Atriplex canescens* (Amaranthaceae) under increasing salinity. Plant Ecology, 217, 1069–1079.

Bilz M, Kell SP, Maxted N and Lansdown RV (2011) European Red List of Vascular Plants. Luxembourg, Publications Office of the European Union

Camacho Villa TC, Maxted N, Scholten M, Ford-Lloyd B (2006) Defining and identifying crop landraces. Plant Genetic Resources, Characterization and Utilization, 3, 373–384.

Casañas F, Simó J, Casals J, Prohens J (2017) Toward an evolved concept of landrace. Frontiers in Plant Science 8: Article 145 DOI 10.3389/fpls.2017.00145.

Chomiki G, Renner SS (2015) Watermelon origin solved with molecular phylogenetics including Linnaean material: another example of museomics. New Phytologist, 205, 526–532.

Cohen R, Burger Y, Horev C, Koren A, Edelstein M (2007) Introduced grafted cucurbit to modern agriculture. The Israeli experience. Plant Disease, 91, 916–923.

Datta SC, Evenari M, Gutterman Y (1970) Heteroblasty of *Aegilops ovata* L. Israel Journal of Botany, 19, 463–483.

Davies LR, Allender CJ (2017) Who is sowing our seeds? A systematic review of the use of plant genetic resources in research. Genetic Resources and Crop Evolution, 6, 1–10.

Dempewolf H, Eastwood RJ, Guarino L, Khoury CK, Müller JV, Toll J (2014) Adapting agriculture to climate change: a global initiative to collect, conserve, and use crop wild relatives. Agroecology and Sustaiable Food System, 38, 369–377.

Dodig D, Zorić M, Kandić V, Perović D, Urlan-Momirović G (2012) Comparison of responses to drought stress of 100 wheat accessions and landraces to identify opportunities for improving wheat drought resistance. Plant Breeding, 131, 369—379.

Duràn JM, Retamal N (1989) Coat structure and regulation of dormancy in *Sinapis arvensis* L. seeds. Journal of Plant Physiology 135, 218–222.

Dyer AR (2004) Maternal and sibling factors induce dormancy in dimorphic seed pairs of *Aegilops triuncialis*. Plant Ecology, 172, 211–218.

Dyer AR (2017) The seed ecology of Aegilops triuncialis: linking trait variation to growing conditions. Seed Science Research, 27, 183–198.

El Keblawy A, Shabana HA, Navarro T, Soliman S (2017) Effect of maturation time on dormancy and germination of *Citrullus colocynthis* (Cucurbitaceae) seeds from the Arabian hyper-arid deserts. BMC Plant Biology, 17:263 DOI 10.1186/s12870-017-1209-x.

Esquinas-Alcázar J (2005) Protecting crop genetic diversity for food security: political, ethical and technical challenges. Nature, 6, 946–953.

Evans CE, Etherington JR (1990) The effect of soil water potential on seed germination of some British plants. New Phytologist, 115, 539–548.

FAO (2010) The second report on the state of the world’s plant genetic resources for food and agriculture. Rome. http://www.fao.org/docrep/013/i1500e/i1500e.pdf. Accessed 20 July 2017.

FAOSTAT (2017) Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/en/#data. Accessed 20 July 2017

Ford-Lloyd BV, Schmidt M, Armstrong SJ, Barazani O, Engels J, Hadas R, Hammer K, Kell SP, Kang D, Khoshbakht K, Li Y, Long C, Lu B-R, Ma K, Nguyen VT, Qiu L, Ge S, Wei W, Zhang Z, Maxted N (2011) Crop wild relatives—undervalued, underutilized and under threat? Bioscience, 61, 559–565.

Galluzzi G, Halewood M, Noriega IL, Vernooy R (2016) Twenty-five years of international exchanges of plant genetic resources facilitated by the CGIAR genebanks: a case study on global interdependence. Biodiversity and Conservation, 25, 1421–1446.

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food Security: the challenge of feeding 9 billion people. Science, 327, 812–818.

Guarino L, Lobell DB (2011) A walk on the wild side. Nature Climate Change, 1, 374–375.

Gutterman Y (2000) Environmental factors and survival strategies of annual plant species in the Negev Desert, Israel. Plant Species Biology, 15, 113–125.

Guzzon F, Müller JV, Abeli T, Cauzzi P, Ardenghi NMG, Balestrazzi A, Rossi G, Orsenigo S (2015) Germination requirements of nine European *Aegilops* species in relation to constant and alternating temperatures. Acta Botanica Gallica, Botany Letters, 162, 349–354.

Hajjar R, Hodgkin T (2007) The use of Wild Relatives in crop improvement: A survey of developments over the last 20 years. Euphytica, 156, 1-13.

Hammer K, Knupffer H, Xhuveli L, Perrino P (1996) Estimating genetic erosion in landraces—two case studies. Genetic Resources and Crop Evolution, 43, 329–336.

Hay FR, Probert RJ (2013) Advances in seed conservation of wild plant species: a review of recent research. Conservation Physiology, 1, cot030.

Heywood V, Casas A, Ford-Lloyd B, Kell S, Maxted N (2007) Conservation and sustainable use of crop wild relatives. Agriculture, Ecosystems and Environment, 121, 245–255.

Hwang JH, Ahn SG, Oh JY, Choi YW, Kanga JS, Park YH (2011) Functional characterization of watermelon (*Citrullus lanatus* L.) EST–SSR by gel electrophoresis and high resolution melting analysis. Scientia Horticulturae 130, 715–724.

Jarret RL (2014) Observations on anatomical aspects of the fruit, leaf and stem tissues of four *Citrullus* spp. African Journal of Plant Science, 8, 521–527.

Kawasaki S, Miyake C, Kohoci T, Fujii S, Uchida M, Yokota A (2000). Responses to wild watermelon to drought stress: accumulation of an ArgE homologue and citrulline inleaves during water deficit. Plant and Cell Physiology, 41, 864–873.

Khoury C, Laliberté B, Guarino L (2010) Trends in ex situ conservation of plant genetic resources: a review of global crop and regional conservation strategies. Genetic Resources and Crop Evolution, 57, 625–639.

Kilian B, Mammen K, Millet E, Sharma R, Graner A, Salamini F, Hammer K, Özkan H (2011). *Aegilops*, pp. 1–76 in Kole C (ed.), Wild Crop Relatives: Genomic and Breeding Resources. Cereals. Berlin Heidelberg, Springer-Verlag.

Laghetti G, Hammer K (2007) The Corsican citron melon (*Citrullus lanatus* (Thunb.) Matsum. et Nakai subsp. *lanatus* var. *citroides* (Bailey) Mansf. ex Greb.) a traditional and neglected crop. Genetic Resources and Crop Evolution, 54, 913–916.

Last L, Arndorfer M, Balázs K, Dennis P, Dyman T, Fjellstad W, Friedel JK, Herzog F, Jeanneret P, Lüscher G, Moreno G, Kwikiriza N, Gomiero T, Paoletti MG, Pointereau MG, Sarthou J-P, Stoyanova S, Wolfrum S, Kölliker R (2014) Indicators for the on-farm assessment of crop cultivar and livestock breed diversity: a survey-based participatory approach. Biodiversity and Conservation, 23, 3051–3071.

Lesk C, Rowhani P, Ramankutty N (2016) Influence of extreme weather disasters on global crop production. Nature, 529, 84–87.

Li DZ, Pritchard HW (2009) The science and economics of ex situ plant conservation. Trends in Plant Sciences, 14, 614–621.

Mabhaudhi T, O'Reilly P, Walker S, Mwale S (2016) Opportunities for underutilised crops in Southern Africa's post-2015 development agenda. Sustainability, 8, Article 302, DOI 10.3390/su8040302.

McCouch S, Baute GJ, Bradeen J, Bramel P, Bretting PK, Buckler E, Burke JM, Charest D, Cloutier S, Cole G, Dempewolf H, Dingkuhn M, Feuillet C, Gepts P, Grattapaglia D, Guarino L, Jackson S, Knapp S, Langridge P, Lawton-Rauh A, Lijua Q, Lusty C, Michael T, Myles S, Naito K, Nelson RL, Pontarollo R, Richards CM, Rieseberg L, Ross-Ibarra J, Rounsley S, Hamilton RS, Schurr U, Stein N, Tomooka N, van der Knaap E, van Tassel D, Toll J, Valls J, Varshney RK, Ward J, Waugh R, Wenzl P, Zamir D (2013) Feeding the future. Nature, 499, 23–24.

Meehl GA, Tebaldi C (2004) More intense, more frequent, and longer lasting heat waves in the 21st century. Science, 305, 994–997.

Modi AT, Zulu NS (2012) Watermelon landrace seedling establishment and field performance in response to differing water regimes. African Journal of Agricultural Research, 7, 6016–6021.

Mujaju C, Zborowska A, Werlemark G, Garkava-Gustavsson L, Andersen SB, Nybom H (2011) Genetic diversity among and within watermelon (Citrullus lanatus) landraces in Southern Africa. Journal of Horticultural Science and Biotechnology, 86, 353–358.

Orlowsky B, Seneviratne SI (2013) Elusive drought: uncertainty in observed trends and short- and longterm CMIP5 projections. Hydrology and Earth System Sciences, 17, 1765–1781.

Orsenigo S, Mondoni A, Rossi G, Abeli T (2014) Some like it hot and some like it cold, but not too much: plant responses to climate extremes. Plant Ecology, 215, 677–688.

Paris HS (2015) Origin and emergence of the sweet dessert watermelon, *Citrullus lanatus*. Annals of Botany, 116, 133–148.

Probert RJ, Daws MI and Hay FR (2009) Ecological correlates of ex situ seed longevity: a comparative study on 195 species. Annals of Botany 104, 57–69.

Rivière S, Müller JV (2017) Contribution of seed banks across Europe towards the 2020 Global Strategy for plant conservation targets, assessed through the ENSCONET database. Oryx. https://doi.org/10.1017/S0030605316001496.

Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. Nature, 418, 671–677.

van de Wouw M, Kik C, van Hintum T, van Treuren R, Visser B (2010) Genetic erosion in crops: concept, research results and challenges. Plant Genetic Resources, 8, 1–15.

van Slageren MW (1994) Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. and Spach) Eig (Poaceae). Wageningen, Agricultural University Papers.

Venable DL (1985) The evolutionary ecology of seed heteromorphism. American Naturalist, 126, 577–595.

Veteläinen M, Negri V, Maxted N (2009) European landraces: on-farm conservation managment and use. Bioversity Technical Bulletin No. 15. Bioversity International, Rome.

Vincent H, Wiersema J, Kell SP, Dobbie S, Fielder H, Castañeda-Álvarez NP, Guarino L, Eastwood R, Leon B, Maxted N (2013) A prioritised crop wild relative inventory as a first step to help underpin global food security. Biological Conservation, 167, 265–275.

Walters C, Wheeler LM and Grotenhuis JM (2005) Longevity of seeds stored in a genebank: species characteristics. Seed Science Research, 15, 1–20.

Warschefsky E, Varma Penmetsa R, Cook DR, von Wettemberg EJB (2014) Back to the wild: tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. American Journal of Botany, 101, 1791–1800.

Zeven CA (1998) Landraces: a review of definitions and classifications. Euphytica, 104, 127-139.

Zhang H., Gong G., Guo D., Ren Y., Xu Y., Ling K.S. (2011) Screening the USDA watermelon germplasm collection for drought tolerance at the seedling stage. Hortscience, 46, 1245–1248.

Original papers

Article I

Orsenigo S, **Guzzon F**, Abeli T, Rossi G, Vagge I, Balestrazzi A, Mondoni A, Müller JV (2017) Comparative germination responses to water potential across different populations of *Aegilops geniculata* and cultivar varieties of *Triticum durum* and *T. aestivum*. Plant Biology, 19(2), 165-171.

Comparative germination responses to water potential across different populations of *Aegilops geniculata* and cultivar varieties of *Triticum durum* and *T. aestivum*

Abstract

Crop Wild Relatives (CWR) are often used to improve crop quality and yields, because they contain genetically important traits that can contribute to stress resistance and adaptation. Here, seed germination of different populations of *Aegilops geniculata* Roth collected along a latitudinal gradient was studied under different drought stress in order to find populations suitable for improving drought tolerance in wheat. Different accessions of *Aegilops neglecta* Req. ex Bertol., *Triticum aestivum* L. and *T. durum* Desf. were used as comparison.

Under full hydration, germination was high in all populations, but increasing drought stress led to reduced and delayed germination. Significant differences in final germination and mean time to germinate were detected among populations. Wheat, durum wheat and the southern population of *Aegilops geniculata* were not significantly affected by drought stress, germinating similarly under all treatments. However, seed germination of the northern populations of *Aegilops geniculata* was significantly reduced under high water stress treatment. Differences between populations of the same species could not be explained by annual rainfall across populations' distributions, but by rainfall during seed development and maturation. Differences in the germination responses to drought found here highlight the importance of source populations as criteria for genotype selection for pre-breeders.

Key words: *Aegilops*; Crop Wild Relatives; drought tolerance; seed germination; climate change; adaptation; crop improvement.

Introduction

Crop domestication resulted in significant changes in morphological, physiological and reproductive aspects compared to wild relatives (Hawkes 1983). Because of genetic drift, population bottlenecks and selection during breeding, many crops lack genetic diversity and show reduced fitness in suboptimal cultivation conditions (Warschefsky et al. 2014). In the future higher demand for food supply and extreme climatic conditions will pose new challenges to agriculture. According to future climate predictions, frequency and intensity of extreme climatic events, such as heat waves, drought and heavy and irregular rainfalls are expected to increase in frequency and intensity (Meehl and Tebaldi 2004; Orlowsky and Seneviratne 2013), with important consequences on agriculture and natural ecosystems (Challinor et al. 2014; Orsenigo et al. 2014; Lesk et al. 2016). To improve crop quality, achieve higher yields, or create pest and stress-tolerant varieties of crops, breeders often used Crop Wild Relatives (CWR) as gene or trait sources (Heywood et al. 2007; Maxted et al. 2008). In fact, CWR are wild species that have relatively close genetic relationships to crops, but that are not domesticated themselves (Maxted et al. 2006). They contain a wealth of genetically important traits that can contribute to improve biotic (e.g. pests) and abiotic (e.g. drought) stress resistance in crops, due to their adaptation to a diverse range of habitats and climatic conditions (FAO 2008; Vincent et al. 2013). Plant ecologists can provide useful information for breeding programs, contributing to connect with harmony agriculture and the environment (Brummer et al. 2011). Infact, wild-to-crop gene flow, requires an in-deep knowledge of wild species and their ecological and morphological characteristics, also in relation to climate change adaptation (Dempewolf et al. 2014). As part of the pre-breeding activities, an ecological characterization of seed germination behaviour and requirements of CWR is of fundamental importance, since germination is a key event in the plant life cycle. In this respect, determination of germination potential of seeds under drought conditions is an important parameter, because germination usually occurs at the soil surface, where drought conditions may compromise radicle emergence and then seedling establishment (Albuquerque and Carvalho 2003).

Wheat is one of the most important and widely cultivated cereal crops, with over 600 million tones harvested annually (Shewry 2009) and the genus *Aegilops* L. is one of the most important wheat ancestors, so similar in certain traits that it has been often considered as part of the genus *Triticum* (Petersen et al. 2006). The cultivation of allohexaploid bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.) originated several times about 10,000 years ago in the Fertile Crescent from independent hybridization of cultivated emmer with *Aegilops* (Feldman 2001). Kilian et al. (2011)

provide details of this strong relationship that makes *Aegilops* potentially interesting for wheat improvement (Nevo 2011).

For instance, *Ae. geniculata* was used to transfer rust resistance genes into cultivated wheat (Dhaliwal et al. 2002; Kuraparthy et al. 2007) and a complete set of *T. aestivum* – *Ae. geniculata* chromosome addition lines was obtained (Friebe et al. 1999; see also Schneider et al. 2008 for a survey of *Triticum*–*Aegilops* and *Aegilops*–*Triticum* hybrids developed under artificial conditions).

However, many studies evaluated the drought tolerance (Dhanda et al. 2004; Zhao et al. 2013) and germination under drought of *Triticum aestivum* and *T. durum* (Hampson and Simpson 1990; Al-Karaki 1998; Almansouri et al. 2001), but only few, mostly considering adult plants, have compared the performance of *Triticum* with *Aegilops* (Molnár et al. 2004; Molnár et al. 2008). Great efforts for improving drought tolerance in wheat were directed towards the use of the *Triticum* ancestor *Aegilops tauschii* Coss. (Sohail et al. 2011), using only a limited number of lines coming from *ex situ* collections. But other *Aegilops* species could be of interest to improve drought tolerance in wheat. Moreover, populations of the same species growing in different environmental conditions could respond differently to environmental stress, as demonstrated by Molnár et al. (2004) in *Aegilops biuncialis* Vis., indicating that even within species the choice of breeding source population is very important.

The aim of this study was to evaluate the germination response of different populations of *Ae. geniculata* selected along a rainfall gradient to six levels of water potential, in order to: a) identify population-specific water potential thresholds at which germination of *Ae. geniculata* is inhibited; b) compare *Ae. geniculata* germination response under drought stress to different *Triticum* varieties and c) develop a population, or environmental gradient approach, to use CWR for crop improvement.

Materials and Methods

Species used and seed collection

Aegilops geniculata Roth is a tetraploid species (2n = 4x =28) that grows in Southern Europe, North Africa and Middle East in dry, somewhat disturbed habitat such as wastelands, roadsides and dry rocky slopes of hills and mountains predominantly on limestone bedrock (Van Slageren 1994; Kilian et al. 2011). Together with *Ae. triuncialis* L. it is the most widespread species in the genus and grows under a wide latitudinal and annual rainfall amplitude. Importantly, *Ae. geniculata* belongs to the section *Aegilops*, which is of all sections in the genus *Aegilops* the most closely related to the genus *Triticum*.

Similarly, *Aegilops neglecta* Req. ex Bertol. (2n = 4x = 28 and 2n = 6x = 42) is widespread in Southern Europe and Turkey and grows in similar habitats as *Ae. geniculata* (Van Slageren 1994; Kilian et al. 2011), but is considered more frost resistant (Zaharaieva et al. 2004).

Seeds of *Ae. geniculata* were collected at the time of natural dispersal (Hay and Smith, 2003) in June and July 2014 from three different populations in Italy (Table 1). Populations were selected along a decreasing rainfall gradient that also corresponded to a north-south latitudinal gradient. Moreover, as demonstrated by Fandrich and Mallory-Smith (2006), the spikelet position on the spike could affect germination in *Aegilops*, because seeds from the upper fertile spikelets (0/1 in *Ae. neglecta*, 0/2 in *Ae. geniculata*) are in general smaller and less numerous than seeds from the lower fertile spikelet (Marañon 1989). Thus, we separated seeds of the lower spikelets from those of the upper spikelets and we considered them as separated seed lots during the germination tests.

Table 1. Species name, geographical and climatic data and population code of the four Aegilops accessions used in the study.

Species	Locality	Latitude	Longitude	Altitude (m a.s.l.)	Annual precipitation	May-June Precipitation	Pop. code
Ae. geniculata Roth	Casa d'Agosto (PV), Lombardy	N 44°53'	E 09°14'	520	1005 mm	154 mm	genPV
Ae. geniculata Roth	Monte Mauro (RA), Emilia-Romagna	N 44°14'	E 11°41'	320	803 mm	124 mm	genRA
Ae. geniculata Roth	Frascineto (CS), Calabria	N 39°50'	E 16°14'	570	839 mm	78 mm	genCS
Ae. neglecta Req. ex Bertol.	Barborina (PV), Lombardy	N 44°54'	E 09°02'	220	998 mm	148 mm	negl

The germination performance under different water potentials of *Ae. geniculata* was compared with *Ae. neglecta* and with three varieties of *Triticum* sp.pl.. Seeds of *Ae. neglecta* were collected from Northern Italy and used as intra-generic comparison. Seeds of three varieties of domesticated *Triticum* were used as comparisons. WL711 is a variety of *T. aestivum*, selected in India and known to be a drought sensitive cultivar (Devi et al. 2012). Seeds of WL711 were provided by the John Innes Centre (Norwich, UK). Senatore Cappelli and Ofanto are two Italian varieties of *T. durum*. Senatore Cappelli is a line originated at the beginning of the XX century in Southern Italy (landrace) while the cultivar Ofanto is a modern variety. The seeds of the two Italian cultivars were provided by CRA-CER (Foggia, Apulia, Italy).

Germination experiments

Germination experiments were conducted by one of the authors (F. Guzzon) in the laboratories of the Millennium Seed Bank (Royal Botanic Gardens, Kew, United Kingdom). Germination tests of the aforementioned accessions involved sowing three replicates of 20 seeds each at 20°C (constant temperature) and six different osmotic potentials (0 MPa, -0.8 MPa, -1 MPa, -1.3 MPa, -1.5 MPa and -1.7 MPa) for each temperature treatment. 20°C was chosen as the most appropriate temperature treatment to perform the current experiment based on the results of Guzzon et al. (2015), that suggested this temperature as the optimal constant temperature for the germination of different *Aegilops* species. Preliminary trials at -2 MPa revealed that this was the germination limit for most of the accessions, although occasionally few seeds germinated at this water potential too.

The treatments at full hydration (0 MPa) were carried out sowing the seeds on 1% distilled water–agar held in 90 mm diameter Petri dishes.

The water potential treatments involved sowing seeds on filter papers soaked with 10 ml of solution of polyethylene glycol 8000 (PEG) (Fisher BioReagents, UK), at a concentration appropriate to the intended treatment (Michel and Kauffmann 1973).

Petri dishes were placed in temperature and light-controlled incubators (LMS Ltd, Sevenoaks, UK) using a 12-h daily photoperiod (photosynthetically active radiation 40– 50 μmol m^{-2} s^{-1}) for 28 days. Seeds were daily checked for germination and germination was defined as visible radicle protrusion and elongation greater than 2 mm.

We calculated the mean germination time following the equation developed by Ellis and Roberts (1980):

$$MGT=\Sigma\ (n\ T)/\ N$$

where:

n= number of seeds that germinated at time T; T= are the days between the beginning of the test and the measurement; N= is the total number of seeds that germinated.

At the end of the tests the seeds were cut to evaluate if the non-germinated seeds after the treatment were viable or not.

Precipitation data

In the absence of local meteorological stations close to the selected populations, precipitation data for the study populations were obtained by querying WorldClim (version 1.4 release 3, www.worldclim.org) at the maximum resolution available of 30 arc-s (about 1 km; Hijmans et al., 2005). Data were extracted using the "point sampling tool" in QGIS 1.8.0 Lisboa.

Data analysis

Linear regressions were performed to highlight significant relationships between water potential and germination in *Aegilops* and *Triticum* accessions.

Differences in the response of germination to water potential between populations and species were investigated by comparing the regression parameters (slope and intercept). In this case, the regression slope provides an information on the strength of the effect of water potential on germination, while intercept corresponds to the germination percentage of the control (0 MPa). The specific H_0 tested is that two samples belong to two populations with the same slope and intercept (= same response to the water potential and same germination performance in the control). Because both, the slope and intercept, follow a *t* distribution, *t* was computed as shown below according to Soliani (2005), adjusted following Paternoster et al. (1998),

$b_1 - b_2 / S_{b1\text{-}b2}$ (slope)

and

$a_1 - a_2 / S_{a1\text{-}a2}$ (intercept)

where $b_1 - b_2$ ($a_1 - a_2$) is the difference between the slopes (intercepts) and $S_{b1\text{-}b2}$ ($S_{a1\text{-}a2}$) is the standard error of the differences between slopes (intercepts).

The *t*-values were then compared with a *t* distribution table. Degrees of freedom have been computed as

$df = n_1 + n_2 - 4df$

Where n_1 and n_2 represent the number of observations in each sample.

Regressions were performed in SPSS 21.0, while the comparison between regression slopes and intercepts were performed in Microsoft Excel. $S_{b1\text{-}b2}$ ($S_{a1\text{-}a2}$) were computed with the function STEYX in Excel.

Moreover, in order to evaluate if the germination behaviour found in the three populations of *Ae. geniculata* is the expression of an adaptation to local environmental conditions, the germination percentage at the lowest water potential tested (-1.7 mPa) was regressed against the total annual precipitation and the average precipitation of May and June (when the seeds ripen and are dispersed) of the sampling sites.

Results

Relationship between germination and water potential in Aegilops *and* Triticum

A significant positive relationship between germination and water potential was found in *Ae. geniculata* and *Ae. neglecta*, with high germination percentage at full moisture and a significant reduction and delay of germination in two out of three populations. On the contrary, no significant relationships between water potential and germination were found in *T. durum* and *T. aestivum* (Table 2). In the drought-sensitive cultivar WL711 the relationship became significant only when considering water potential from -1 downward (R^2 = 0.920; df = 3; F = 22.976; P = 0.041). The effect of water potential on seed germination and the germination in the control did not differ between northern populations of *Ae. geniculata* and *Ae. neglecta* (data not shown).

Table 2. Results from the linear regression between water potential and germination in *Ae. geniculata, Ae. neglecta, T. aestivum* and *T. durum.*

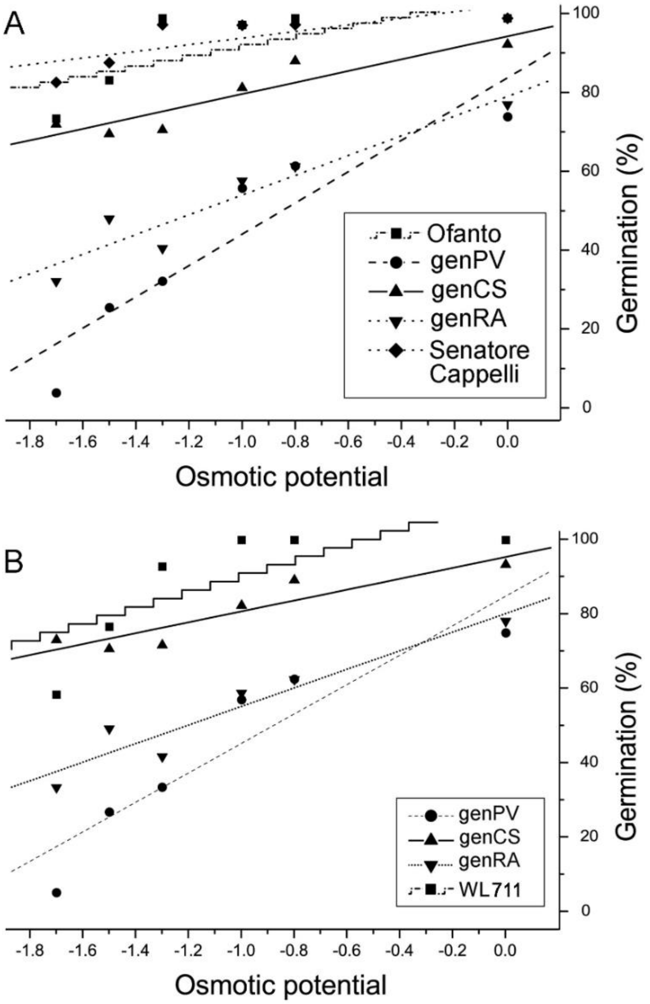
Species	Population (cv.)	R^2	df	F	P
Ae. geniculata	genPV	0.858	5	24.346	0.008
Ae. geniculata	genRA	0.907	5	38.927	0.003
Ae. geniculata	genCS	0.845	5	21.806	0.010
Ae. neglecta	negl	0.891	5	32.827	0.005
T. durum	(Ofanto)	0.505	5	4.074	n.s.
T. durum	(Sen. Cappelli)	0.586	5	5.656	n.s.
T. aestivum	(WL711)	0.571	5	5.319	n.s.

With respect to *Ae. geniculata*, the effect of water potential on seed germination was similar in northern Italian populations genPV and genRA, with a strong drop of germination with decreasing water potential (i.e. from 75% at 0 MPa to 35% at -1.3 MPa), as revealed by the high regression slopes. Seed germination of the southern Italian population genCS also decreased, but with a less abrupt trend (i.e. from 93% at 0mPa to ca. 70% at -1.7 mPa), as revealed by the comparison between the regression slopes (Table 3; Figure 1). This is reflected in a significant difference of the slopes between northern (genPV and genRA) and southern populations (genCS; t = 2.279; df = 8; $P < 0.05$), with northern populations showing a more negative effect (higher slope value) of water potential on germination than the southern population.

Table 3. Comparison of regression slopes from the regression between germination percentage and water potential in three populations of *Ae. geniculata* from different localities. The level of significance is above the diagonal, while the t-value is below the diagonal (Degrees of freedom = 8 in all cases).

	genCS	genRA	genPV
a) Slope			
genCS	-	<0.05	< 0.01
genRA	2.0334	-	n.s.
genPV	2.8973	1.6309	-
b) Intercept			
genCS	-	n.s.	<0.05
genRA	1.0281	-	n.s.
genPV	2.5177	0.4395	-

Figure 1. Comparison of the linear regression between osmotic potential and mean germination percentage in *Aegilops geniculata* and *Triticum durum* varieties (A) and *Triticum aestivum* (B). The figure highlights the difference in slope and intercept between genPV, genRA and genCS.



Differences between the first and second spikelet in Aegilops

The effect of water potential on germination percentage did not differed between seeds from the 1st and 2nd spikelet in all species and populations. However, the germination percentage of seeds from the 1st and 2nd spikelet significantly differed in negl (t = 2.211; P < 0.05; df = 8); genPV (t = 2.436; P < 0.05; df = 8) and genRA (t = 3.205; P < 0.01; df = 8), with the seeds from the 1st spikelets that germinate better than the seeds from the 2nd spikelets, except in genCS, with no significant differences between spikelets (Figure 2).

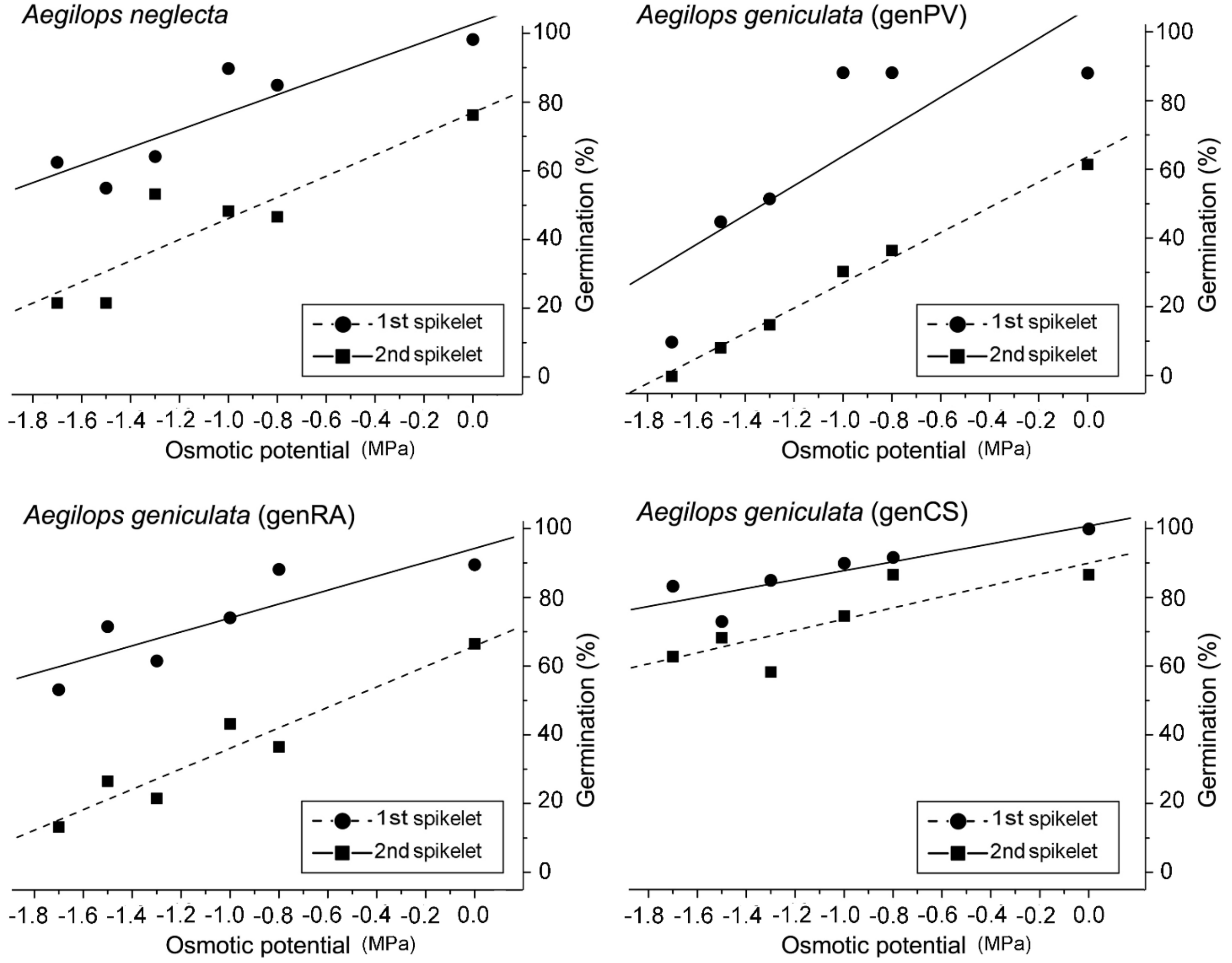


Figure 2. Comparison of the linear regression between osmotic potential and mean germination percentage *Aegilops geniculata* populations and *Aegilops neglecta*. The figure highlights a similar effect of the osmotic potential on germination (slope) and a different germination performance (intercept) between first and second spikelet.

Mean Time to Germination

A significant positive relationship between water potential and Mean Time to Germination (MTG) was found in all *Triticum* species and cultivars and in *Ae. geniculata* genCS and genPV, but not in genRA. MTG in *Ae. neglecta* did not show any relationship with water potential (Table 4).

Table 4. Results of the linear regression between water potential and Mean Time to Germination (MTG) in *Ae. geniculata*, *Ae. neglecta*, *T. aestivum* and *T. durum*.

Species (cv.)	Population	R^2	df	F	P
Ae. geniculata	genPV	0.695	5	9.124	0.039
Ae. geniculata	genRA	0.525	5	4.412	n.s.
Ae. geniculata	genCS	0.703	5	9.478	0.037
Ae. neglecta	negl	0.296	5	1.680	n.s.
T. durum (Ofanto)	-	0.819	5	18.140	0.013
T. durum (Sen. Cappelli)	-	0.844	5	21.557	0.010
T. aestivum (WL711)	-	0.834	5	20.148	0.011

Relation between drought tolerance and precipitation

Regressions between the germination percentage at the lowest water potential (-1.7 MPa) and rainfall of the sites of occurrence of *Ae. geniculata* yielded different results depending on the considered precipitation parameters. In fact, the germination at -1.7 MPa was not related to the total annual precipitation. A significant negative relationship was found between germination at -1.7 MPa and the average precipitation of May and June ($R^2 = 0.999$; df = 2; F = 1668.276; P = 0.016) was found (Figure 3).

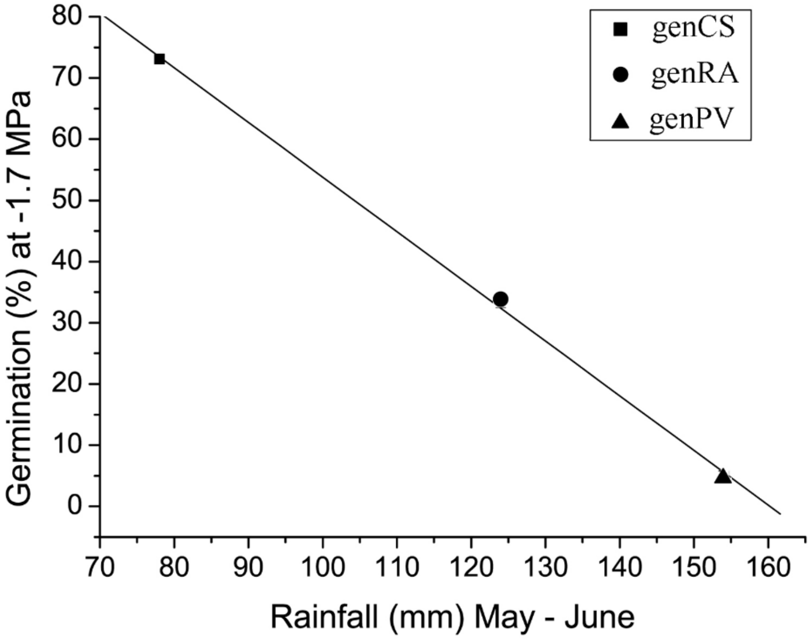


Figure 3. Linear regression between the mean germination percentage at the lowest osmotic potential (-1,7 MPa) and local precipitation recorded at each site of occurrence of *Aegilops geniculata*.

Discussion

In this study we investigated the response of seed germination of *Aegilops geniculata* under different levels of drought stress compared to cultivated wheat. The aim of this study was to determine if *Ae. geniculata* can be a useful donor of tolerance/resistance traits for improving germination drought tolerance in wheat crop improvement. The study of gene flow between cultivated wheat and wild *Aegilops* species revealed only a limited gene flow from wheat to *Ae. geniculata*, while *Ae. neglecta* and *Ae. triuncialis* showed substantial evidence of introgression (Arrigo et al. 2011); however, the hybridization between *Ae. geniculata* and *Triticum aestivum* is quite common, as witnessed by the natural hybrid *Aegilotriticum* × *requienii* (Ces., Pass. & Gibelli) P.Fourn. already described in XIX century (Veldkamp 2009).

Previous studies on adult plants demonstrated that certain *Aegilops* species (e.g. like *Ae. biuncialis* and *Ae. tauschii*) can indeed be used to improve the drought tolerance in wheat (Molnár et al. 2004; Sohail et al. 2011). Our results on germination did not completely support this evidence, because the germination performance of *Ae. geniculata* was always lower (or similar for one accession) to the germination performance of wheat, including the drought stress sensitive variety WL711.

The capability of the tested *Triticum* cultivars to germinate at low osmotic potentials is in line with previous findings from the literature. For instance, several *Triticum aestivum* lines achieve 75% of final germination at osmotic potentials lower than those tested here (e.g. <-2 mPa, Wuest and Lutcher 2012). Similarly, cultivars of *Triticum durum* can germinate at low water potentials as well (Almansouri et al. 2001; Singh et al. 2013).

The comparison between *Ae. geniculata* and *Ae. neglecta* did not show any difference in the germination at full moisture, or in the effect of water potential on germination. Interestingly, our results highlight that different source populations of a given species may have different potentials for tolerance/resistance trait scouting. In fact, germination of *Ae. geniculata* populations showed different response to decreasing water potentials.

The performance of the southern genCS differ from that of northern Italian population (genPV and genRA) and is similar to the performance of cultivated varieties of *Triticum*, highlighting that some wild *Ae. geniculata* populations may perform equally well as selected cultivated varieties. These differences in germination behaviour between populations of the same species could be explained by the large ecological amplitude of *Ae. geniculata*, that grows from sea level to 1,750 m a.s.l. and in a very wide range (Kilian et al. 2011). Moreover, *Ae. geniculata* is known to have the largest variability in seed germination at different temperatures compared to other *Aegilops* species (Guzzon et al. 2015).

Differences in the germination percentage between populations of *Ae. geniculata* at low water potentials could be due to the local environmental conditions under which seeds developed. Important factors affecting drought tolerance in seed germination are temperatures and rainfall during the period of seed formation and maturation (Nicotra et al. 2010; Bernareggi et al. 2015). According to our results, the highest germination percentage at -1.7 MPa was found in the southern population genCS, which is characterized by the lowest rainfall during the period of seed maturation and dispersal (May and June). Hence, the possibility that this population is probably more adapted to cope with drought conditions during germination phase seems likely.

Moreover, seeds of the same population collected in different years may show different germination behaviour, due to an effect of contingent local environmental conditions (Nicotra et al. 2010). Functionally adaptive transgenerational effects, elicited by environmental stress, are known to affect several plant traits in the offspring (see e.g. Herman et al. 2012), including seed germination and longevity (Meyer and Allen 1999; Mondoni et al. 2014). It follows that, plastic responses to the local environment or seasonality mediated by parental effects, could confer different germination responses to drought in the offspring. However, such interestingly possibility still need to be investigated.

With respect to the germination of seeds from the upper and lower spikelets, our results confirm that generally the germination of seeds from the upper spikelets is significant lower (Fig. 1), except for genCS. However, the effect of water potential on seed germination percentage did not differ between seeds from different spikelets in all species and populations.

Finally, water stress significantly enhanced MTG in *Triticum*, genPV and genCS seeds, while did not affect MTG in genRA and negl. This further highlights that different populations may show different responses to a given stress.

It is demonstrated that those *Triticum durum* cultivars that achieve best germination performance under osmotic stress also show high emergence in dry soil (Singh et al. 2013). This fact highlights the importance to study germination under osmotic stress in wheat wild relatives, in order to understand their drought resistance. However, in many wild plants species the germination response under low water potential is not directly correlated with the habitat in which species grow (e.g. drought avoidance mechanisms; Evans and Etherington 1990). Therefore, results from germination tests should be complemented with evidence from emergence tests, survival and establishment of seedlings at low osmotic potentials.

In conclusion, our results indicate that the characterization of CWR for useful trait scouting for crop improvement should take into account the population-specific characteristics of a given CWR. In the case of *Aegilops geniculata*, populations experiencing drier conditions during seeds maturation showed a high germination even at very low water potential, although unfortunately we could not detect a population performing better than selected wheat varieties. However, the possibility that other *Ae. geniculata* populations that live in more arid areas (e.g. North Africa, Middle East) can germinate at even lower osmotic potentials can’t be ruled out. Future researches on drought tolerance in *Aegilops* species should investigate also neglected species inside the section *Aegilops* (e.g. *Aegilops peregrina* (Hack.) Maire & Weiller, *Aegilops uniaristata* Vis.) or species growing in arid/semi-arid environments like desert edges or sandy soils (e.g. *Aegilops crassa* Boiss., *Aegilops kotschyi* Boiss., *Aegilops sharonensis* Eig). Moreover, useful traits to improve drought tolerance in *Triticum* may be found in other wild species, belonging to the tertiary genepool of wheat. For example, within the genus *Elytrigia* Desv. several species show interesting pathogens resistance, salt stress and drought tolerance traits (Jiang et al. 2005; Colmer et al. 2006). In particular, *E. pontica* (Podp.) Holub and *E. intermedia* (Host) Nevski grow in dry areas (the latter in areas with annual precipitations below 300mm, Barkworth 2007). Future studies investigating the suitability of wild accessions for crop improvement must necessarily take into account an ecological and population-specific approach.

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References

Albuquerque FMC, de Carvalho NM (2003) Effect of type of environmental stress on the emergence of sunflower (*Helianthus annuus* L.), soyabean (*Glycine max* (L.) Merril) and maize (*Zea mays* L.) seeds with different levels of vigor. Seed Science and Technology, 31, 465-467.

Al-Karaki GN (1998) Response of wheat and barley during germination to seed osmopriming at different water potential. Journal of Agronomy and Crop Science, 181, 229-235.

Almansouri M, Kinet JM, Lutts S (2001) Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). Plant and Soil, 231, 245-256.

Arrigo N, Guadagnuolo R, Lappe S, Pasche S, Parisod C, Felber F (2011) Gene flow between wheat and wild relatives: empirical evidence from *Aegilops geniculata*, *Ae. neglecta* and *Ae. triuncialis*. Evolutionary Applications, 4, 685-695.

Barkworth M.E. (2007) *Thinopyrum*. In: Barkworth M.E. et al. (Eds), Flora of North America vol.24. Viewed at http://herbarium.usu.edu/webmanual on 17/02/2016.

Bernareggi G, Carbognani M, Petraglia A, Mondoni A (2015) Climate warming could increase seed longevity of alpine snowbed plants. Alpine Botany, 125, 69-78.

Brummer EC, Barber WT, Collier SM, Cox TS, Johnson R, Murray SC, Olsen RT, Pratt RC, Thro AM (2011) Plant breeding for harmony between agriculture and the environment. Frontiers in Ecology and the Environment, 9, 561-568.

Challinor AJ, Watson J, Lobell DB, Howden SM, Smith DR, Chhetri N (2014) A meta-analysis of crop yield under climate change and adaptation. Nature Climate Change, 4, 287-291.

Colmer TD, Flowers TJ, Munns R (2006). Use of wild relatives to improve salt tolerance in wheat. Journal of Experimental Botany, 57, 1059-1078.

Dhaliwal HS, Harjit-Singh, William M (2002) Transfer of rust resistance from *Aegilops ovata* into bread wheat (*Triticum aestivum* L.) and molecular characterisation of resistant derivatives. Euphytica, 126, 153-159.

Dhanda SS, Sethi GS, Behl RK (2004) Indices of drought tolerance in wheat genotypes at early stages of plant growth. Journal of Agronomy and Crop Science, 190, 6-12.

Dempewolf H, Eastwood RJ, Guarino L, Khoury CK, Müller JV, Toll J (2014) Adapting agriculture to climate change: a global initiative to collect, conserve, and use Crop Wild Relatives. Agroecology and Sustainable Food Systems, 38, 369-377.

Devi R, Kaur N, Gupta AK (2012) Potential of antioxidant enzymes in depicting drought tolerance of wheat (*Triticum aestivum* L.). Indian Journal of Biochemistry and Biophysics, 49, 257-265.

Ellis RH, Roberts EH (1980) Improved equations for the prediction of seed longevity. Annals of Botany, 45, 13-30.

Evans CE, Etherington JR (1990) The effect of soil water potential on seed germination of some British plants. New Phytologist, 115, 539-548.

Fandrich L, Mallory Smith CA (2006) Factors affecting germination of jointed goatgrass (*Aegilops cylindrica*) seed. Weed Science, 54(4), 677-684.

FAO (2008) *Climate Change and Biodiversity for Food and Agriculture*. Food and Agriculture Organization of the United Nations, Rome.

Feldman M (2001) Origin of cultivated wheat. In: Bonjean A.P., Angus W.J. (Eds), The world wheat book: a history of wheat breeding. Lavoisier Publishing, Paris, France: 3-56.

Friebe B, Tuleen NA, Gill BS (1999) Development and identification of a complete set of *Triticum aestivum–Aegilops geniculata* chromosome addition lines. Genome, 42, 374-380.

Guzzon F, Müller JV, Abeli T, Cauzzi P, Ardenghi NMG, Balestrazzi A, Rossi G, Orsenigo S (2015) Germination requirements of nine European *Aegilops* species in relation to constant and alternating temperatures. Acta Botanica Gallica, Botany Letters, 162(4), 349-354.

Hay FR, Smith RD (2003) Seed maturity: when to collect seeds from wild plants. In: Smith R.D., Dickie J.B., Linington S.H., Pritchard H.W., Probert R.J. (Eds), Seed conservation: turning science into practice. Royal Botanic Gardens, Kew; London, UK: 97-133.

Hampson CR, Simpson GM (1990) Effects of temperature, salt and osmotic potential on early growth of wheat (*Triticum aestivum*). I. Germination. Canadian Journal of Botany, 68, 524-528.

Hawkes JG (1983) The diversity of crop plants. Harvard University Press, London, UK: 184 pp.

Herman JJ, Sultan SE, Horban-Kobelski T, Riggs C (2012) Adaptive transgenerational plasticity in an annual plant: grandparental and parental drought stress enhance performance of seedlings in dry soil. Integrative and Comparative Biology, 52, 77-88.

Heywood V, Casas A, Ford-Lloyd B, Kell S, Maxted N (2007) Conservation and sustainable use of crop wild relatives. Agriculture Ecosystems and Environment, 121, 245-255.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology, 25, 1965-1978.

Jiang SM, Hu J, Yin WB, Chen YH, Wang RRC, Hu ZM (2005). Cloning of resistance gene analogs located on the alien chromosome in an addition line of wheat-*Thinopyrum intermedium*. Theoretical and Applied Genetics, 111, 923-931.

Kilian B, Mammen K, Millet E, Sharma R., Graner A., Salamini F., Hammer K., Özkan H. (2011): Chapter 1 *Aegilops*. In: C.Kole (Ed), *Wild Crop Relatives Genomic and Breeding Resources, Cereals*. Springer-Verlag Berlin Heidelberger: 1-76.

Kuraparthy V., Chhuneja P., Dhaliwal H.S., Kaur S., Bowden R.L., Gill B.S. (2007) Characterization and mapping of cryptic introgression from *Ae. geniculata* with new leaf rust and stripe rust resistance genes Lr57 and Yr40 in wheat. Theoretical and Applied Genetics, 114, 1379-1389.

Lesk C, Rowhani P, Ramankutty N (2016) Influence of extreme weather disasters on global crop production. Nature, 529, 84-87.

Marañon T (1989) Variations in seed size and germination in three *Aegilops* species. Seed Science and Technology, 17, 583-588.

Maxted N, Ford-Lloyd BV, Jury S, Kell S, Scholten M (2006) Towards a definition of a crop wild relative. Biodiversity and Conservation, 15, 2673-2685.

Maxted N, Kell SP, Ford-Lloyd BV (2008) Crop Wild Relative conservation and use: establishing the context. In: Maxted N, Ford-Lloyd BV, Kell SP, Iriondo JM, Dulloo ME, Turok J (Eds), Crop Wild Relative conservation and use. CABI Publishing, Wallingford, UK: 3-30.

Meelh GA, Tebaldi C (2004) More intense, more frequent, and longer lasting heat waves in the 21st century. Science, 305, 994-997.

Meyer SE, Allen PS (1999) Ecological genetics of seed germination regulation in Bromus tectorum L. II. Reaction norms in response to a water stress gradient imposed during seed maturation. Oecologia, 20, 35-43.

Michel BE, Kauffmann MR (1973) The osmotic potential of polyethylene glycol 6000. Plant Physiology, 51, 914-916.

Molnár I, Gáspár L, Sárvári E, Dulai D, Hoffmann B, Molnár-Láng M, Galiba G (2004) Physiological and morphological responses to water stress in Aegilops biuncialis and Triticum aestivum genotypes with differing tolerance to drought. Functional Plant Biology, 31, 1149-1159.

Molnár I, Dulai S, Molnár-Láng M (2008) Can the drought tolerance traits of *Ae. biuncialis* manifest even in the wheat genetic background? Acta Biologica Szegediensis, 52, 175-178.

Mondoni A, Orsenigo S, Donà M, Balestrazzi A, Probert R, Hay FR, Petraglia A, Abeli T (2014) Environmentally induced transgenerational changes in seed longevity: maternal and genetic influence. Annals of Botany, 113, 1257-1263.

Nevo E (2011): Chapter 10 *Triticum* in: Kole C. (Ed), Wild Crop Relatives Genomic and Breeding Resources, Cereals. Springer-Verlag Berlin Heidelberger: 407-456.

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, van Kleunen M (2010) Plant phenotypic plasticity in a changing climate. Trends in Plant Science, 15, 684-690.

Orlowsky B, Seneviratne S I (2013) Elusive drought: uncertainty in observed trends and short and long-term CMIP5 projections. Hydrology and Earth System Sciences, 17, 1765-1781.

Orsenigo S, Mondoni A, Rossi G, Abeli T (2014) Some like it hot and some like it cold, but not too much: plant responses to climate extremes. Plant Ecology, 215 (7), 677-688.

Paternoster R, Brame R, Mazerolle P, Piquero A (1998) Using the correct statistical test for the equality of regression coefficients. Criminology, 36, 859-866.

Petersen G, Seberg O, Yde M, Berthelsen K (2006). Phylogenetic relationships of *Triticum* and *Aegilops* and evidence for the origin of the A, B, and D genomes of common wheat (*Triticum aestivum*). Molecular Phylogenetics and Evolution, 39, 70-82.

Schewry PR (2009) Wheat. Journal of Experimental Botany, 60, 1537-1553.

Schneider A, Molnár I, Molnár-Láng M (2008) Utilisation of *Aegilops* (goatgrass) species to widen the genetic diversity of cultivated wheat. Euphytica, 163, 1-19.

Singh P, Ibrahim HM, Flury M, Schillinger F, Knappenberger T (2013) Critical water potentials for germination of wheat cultivars in the dryland Northwest USA. Seed Science Research, 23, 189-198.

Sohail Q, Inoue T, Tanaka H, Eltayeb AE, Matsuoka Y, Tsujimoto H (2011) Applicability of *Aegilops tauschii* drought tolerance traits to breeding of hexaploid wheat. Breeding Science, 61, 347-357.

Soliani L (2005) *Manuale di statistica per la ricerca e la professione*. Università di Parma, Parma, Italy. Viewed at http://www.dsa.unipr.it/soliani/soliani.html on 01/11/2015.

Van Slageren MW (1994) Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (*Poaceae*). Agricultural University Papers, Wageningen, Netherlands.

Veldkamp JF (2009) *Aegilotriticum* × *requienii*, the correct name for *Aegilops* × *triticoides* (*Gramineae*). Webbia, 64, 13-15.

Vincent H, Wiersema J, Kell SP, Dobbie S, Fielder H, Castañeda Álvarez NP, Guarino L, Eastwood R, Leon B, Maxted N (2013) A prioritised crop wild relative inventory as a first step to help underpin global food security. Biological Conservation, 167, 265-275.

Warschefsky E, Penmetsa RV, Cook DR, von Wettberg EJB (2014) Back to the wilds: evolutionary adaptations for resilient crop through systematic hybridization with crop wild relatives. American Journal of Botany, 101, 1791-1800.

Wuest SB, Lutcher LK (2012) Soil water potential requirement for germination of winter wheat. Soil & Water Management & Conservation, 77, 279-283.

Zaharieva M, Prosperi J, Monneveux P (2004) Ecological distribution and species diversity of *Aegilops* L. genus in Bulgaria. Biodiversity and Conservation, 13, 2319-2337.

Zhao H, Zhang W, Wang J, Li F, Cui F, Ji J, Wang D, Li J (2013) Comparative study on drought tolerance of wheat and wheat-*Aegilops biuncialis* 6U^b addition lines. Journal of Food, Agriculture & Environment, 11, 1046-1052.

Article II

Guzzon F, Müller JV, do Nascimiento Araujo M, Cauzzi P, Orsenigo S, Mondoni A, Abeli T (2017) Drought avoidance adaptive traits in seed germination and seedling growth of *Citrullus amarus* landraces. South African Journal of Botany, 113, 382-388.

Drought avoidance adaptive traits in seed germination and seedling growth of *Citrullus amarus* landraces

Abstract

Citrullus lanatus cultivation is affected by drought stress. *Citrullus* species that grow wild and domesticated in arid areas, are considered potential useful donors of drought tolerance traits. Here, we evaluated the response of seed germination, seedling establishment and growth to different water availabilities in eleven landraces of *C. amarus*, from most of their cultivation range and in one *C. lanatus* commercial cultivar (‘Sugar Baby’). ‘Sugar Baby’ germinated to lower water potential better than all *C. amarus* landraces, while seedling establishment was much higher in *C. amarus* than in *C. lanatus*. Finally, seedling growth of *C. amarus* landraces and the *C. lanatus* cultivar followed different patterns depending on water availability, showing enhanced biomass growth under wet conditions and no changes between dry and wet growing treatments, respectively. The different water use strategies in seed germination and seedling growth found in the two crops highlight drought avoidance strategies linked to the species growing environment in *C. amarus*, not present in the *C. lanatus* cultivar. The high seedling establishment, the plastic responses to water availabilities and the strong root system, indicate that genetic resources of *C. amarus* may have important applications in breeding programmes and in the selection of water-use efficient rootstock lines.

Key words: Citron watermelon; *Citrullus lanatus*; root/shoot ratio; sweet dessert watermelon; neglected crop; seedling establishment.

Introduction

In 2012, 3.5 million hectares of agricultural land were used to cultivate watermelons (*Citrullus* Schrad). The annual production reached 105 million metric tons, which is 9.5% of the global vegetable production on 6% of the area used globally for the cultivation of vegetables (FAOSTAT 2014). In particular in arid and semi-arid regions, the cultivation of *Citrullus lanatus* (Thunb.) Matsum. & Nakai, the sweet dessert watermelon, relies heavily on irrigation (Cohen et al. 2007) and is affected by water stress. In recent years, severe drought events caused a sharp decline of watermelon production in some areas (Zhang et al. 2011a). The selection of drought tolerant cultivars or rootstock lines with a more efficient water use might offer solutions to cope with water scarcity (Cohen et al. 2007; Davis et al. 2008; Zhang et al. 2011a). Despite the economic importance of *Citrullus lanatus*, so far only few research studies and breeding efforts dealt with the water and other abiotic stress resistances in watermelons (Wehner 2008). Other species in the genus *Citrullus* which grow in arid areas, either as native, undomesticated crop wild relatives or as domesticated crops, are considered potential donors of useful traits to improve abiotic and also biotic stress resistances of modern *C. lanatus* cultivars (Hwang et al. 2011; Jarret 2014). Underutilized crops and crop landraces, in particular, are known to be adapted to a cultivation in marginal environments and to tolerate high levels of abiotic stress (Mabhaudhi et al. 2016; Camacho Villa et al. 2006; Zeven 1998). Landraces are also considered important resources to broaden the reduced crop gene pool in a climate change scenario, and to enhance low-input agricultural systems (e.g. organic farming) (Veteläinen et al. 2009).

All over the world, different landraces of *Citrullus amarus* Schrad., commonly known as citron watermelon, are locally cultivated as a minor crop (Laghetti and Hammer 2007; Paris 2015). Its fruits are used to produce jams, mustards and preserves, pectin for citron peel, and as livestock fodder (Laghetti and Hammer 2007). For a long time, *C. amarus* had been classified as a botanical variety of *Citrullus lanatus* as *Citrullus lanatus* var. *citroides* Bailey or as *Citrullus lanatus* (Thunb.) Matsum. et Nakai subsp. *lanatus* var. *citroides* (Bailey) Mansf. ex Greb. It had often been considered the progenitor or an early domestication form of the sweet dessert watermelon (Vavilov 1987; Jarret et al. 1997; Rubatzky 2001). However, a recent molecular study revealed that citron watermelon is a different species from *C. lanatus*, which had been independently domesticated. The natural area of distribution and its centre of domestication lies in southern Africa (Chomiki and Renner 2015). However, the two species are very similar, in fact *C. amarus* crosses readily with *C. lanatus* in the field (Wehner 2008), even if reduced pollen fertility and massive preferential segregation were found in the hybrids (Levi et al. 2003; McGregor and Waters, 2013). *C. amarus* is also being used as rootstock for *C. lanatus* cultivars (Huitrón et al. 2007; King et al. 2010; Thies et al. 2015b). Mc Gregor (2012) accounted for the different traditional uses of *Citrullus amarus* in southern Africa where the fruits are a source of water in arid regions and where the pulp, seeds and leaves are used as food, therefore this species also holds a certain importance for food security in those areas (Mujaju et al. 2011; Modi 2012).

C. amarus landraces show three distinct traits which are interesting for *Citrullus lanatus* breeding and crop improvement, namely pest resistances (Levi et al. 2001b; Davis et al. 2007; Zhang et al. 2011b; Wechter et al. 2012; Thies et al. 2015a) a high genetic diversity (Levi et al. 2001a; Hwang et al. 2011) and adaptations to arid conditions. Regarding this latter important characteristic, it is known that wild accessions of *C. amarus* have higher tolerance to drought stress than cultivated accessions of *C. lanatus* (Kawasaki et al. 2000; Yoshimura et al. 2008). Interestingly, Zhang et al. (2011a) screened 820 *C. lanatus* and *C. amarus* accessions as well as sweet dessert watermelon breeding lines for drought tolerance at seedling stage using a mean drought tolerance rating and ranking scale. Among the 25 accessions that showed the highest drought tolerance, 12 were *C. amarus* from southern Africa. Mo et al. (2015) confirmed that wild watermelon (wild *C. amarus*, referred to as *C. lanatus* var. *citroides*) is more drought tolerant than a *C. lanatus* inbred line. In particular, under drought conditions, *C. amarus* showed higher root/shoot ratio and higher root length, when compared to the domesticated *C. lanatus* lines. In other words, the wild accession showed a greater biomass allocation to the root system when exposed to drought than the inbred line.

The study presented here is the first work to evaluate different landraces, covering most of the wide production area of *C. amarus*, for drought resistance in the first key stages of the life cycle, and comparing them with a commercial high-yield cultivar of *C. lanatus*. In particular, we evaluated the drought responses in seeds and seedlings, in 11 landraces of *C. amarus* compared to a commercial cultivar of *C. lanatus*. The aim of the study was to 1) understand the degree and the mechanisms of drought stress tolerance in the the tested *Citrullus* accessions in the first stages of plant development ; 2) screen if any of the tested *C. amarus* landraces show interesting traits that could be used for a crop improvement programme of *C. lanatus* cultivars, aiming for drought resistance in the early stages of the life cycle or for the selection of water use efficient rootstock lines; 3) compare the drought tolerance strategies in *C. lanatus* and *C. amarus*.

Materials and methods

Seed accessions and germination study.

Twelve *Citrullus* accessions were used in this study (Table 1). Seeds of six landraces (RSA, RUS, UKR, USA, UZB, CAN) of *C. amarus* were provided by the Vavilov Centre of Plant Industry (Saint Petersburg, Russia), five seed accessions of northern Italian *C. amarus* landraces (ITA2, ITA3, ITA4, ITA5, ITA6) were provided by the Germplasm Bank of the University of Pavia (Italy). One commercial cultivar of *C. lanatus* called 'Sugar Baby' was purchased from a French seed company (B&T World Seeds). All germination experiments were carried out in the laboratories of the Millennium Seed Bank (Royal Botanic Gardens, Kew, United Kingdom). Seed of all accessions were weighted and the area estimated. Seed weight was determined by weighting 25 individual seeds from each accession using a microbalance (UMT2 Mettler Toledo), while the area was measured on 20 seeds per accession, using a microscope (Zeiss Stemi SV 11) with camera (Zeiss AxioCam). To evaluate the seed area, a two dimensional projection of the length was used with width as a size metric. Seed germination tests at five osmotic potentials (0, -0.1, -0.2, -0.3, -0.4 MPa), involved sowing seeds in 90 mm diameter Petri dishes containing two layers of germination paper soaked with 10 ml polyethylene glycol (PEG) 8000 solution (Fisher BioReagents, UK), at a concentration appropriate to the intended treatment (Michel 1983, Hardegree and Emmerich 1989). Five replicates of 15 seeds per population and osmotic treatment were incubated at 30°C in temperature and controlled incubators (LMS Ltd, Sevenoaks, UK). The treatment at full hydration (0 MPa) was performed by soaking germination paper with 10 ml of distilled water. To prevent changes in water potential, Petri dishes were refilled with the appropriate solution when needed. Since *Citrullus* species are known to be dark germinators (Thanos and Mitrakos 1992), the Petri dishes were covered with aluminium foil and put in black plastic bags to avoid any contact with light. Black plastic bags were randomly interspersed within the germinator. The germination scoring was performed in a dark room using green safe light. Seeds were daily checked for germination for four weeks. Germination was defined as a radicle emergence of, at least, 2 mm. We calculated the mean germination time (MGT) following the formula using the formula (Ellis and Roberts 1980):

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

where: n= number of seeds that germinated at time T; T= the days between the beginning of the test and the measurement; N= the total number of seeds that germinated.

Table 1.

The twelve Citrullus accessions tested. UNIPV = Plant Germplasm Bank of the University of Pavia (Italy), VIR = Vavilov Centre of Plant Industry (Saint Petersburg). Seed weight is the mean of the weights of 25 individual seeds, Seed area is the mean of the areas of 20 individual seeds.

Species	Year of production	Origin	Name	Provenience	Seeds colour	Code	Seed weight (dg)	Seed weight st.dev. (dg)	Seed area mm^2	Seed area st.dev. mm^2
C. amarus	2013	Italy (Reggio Emilia)	Anguria da marmellata/mostarda	UNIPV	Red	ITA2	1.34083	0.10666	66.80	4.68
C. amarus	2014	Italy (San Pancrazio-PR)	Anguria da mostarda	UNIPV	Red	ITA3	1.40650	0.10098	68.62	3.02
C. amarus	2011	Italy (Cremona)	Anguria da mostarda da semi verdi	UNIPV	Green	ITA4	1.41619	0.09471	76.98	3.67
C. amarus	2013	Italy (Reggio Emilia)	Cocomero da marmellata/mostarda seme rosso di Colorno (PR)	UNIPV	Red	ITA5	1.51823	0.08822	70.76	3.89
C. amarus	2014	Italy (Parma)	Anguria da mostarda da semi rossi	UNIPV	Red	ITA6	1.41023	0.15959	67.93	3.87
C. amarus	2014	Ukraine	Kormovoy 4–73	VIR	Light Grey	UKR	1.81772	0.09644	80.72	5.76
C. amarus	2014	South Africa	Bethulie	VIR	Red	RSA	1.21717	0.10956	60.61	5.49
C. amarus	2014	Uzbekistan	Bogarnyi 112	VIR	Light Grey	UZB	1.71796	0.15351	77.74	5.44
C. amarus	2014	United States	Colorado green seeds	VIR	Light Grey	USA	1.43519	0.13945	76.89	4.08
C. amarus	2014	Russia	Saratovskyi	VIR	Light Grey	RUS	1.77699	0.12946	82.62	2.22
C. amarus	2014	Canada	Colorado Prosor	VIR	Dark Grey	CAN	1.58921	0.09547	73.6	4.19
C. lanatus			Sugar Baby	Private seed company	Brown	S.Baby	0.40302	0.08614	30.71	2.85

Growth experiment.

Eight accessions (CAN, ITA6, RSA, RUS, S.Baby, UKR, USA, UZB), chosen to consider the widest geographical gradient possible, were used to perform a plant growth experiment lasting three weeks. Eighty seeds per accession, which germinated after two days of incubation (in the dark at 30°C on 90 mm diameter Petri dishes with 1% agar), were sown in 350 ml pots filled with fine (1.00 – 3.00 mm) vermiculite and sand (1:1). The germinated seeds were sown under a thin soil layer. The experiment was performed in a glasshouse of the Millennium Seed Bank. Two data loggers checked temperature and humidity homogeneity (mean temperature ± st. dev.: 23.5°C ± 5.6; mean relative humidity ± st.dev.: 69% ± 20.8). Soil moisture was kept at full saturation during the first week, putting the pots in water-filled trays, so that a water table was always available under the pots. At the sixth day of the experiment, a water-soluble fertilizer mixture at the injection rate of 1:200 (19% nitrogen, 19% phosphorus pentoxide, 19% potassium oxide and 0.15% magnesium oxide with iron, copper, boron, manganese and molybdenum as trace elements) was supplied for 24 hours. During the remaining two weeks of the experiment, two different water treatments were used. In the control treatment (= wet treatment), full water saturation was continued as described above. In the dry treatment, the water in the trays was removed and plants left without water until the end of the experiment two weeks later. The pots were put in a randomized order in the glasshouse and their position was changed randomly twice a week. The seedling establishment was checked every 12 hours for the first week. We considered a seedling established when the two cotyledons were fully unfolded and opened. We calculated the mean establishment time (MET) following the formula cited by Ellis and Roberts (1980) for MGT. After three weeks at the end of the experiment, 15 plants per accession and treatment were harvested. The soil was carefully removed from the roots by washing them. The roots were then separated from the shoots. The lengths of the harvested seedling shoots and the third leaves (= first true leaves) were measured. Both roots and shoots were put in aluminium foils and oven-dried at 103°C for 24h to evaluate the dry biomass weight. From these data we computed the root/shoot ratio. Three samples of soil for both the treatments were collected and then oven-dried at 103°C for 24h to measure the water content of the soil. At the end of the experiment, the water content (in percent) in the control (wet) treatment was 55.35% ± 10.07 (mean ± st. dev.), while in the dry treatment it was 4.84% ± 0.5.

Data analysis

Seed germination and seedling establishment were analysed by means of General Linear Models (GLMs), using a binomial probability distribution and a logit link function. MGT and MET were analysed using univariate ANOVA. All other data (dry biomass, shoot length, leaf length, root/shoot ratio) were analysed through two-ways ANOVAs. Bonferroni post hoc tests were performed to identify differences between accessions. Mann-Whitney U-tests were performed to identify differences between treatments. Data were checked for normality and homogeneity of variance. Data were analysed in SPSS 21.0.

Results

Germination

Final germination percentages were significantly affected by treatment with a significant interaction between accession and treatment (Table 2). Highest germination at the two lowest osmotic potentials was found in the standard *C. lanatus* cultivar 'Sugar Baby'. Among the *C. amarus* landraces, the South African accession (RSA) achieved the best final germination at -0.3MPa and -0.4MPa (Figure 1). Osmotic treatments had a significant effect on MGT (df = 3, F = 194.637, P <0.001). The osmotic treatment slowed the MGT significantly in all populations (P <0.001) except for ITA2 (P = 0.259). The population that showed the fastest MGT was 'Sugar Baby' (Table 3).

Table 2. Results of the general linear model (GLM) for germination (* P <0.05).

Factor	Wald Chi-Square	df	Sig.
Osmotic potential	125.769	4	0.000*
Accession	18.894	11	0.063
OsmPotential * Acc	162.139	44	0.000*

Seedling establishment

The difference in seedlings establishment among cultivars was significant (Wald Chi-square = 75.788, df = 7, P <0.001) 'Sugar Baby' and RUS had a final seedling establishment significantly lower than the other landraces (Figure 2), in which seedling establishment reached almost 100%. In particular, in 'Sugar Baby' seedling establishment was less than half than in the other accessions (Figure 2). Similarly, MET was significantly different among accessions (df = 7, F= 22.406, P <0.001). In 'Sugar Baby' the establishment occurred faster than in the *C. amarus* landraces (Table 3).

Table 3. Mean Germination Time (MGT) for the twelve accession used in the germination study (data from 0MPa to -0.3MPa pooled together) and Mean Establishment Time (MET) for the eight accession used in the growth study

Accession	MGT (days, mean ± st.dev.)	MET (hours, mean ± st.dev.)
CAN	3.72 ± 1.82	67.12 ± 4.61
ITA2	4.28 ± 1.98	-
ITA3	3.76 ± 1.07	-
ITA4	4.40 ± 1.67	-
ITA5	5.97 ± 2.15	-
ITA6	4.77 ± 2.40	74.1 ± 8.01
S.Baby	2.31 ± 0.39	45.85 ± 6.30
RSA	2.44 ± 0.63	59.1 ± 2.70
RUS	4.21 ± 2,32	67.67 ± 6.53
UKR	3.36 ± 1.43	74.15 ± 7.07
USA	6.1 ± 2.53	62.85 ± 3.45
UZB	3.71 ± 1.88	69 ± 1.92

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Final dry biomass

Total dry biomass was significantly affected by treatment, accession and the interaction between these two factors (Table 4). The biomass was always greater in landraces of *C. amarus* than in 'Sugar Baby', with values about threefold higher in both dry and wet treatment. The response of *C. amarus* landraces in terms of final biomass significantly differed in wet and dry treatments, while in 'Sugar Baby' there were no statistical differences (P = 0.611; Figure 3). Considering the shoot and the root dry biomass separately in all *C. amarus* landraces both, the root and the shoot were heavier in the control (wet) treatment than in the dry treatment (P <0.05). At the same time, there was no statistically significant difference between the two water treatments for 'Sugar Baby', both in the shoot dry weight (P = 0.137) or in the root dry weight (P = 0.503).

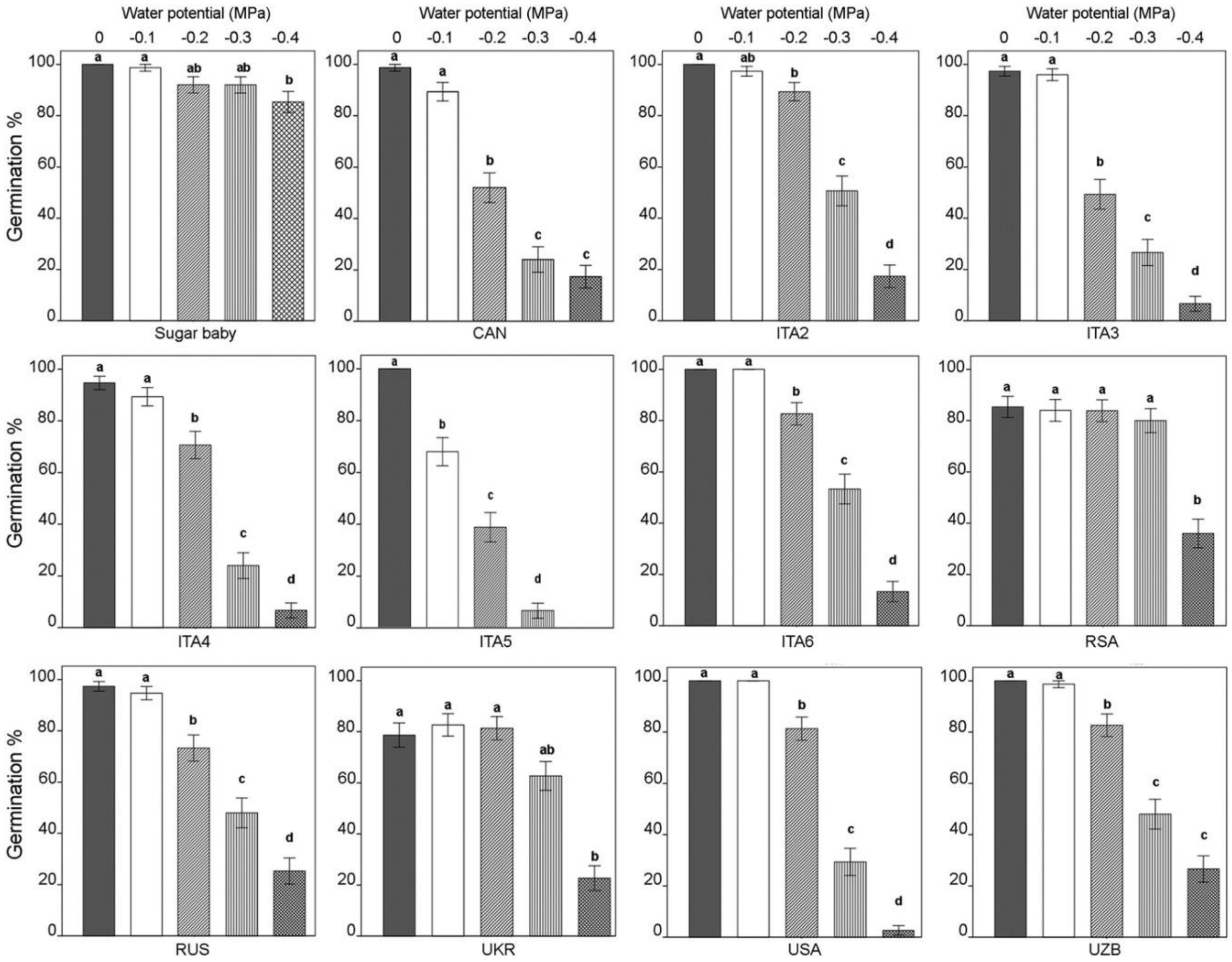

Figure 1. Final germination percentages (mean ± st.err.) at the five water potentials tested for the twelve accessions. Lowercase letters indicate statistically significant differences between different osmotic potentials; same letters indicate non-significant differences; different letters indicate differences significant at P<0.05.

Table 4. Results of the two-ways ANOVAs for mean total biomass, mean leaf length and mean root/shoot ratio (RSR) at the end of the experiment. Levels of significance: *** P < 0.001; ** P < 0.01; * P < 0.05.

Factors	df	F		
		Biomass	Leaf length	RSR
Treatment	1	315.258***	250.888***	0.142
Accession	7	177.032***	34.059***	12.583***
Acc*Treatment	7	8.426***	2.750**	6.744***

Shoot length, leaf length and root/shoot ratio (RSR)

Treatment had no statistically significant effects on the shoot length (df = 1, F = 2.167, P = 0.142). However for the leaf length, all accessions had statistically significant higher values in the control (wet) treatment than in the dry treatment (Table 4, Figure 4).

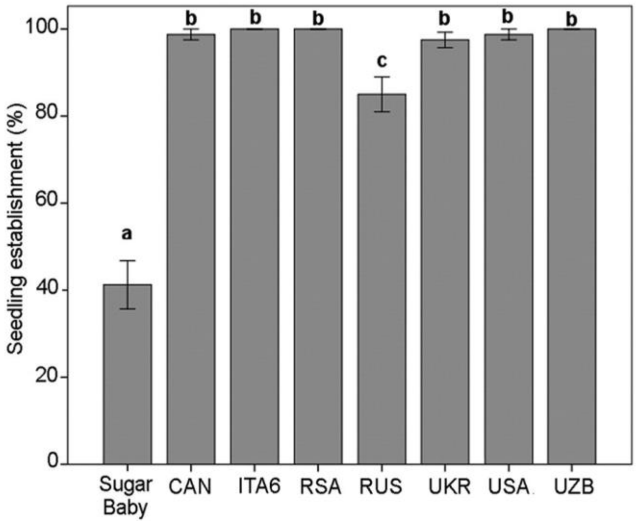

Figure 2. Final seedling establishment percentages (mean ± st.err.) for the eight accessions tested. Lowercase letters indicate statistically significant differences between species; same letters indicate non-significant differences; different letters indicate differences significant at P < 0.05.

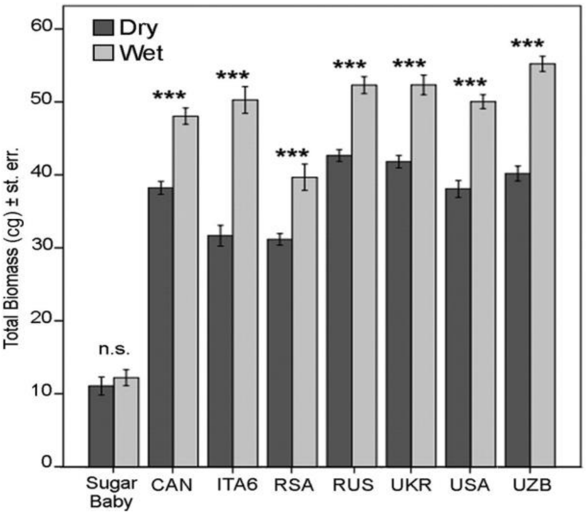

Figure 3. Final total dry biomass (mean ± st.err.) for the eight accessions tested. Levels of significance of the treatment effect for each accession: *** P < 0.001; ** P < 0.01; * P < 0.05; n.s.= not significant.

Accession and the interaction between accession and treatment had significant effect on RSR, while the treatment had not (Table 4). There was a statistically significant difference between wet and dry treatments for RSR in ‘Sugar Baby’, ITA6, RSA, and UZB. In ‘Sugar Baby’ the mean RSR was higher in the dry treatment than in the wet treatment while, on the contrary, in ITA6, RSA and UZB the mean RSR was higher in the control (wet) than in dry treatment (Figure 5).

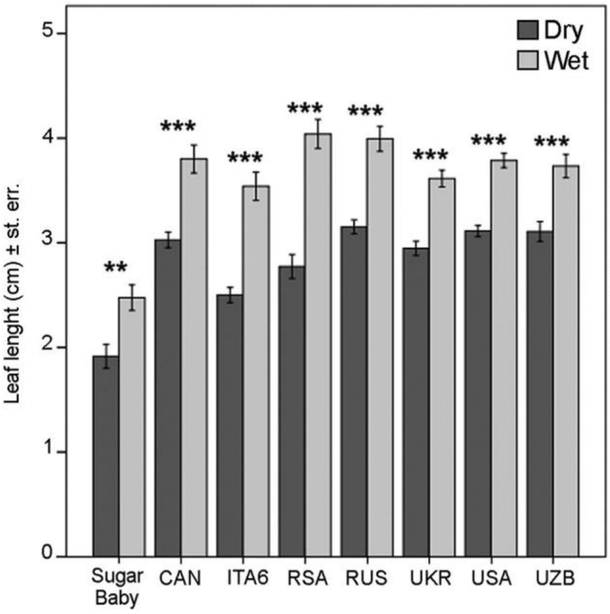

Figure 4. Length of the first true leaf at the end of the experiment (mean ± st.err.) for the eight accessions tested. Levels of significance of the treatment effect for each accession: *** P < 0.001; ** P < 0.01; * P < 0.05; n.s.= not significant.

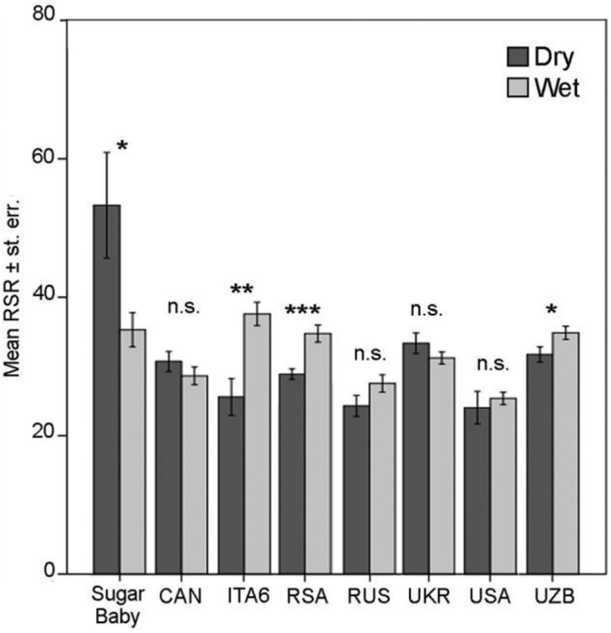

Figure 5. Root/Shoot ratio (RSR) at the end of the experiment (mean ± st.err.) for the eight accessions tested. Levels of significance of the treatment effect for each accession: *** P < 0.001; ** P < 0.01; * P < 0.05; n.s.= not significant.

Discussion

In this study, we investigated the response of seed germination and seedling growth of *C. amarus* landraces under different levels of water availability compared to a *C. lanatus* standard line. The aims of this study were to understand the drought responses in the two crops and to determine if *C. amarus* landraces can be useful donors of resistance traits for improving drought tolerance in *C. lanatus* cultivars in the earliest and crucial stages of plant development (i.e. seed germination and seedling establishment). 'Sugar Baby' seeds are smaller than all *C. amarus* accessions (Table 1). This confirms that watermelons intended for confectionery food production produce big seeds while those watermelon cultivars intended for consumption possess small or medium sized seeds (Wehner 2008). Our results suggest that germination in 'Sugar Baby' is less affected by low osmotic potentials than in *C. amarus* landraces, achieving high germination percentages also at the lowest potential (-0.4MPa) (Figure 1). The citron watermelon landraces tested in this study behaved in a similar fashion as the seeds of feral *C. amarus* from Florida tested by Ramirez et al. (2014), which showed a final germination percentage of 37% at -0.3MPa. Interestingly, the *C. amarus* landrace from South Africa (RSA), native to the centre of domestication of this crop, reached the highest germination at -0.4MPa (36%). Considering the germination percentages, MGT and MET, the behaviour of ‘Sugar Baby’ is typical of a crop, which had been artificially bred to achieve a prompt, uniform germination after sowing and even under non-optimal environmental conditions (Fuller and Allaby 2009). On the contrary, the higher water requirement for germination observed in *C. amarus* landraces could be related to patterns of wild species, which tend to germinate only under optimal conditions. Indeed, seed germination of the wild *C. amarus* growing in arid areas of southern Africa occurs during the short rainy season (Mc Gregor 2012) and similarly *C. amarus* landraces are watered only during sowing (Jensen et al. 2000; M. Fontana personal communication). Hence, the scarce germination of *C. amarus* at low water availability could be considered as a drought avoidance strategy to prevent seedlings emergence during dry conditions, unfavourable for establishment, a common phenomenon in dryland plants (Evans and Etherington 1990; Gutterman 2000). This is in accordance with the fact that *C. amarus* seedling emergence does not occur at the soil surface (Ramirez et al. 2014), which is a typical response of semi-desert plants that prevent seedlings from drying out (Ren et al. 2002). Further studies are needed to clarify whether germination behaviours of citron watermelon landraces are the result of a less advanced level of domestication compared to sweet dessert watermelon cultivars.

MGT and MET were lower in the *C. lanatus* accession than in *C. amarus* landraces, with a high variability among the latter. It is of crucial importance for grafting, and therefore for the utilization of *C. amarus* landraces as rootstock, to characterise the timing of germination and emergence, since grafting is performed at seedling stage and the uniformity in germination and growth of the rootstock and scion are critical for the survival rates of grafted material (Davis et al. 2008). In line with the findings of Mavi et al. (2010), the accession that showed the fastest MGT (‘Sugar Baby’), showed also the fastest MET. However, in contrast to the results presented by Mavi et al. (2010), there was no positive correlation between MGT and final establishment. In fact, seed germination was the highest and fastest in ‘Sugar Baby’, though it had the lowest seedling establishment. *C. amarus* landraces, on the other hand, showed a high seedling establishment rate (close to 100% for most accessions, Figure 2). The very high seedlings establishment percentages in *C. amarus* accessions can therefore be considered a useful trait to improve *C. lanatus* lines in order to decrease the high seedling mortality observed in commercial cultivars.

‘Sugar Baby’ demonstrated to be a drought tolerant cultivar. There were not significant differences between the treatments in relation to biomass, which demonstrates the capability of this cultivar to maintain the overall growth under drought stress conditions (Figure 3). The size of the first true leaf of ‘Sugar Baby’ was smaller in the dry treatment than in the wet treatment (Figure 4), showing a typical response to drought intended to limit the water loss by reducing the leaf area and therefore the transpiration rate (Farooq et al. 2009). Regardless the treatment, *C. amarus* plants were consistently bigger than the *C. lanatus* cultivar ‘Sugar Baby’, for all biomass and growth parameters. In *C. amarus*, the biomass was higher in the wet treatment for all accessions (Figure 3), also the shoot biomass was significantly different between treatments. At the same time, shoot length did not change significantly between wet and dry treatments, while the first leaf length was always significantly different among treatments (Figure 4). This means that the variation of shoot biomass in *C. amarus* is due to a different investment in the dimension of the first true leaf between the treatments, to cope with the necessity to decrease the transpiration rate during periods of dry weather.

In ‘Sugar Baby’ the root/shoot ratio was higher in the dry treatment than in the wet treatment (Figure 5), suggesting that when exposed to drought conditions, plants invest more biomass in the root than in the shoot. The development of the root system is a strategy to increase the water uptake in many plant species under water stress (Jaleel et al. 2009). On the contrary the root/shoot ratio was not different between treatments in some *C. amarus* accessions (CAN, UKR, RUS, USA), while it increased significantly in the wet treatment in others (ITA6, RSA, UZB), meaning that the biomass allocation to the root system was higher in the full hydration (wet) treatment (Figure 5). This seems in contrast with a previous study on drought effects in wild *C. amarus* compared to commercial *C. lanatus* (Mo et al. 2015), in which *C. amarus*, under drought stress, showed a higher increase of RSR than commercial *C. lanatus*. The apparently opposite response, detected in this study, can be explained by a simple methodological difference. While in Mo et al. (2015) the plants were watered once a day until reaching the 75 ± 5 % field capacity, in our experiment the plants had in the wet treatment a water table below the pots which kept the soil always at water saturation during the entire three weeks of the experiment. Indeed, the constant availability of water stimulated a continuous biomass increase in *C. amarus* landraces in terms of root biomass, shoot biomass and leaf length. Considering that rain is the most important source of water in *C. amarus* cultivation, the difference in biomass between wet and dry treatments can be considered as adaptive traits. That is, the citron watermelon plant is able to maximise its growth in periods of good water availability. The *C. lanatus* cultivar ‘Sugar Baby’ and the *C. amarus* landraces have clearly different responses to different moisture levels during germination and seedling phases. Nevertheless, given the high variability in the landraces responses, it was not possible to find groups of *C. amarus* accessions exhibiting the same behaviour, considering the different parameters, under the water treatments tested. Referring to the specific aims of this paper, we were not able to find a *C. amarus* accession that showed undoubtedly a better performance under the drought conditions. Nevertheless, the high plasticity of *C. amarus* landraces in response to different water availabilities is worthwhile of further investigations. The high vigour of *C. amarus* seedlings and their strong root systems are all parameters of interest in a rootstock line selection (Davies et al. 2008).

This study confirms the assumption that landraces and neglected crops often show adaptive traits towards marginal conditions and abiotic stresses (Camacho Villa et al. 2006). The finding of these interesting adaptive features towards drought, reinforce the urgent need to continue the pre-breeding characterization of landraces and minor crops in the genus *Citrullus*.

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References:

Camacho Villa TC, Maxted N, Scholten M, Ford-Lloyd B (2006) Defining and identifying crop landraces. Plant Genetic Resources, Characterization and Utilization 3, 373–384.

Chomiki G, Renner SS (2015) Watermelon origin solved with molecular phylogenetics including Linnaean material: another example of museomics. New Phytologist 205, 526–532.

Cohen R, Burger Y, Horev C, Koren A, Edelstein M (2007) Introduced grafted cucurbit to modern agriculture. The Israeli experience. Plant Disease 91, 916-923.

Davis AR, Perkins-Veazie P, Sakata Y, López-Galarza S, Maroto JV, Lee SG, Huh YC, Sun Z, Miguel A, King SR, Cohen R, Lee JM (2008) Cucurbit Grafting. Critical Reviews in Plant Sciences 27, 50–74.

Davis AR, Levi A, Tetteh A, Wehner T, Russo V, Pitrat M (2007) Evaluation of Watermelon and Related Species for Resistance to Race 1W Powdery Mildew. Journal of the American Society for Horticultural Science 132, 790–795.

Ellis RH, Roberts EH (1980) Improved equations for the prediction of seed longevity. Annals of Botany 45, 13-30.

Evans CE, Etherington JR (1990) The effect of soil water potential on seed germination of some British plants. New Phytologist 115, 539-548.

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development 29, 185–212.

FAOSTAT (2014) Food and Agriculture Organization of the United Nations. http://faostat.fao.org/site/291/default.aspx. Accessed 15/03/2016

Fuller DQ, Allaby R (2009) Seed dispersal and crop domestication: shattering, germination and seasonality in evolution under cultivation. Annual Plant Reviews 38, 238–295.

Gutterman Y (2000) Environmental factors and survival strategies of annual plant species in the Negev Desert, Israel. Plant Species Biology 15, 113-125.

Hardegree SP, Emmerich WE (1989) Effect of Polyethylene Glycol Exclusion on the Water Potential of Solution-Saturated Filter Paper. Plant Physiology 92, 462-466.

Huitrón, M.V., Diaz, M., Diánez, F., Camacho, F., 2007. The effect of various rootstocks on triploid watermelon yield and quality. Journal of Food, Agriculture & Environment 5, 344 -348.

Hwang, J.H., Ahn, S.G., Oh, J.Y., Choi, Y.W., Kanga, J.S., Park, Y.H., 2011. Functional characterization of watermelon (*Citrullus lanatus* L.) EST–SSR by gel electrophoresis and high resolution melting analysis. Scientia Horticulturae 130, 715–724.

Jaleel, C.A., Paramasivam, M., Wahid, A., Farooq, M., Al-Juburi, H.J., Somasundaram, R., Panneerselvam, R., 2009. Drought stress in plants: a review on morphological characteristics and pigments composition. International Journal of Agriculture and Biology 11, 100–105.

Jarret, R.L., Merrick, L.C., Holms, T., Evans, J., Aradhya, M.K., 1997. Simple sequence repeats in watermelon (Citrullus lanatus (Thunb.) Matsum. &Nakai). Genome 40, 433-441.

Jarret, R.L. 2014. Observations on anatomical aspects of the fruit, leaf and stem tissues of four *Citrullus* spp. African Journal of Plant Sciences 8, 521-527.

Jensen, B.D., Tourè, F.M., Hamattal, M.A., Tourè, F.A., Nantoumè, A.D., 2011. Watermelons in the Sand of Sahara: Cultivation and use of indigenous landraces in the Tombouctou Region of Mali. Ethnobotany Research and Applications 9, 151-162.

Kawasaki, S., Miyake, C., Kohoci, T., Fujii, S., Uchida, M., Yokota, A., 2000. Responses to wild watermelon to drought stress: accumulation of an ArgE homologue and citrulline in leaves during water deficit. Plant and Cell Physiology 41, 864-873.

King, S.R., Davis, A.R., Liu, W., Levi, A., 2008. Grafting for Disease Resistance. HortScience 43, 1673-1676.

Levi, A., Thomas, C.E., Keinath, A.P., Wehner, T.C., 2001a. Genetic diversity among watermelon (*Citrullus lanatus* and *Citrullus colocynthis*) accessions. Genetic Resources and Crop Evolution 48, 559-566.

Levi, A,. Thomas, C.E., Wehner, T.C., Zhang, T.C., 2001b. Low genetic diversity indicates the need to broaden the genetic base of cultivated watermelon. HortScience 36, 1096–1101.

Levi, A., Thomas, C.E., Newman, M., Zhang, X., Xu, Y., Wehner, T.C., 2003. Massive preferential segregation and non random assortment of linkage groups produce quasi-linkage in an F2 mapping population of watermelon. HortScience 38, 782.

Laghetti, G., Hammer, K., 2007. The Corsican citron melon (*Citrullus lanatus* (Thunb.) Matsum. et Nakai subsp. *lanatus* var. *citroides* (Bailey) Mansf. ex Greb.) a traditional and neglected crop. Genetic Resources and Crop Evolution 54, 913-916.

Mabhaudhi, T., O'Reilly, P., Walker, S., Mwale, S. 2016. Opportunities for Underutilised Crops in Southern Africa's Post–2015 Development Agenda. Sustainability 8, 302.

Mavi, K., Demir, I., Matthews, S., 2010. Mean germination time estimates the relative emergence of seed lots of three cucurbit crops under stress conditions. Seed Science and Technology 38, 14-25.

Mc Gregor, C., 2012. *Citrullus lanatus* germplasm of southern Africa. Israel Journal of Plant Sciences 60, 403-413.

McGregor, C.E., Waters, V., 2013. Pollen Viability of F1 Hybrids between Watermelon Cultivars and Disease-resistant, Infraspecific Crop Wild Relatives. HortScience, 48, 1428-1432.

Michel, B.E., 1983. Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. Plant Physiology 72, 66-70.

Mo, Y., Yang, R., Liu, L., Gu, X., Yang, X., Wang, Y., Zhang, X., Li, H., 2015. Growth, photosynthesis and adaptive responses of wild and domesticated watermelon genotypes to drought stress and subsequent re-watering. Plant Growth Regulation (in press).

Modi, A.T., Zulu, N.S., 2012. Watermelon landrace seedling establishment and field performance in response to differing water regimes. African Journal of Agricultural Research 7, 6016-6021.

Mujaju, C., Zborowska, A., Werlemark, G., Garkava-Gustavsson, L., Andersen, S.B., Nybom, H. 2011. Genetic diversity among and within watermelon (*Citrullus lanatus*) landraces in Southern Africa. Journal of Horticultural Science and Biotechnology 86, 353-358.

Paris, H.S., 2015. Origin and emergence of the sweet dessert watermelon, *Citrullus lanatus*. Annals of Botany 116, 133–148.

Ramirez, A.H.M., Jhala, A.J., Singh, M., 2014. Factors affecting germination of citronmelon (*Citrullus lanatus* var. *citroides*). Weed Science 62, 45-50.

Ren, J., Tao, L., Liu, X.M., 2002. Effect of sand burial depth on seed germination and seedling emergence of *Calligonum* L. species. Journal of Arid Environments 51, 603–611.

Rubatsky, V.E., 2001. Origin, distribution, and uses. In: *Watermelons: characteristics, production, and marketing*. pp.21-26. Ed. D.N. Maynard. Alexandria, VA, USA: ASHS Press.

Thanos, C.A., Mitrakos, K., 1992. Watermelon seed germination. 1. Effects of light, temperature and osmotica. Seed Science and Research 2, 155-162.

Thies, J.L., Ariss, J.J., Hassel, R.L., Buckner, S., Levi, A., 2015a. Accessions of *Citrullus lanatus* var. *citroides* Are Valuable Rootstocks for Grafted Watermelon in Fields Infested with Root-knot Nematodes. HortScience 50, 4-8.

Thies, J.L., Buckner, S., Horry, M., Hassel, R., Levi, A., 2015b. Influence of *Citrullus lanatus* var. *citroides* Rootstocks and Their F1 Hybrids on Yield and Response to Root-knot Nematode, *Meloidogyne incognita*, in Grafted Watermelon. HortScience 50: 9-12.

Vavilov, N.I., 1987. The phyto-geographical basis for plant breeding. In: *Origin and geography of cultivated plants*. pp: 316-366. Cambridge, UK: Cambridge University Press (first published 1935).

Wehner, T.C., 2008. Watermelon. In: *Handbook of plant breeding, vegetables I*. pp. 381-419. Eds. J. Probens, F. Nuez. New York, NY, USA: Springer.

Wechter, P.W., Kousik, C., McMillan, M., Levi, A., 2012. Identification of resistance to *Fusarium oxysporum* f. sp. *niveum* race 2 in *Citrullus lanatus* var. *citroides* plant introductions. HortScience 47, 334-338.

Yoshimura, K., Masuda, A., Kuwano, M., Yokota, A., Akashi, K., 2008. Programmed proteome response for drought avoidance/tolerance in the root of a C_3 xerophyte (wild watermelon) under water deficits. Plant Cell Physiology 49, 226-241.

Zeven, A.C., 1998. Landraces: A review of definitions and classifications. Euphytica 104:127–139

Zhang H, Gong G, Guo D, Ren Y, Xu Y, Ling KS, 2011a.Screening the USDA watermelon germplasm collection for drought tolerance at the seedling stage. HortScience 46, 1245-1248.

Zhang, H., Guo, D., Gong, G., Ren, Y., Davis, A.R., Xu, Y., 2011b. Sources of Resistance to Race 2WF Powdery Mildew in U.S. Watermelon Plant Introductions. HortScience 46, 1349-1352.

Article III

Guzzon F, Orsenigo S, Gianella M, Müller JV, Vagge I, Rossi G, Mondoni A (2018) Seed heteromorphy influences seed longevity in *Aegilops*. Seed Science Research, 28, 277-285.

Supplementary material is provided after the references section: pp 80-81

Seed heteromorphy influences seed longevity in *Aegilops*

Abstract

The genus *Aegilops* belongs to the secondary gene pool of wheat and has great importance for wheat cultivar improvement. As a genus with only annual species, regeneration from seeds in *Aegilops* is crucial. In several species in *Aegilops*, spikes produce different seed morphs, both in size and germination patterns. However, little is known about the ecology of seed germination, nor about the seed longevity in this genus. Here we investigated the germination phenology of *Ae. neglecta* under laboratory and field conditions and assessed longevity of different seed morphs of five additional *Aegilops* species using controlled ageing tests. Large seeds were short-lived and germinated faster than small seeds in most of the species. Field experiments with *Ae. neglecta* showed that large seeds of the dimorphic pair germinated 3 months after dispersal in contrast to 14 months for smaller seeds. Differences in longevity were detected not only in dimorphic seed pairs, but also among seeds from different positions on the spike. Our results indicate that different longevities in seed morphs of *Aegilops* may reflect a different soil seed bank persistence, with smaller seeds able to maintain a higher viability after dispersal than larger ones, thereby spreading seedling emergence over two years. Differences of seed germination and longevities between seed morphs in *Aegilops* may have important implications for ex situ seed conservation and reinforce the hypothesis of a bet-hedging strategy in the germination ecology of this genus.

Keywords: controlled ageing test; crop wild relatives; germination ecology; seed conservation; seed dimorphism; phenology; soil seed bank.

Introduction

Seed heteromorphy is defined as the production of different types of seeds by a single plant and is a common strategy to cope with unpredictable climatic conditions (Bhatt and Santo 2016). Seed heteromorphy influences seed dispersal ability, germination, seed dormancy and persistence in the soil (Duràn and Retamal 1989; Puga-Hermida et al. 2003; El-Keblawy and Bhatt 2015). Seed dimorphism is considered the main type of seed heteromorphy and it is expressed mostly in annual plants (Venable 1985).

The *Aegilops* genus includes 23 species of annual grasses, occurring in southern Europe, North Africa, the Near East and western and central Asia. *Aegilops* species occur in various dry, thermophile and low nutrient, often disturbed habitats (e.g. pastures, roadsides, field margins, wastelands; van Slageren 1994). Some *Aegilops* L. species (Triticeae Dumort.) produce caryopses (hereafter referred to as seeds) of different size and shape and different testa colours. Seed dimorphism was also detected and shown to be linked to a complex dispersal strategy (Dyer 2004; Marañon 1989; Matilla et al. 2005). In *Aegilops geniculata* Roth, *Ae. kotschyi* Boiss., *Ae. neglecta* Req. ex Bertol. and *Ae. triuncialis* L. dimorphic pairs of seeds are found in the lower fertile spikelets, with one seed larger and lighter coloured than the other one (Datta et al. 1970; Wurzburger et al. 1976; Dyer 2004; Marañon 1989). In *Ae. geniculata*, only the larger seeds of the dimorphic pair within the spikelets readily germinate, whereas the germination of the smaller seeds is inhibited probably due to the presence of an inhibitor in the glumes (Datta et al. 1970). A similar behaviour was detected in *Ae. cylindrica* Host and *Ae. triuncialis* (Fandrich and Mallory-Smith 2005; Dyer 2017). Seed heteromorphism in *Aegilops* is supposed to reflect a bet-hedging strategy to cope with variable environments with unpredictable resource availability (Dyer 2004). Germination delay for some of the seeds increases the chances that at least some offspring survive, reducing also the competition among sibling seedlings (Nave et al. 2016). This strategy could be particularly functional in *Aegilops*, considering its very limited dispersal capacity (Dyer 2004; van Slageren 1994; Volis 2016). It has been hypothesized that in *Aegilops* the largest seeds of the dimorphic pair germinate after the first rainfall at the end of summer, or beginning of autumn, whereas the smallest seeds germinate later (Datta et al. 1970; Marañon 1989), probably during the following spring (Onnis et al. 1995). Consequently, smaller seeds should be able to maintain a high viability for lingering after dispersal than larger ones. Interestingly, the smaller seeds of *Ae. triuncialis* remained viable longer during storage under laboratory conditions compared with larger seeds (Dyer 2017) and similar patterns were found in dimorphic seeds of *Dasypyrum villosum* (L.) P. (De Pace et al. 2011), suggesting that different germination patterns across seed morphs could reflect different seed longevities, i.e. with small seeds being inherently longer-lived than large seeds, since they have to persist longer in the soil before germination. Despite this, little is known about differences of longevity across seed morphs in *Aegilops*.

The longevity of seeds is an important plant trait, allowing not only seed persistence in the soil but also long-term ex situ plant conservation in germplasm banks (Walters et al. 2005; Probert et al. 2009). In orthodox seeds, longevity is mainly determined by seed moisture content and temperature during storage. By experimentally increasing these two factors, it is possible to accelerate ageing processes in the laboratory, thereby estimating seed longevity from survival curves (Ellis and Roberts 1980) and predicting with caution seed persistence in the field (Long et al. 2008). Understanding seed longevity differences among species (see e.g. Walters et al. 2005; Probert et al. 2009; Mondoni et al. 2011; Bernareggi et al. 2015; Davies et al. 2016) is crucial for the management of ex situ seed conservation facilities, as it allows to plan re-collection or define regeneration intervals (Walters 2003).

Ex situ seed conservation is particularly important for crop wild relatives (CWR) whose collections should be representative of the largest possible genetic variability, in order to safeguard it and provide useful traits for crop improvement (Warschefsky et al. 2014). *Aegilops* forms part of the secondary gene pool of wheat, playing a key role in wheat evolution and domestication (van Slageren 1994) and being extensively used in breeding programmes (Maxted et al. 2008; Kilian et al. 2011). Furthermore, some *Aegilops* species are endangered (e.g. *Ae. uniaristata* Vis., *Ae. biuncialis* Vis. and *Ae. tauschii* Coss.; Bilz et al., 2011; Rossi et al., 2013), underlining the need for an effective ex situ conservation of this genus. Therefore, a better understanding of the ecology of these wheat wild relatives is essential for their exploitation in breeding programmes and for their conservation (McCouch et al. 2013; Dempewolf et al. 2014).

The objective of this study was to characterize germination and longevity of heteromorphic seeds in different *Aegilops* species, integrating field and laboratory investigations. In particular, we aimed to: (1) estimate the longevity of different seed morphs in six *Aegilops* species using controlled ageing tests (CAT), and (2) investigate germination phenology in seed morphs of *Ae. neglecta* under laboratory and field conditions.

Materials and methods

Study material

Dispersal units of all *Aegilops* species were collected in 2015 at the time of natural dispersal (May–June) in nature, except those of *Ae. biuncialis* that were collected from a first generation population cultivated in Cyprus (Table 1). After collection, seeds were extracted from the dispersal units. For the *Aegilops* species in which the whole spike is the dispersal unit and therefore spikelets do not disarticulate at maturity (i.e. *Ae. geniculata*, *Ae. neglecta*, *Ae. peregrina* (Hack.) Maire and Weiller and *Ae. uniaristata*), the basal fertile spikelet was separated from the upper ones and the dimorphic seed pairs extracted from it. After seed cleaning, seeds were kept at 15% relative humidity (RH) and 15°C (ISTA, 2018) until use. Germination phenology of *Ae. neglecta* both in the laboratory and in the field were studied using fresh seeds, re-collected from the same population in July 2016 and immediately sown. Seed mass was determined by weighing 20 individual seeds, kept at 15% RH, randomly sampled from each seed lot, using an analytical balance (Kern ALJ 220-4 nm).

Table 1. Geographical locations of the sampled populations and type of the six Aegilops species. The type of dispersal unit (DU) is indicated. The seed lots extracted in each species by the DU are also provided

Species	Country	Latitude	Longitude	Section	DU	Biological status	Seed lots
Ae. cylindrica	Italy	N 44° 57′ 25.02″	E 9° 11′ 53.88″	Cylindropyrum	Spikelet	Wild	a/b/s
Ae. biuncialis	Cyprus	N 34° 55′ 55.96″	E 32° 21′ 01.8″	Aegilops	Spike	Cultivated	1/2/3
Ae. geniculata	Italy	N 44° 57′ 25.02″	E 9° 11′ 53.88″	Aegilops	Spike	Wild	1a/1b
Ae. peregrina	Cyprus	N 34° 43′ 53.69″	E 33° 21′ 01.8″	Aegilops	Spike	Wild	1a/1b/2a
Ae. neglecta	Italy	N 44° 48′ 26.6″	E 8° 54′ 16.7″	Aegilops	Spike	Wild	1a/1b/2a
Ae. uniaristata	Italy	N 40° 37′ 0.73″	E 16° 58′ 9.08″	Comopyrum	Spike	Wild	1a/1b

Ageing experiments and longevity assessment

Seed longevity was estimated using a modified protocol for accelerated ageing tests (Newton et al. 2009). Ageing experiments started in April 2016 and lasted for 6 months. Prior to storage, seeds were first rehydrated for 14 days in open Petri dishes over a non-saturated solution of LiCl in a sealed 300 × 300 × 130 mm sealed electrical enclosure box (Ensto UK Ltd, Southampton, UK) at 47% RH and 20°C. Thereafter, seed equilibrium relative humidity (eRH) was verified with an AW-DI0 water activity probe used in conjunction with a HygroPalm 3 display unit (Rotronic Instruments UK Ltd, Crawley, UK). Once the seed lot was judged to have reached eRH, the initial germination was assessed using triplicates of 20 seeds using the protocol described in the next section. Next, seed lots were stored in sealed box over a non-saturated LiCl solution at 60% RH at 45 ± 2°C in the dark placed in a compact incubator (Binder FD53, Binder GmbH, Tuttlingen, Germany). The RH generated by the LiCl solution in the box was monitored with a data logger inside the enclosure box (Tinytag View 2 Temperature/Relative Humidity Logger, Gemini Data Logger, Chichester, UK). When necessary, the bulk solution was adjusted by adding distilled water, stirring and allowing the solution to equilibrate (Hay et al. 2008; Newton et al. 2009). At three to four intervals during storage, tri-replicates of 20 seeds were removed to assess viability using germination. Different seed lots were exposed to different periods of storage according to their lifespan so that seeds could experience a complete loss of viability.

Seed germination in the laboratory

Triplicates of 20 seeds each per lot were sown on 1% agar in 50 mm diameter Petri dishes that were placed, randomly interspersed, in an alternating temperature (20/10°C) and 12 h daily photoperiod (photosynthetically active radiation 40–50 µmol m^{-2} s^{-1} ; LMS 250A, LMS Ltd, Sevenoaks, UK; Guzzon et al., 2015). Germination was scored twice a day when the emerging radicle had reached approximately 2 mm. At the completion of each germination test (4 weeks after sowing), ungerminated seeds were cut to confirm whether they were empty. Final germination percentages were adjusted based the number of filled seeds.

Seed germination in the field

A triplicate of 70 spikes from *Aegilops neglecta* was sown in July 2016 at the original collection site (Table 1). One plot (50 × 60 cm) per each replicate was prepared by removing the vegetation and flattening the soil. The spikes were inserted in the soil surface to simulate natural dispersal (Perrino et al. 2014). Seedling emergence from each spikelet was recorded every 2 weeks for the first 3 months and thereafter, once a month, for the following 11 months until the end of September 2017. Only the emergence of the dimorphic seed pairs of the basal fertile spikelet was recorded. One year after sowing, 20 spikelets per replicate were retrieved and the viability of ungerminated seeds was assessed using a germination test in the laboratory as described above. Data loggers recording temperature and RH were placed at the soil surface (WatchDog data logger, Spectrum Technologies, Inc.) and buried 2 cm deep in the soil (Supplementary Figure 1).

Statistical analysis

Non-parametric Kruskal–Wallis tests were applied to evaluate if the difference in seed masses was significant among different species and if the mass of the different seed lots varied significantly within species.

To estimate p_{50}, as measure of seed longevity, probit analysis of the seed viability data was carried out using GenStat for Windows, version 9 (VSN International Ltd, Oxford, UK) by fitting the seed viability equation:

$v = K_i - p/s,$

where v is the viability in normal equivalent deviates (NED) at time p (days); K_i is the initial viability or the intercept on the y-axis (NED), and σ is the standard deviation of the normal distribution of seed deaths in time. Previous studies have highlighted that in some Aegilops species the dimorphic seed pairs extracted from the spikelet show differences in the germination timing, with larger seeds germinating faster than the smaller ones (Datta et al. 1970; Marañon 1989). To test this observation, differences in germination timing (mean time to germinate, MTG) were recorded of fresh seeds (i.e. not exposed to CAT). The meantime to germination MTG was calculated as:

$$MTG = \Sigma\,(nT)/N,$$

where n is the number of seeds that germinated within consecutive intervals of time, T is the time between the beginning of the test and the end of a particular interval of measurement, and N is the total number of seeds that germinated. The MTG was calculated using the hour of sowing as initial time.

ANOVA was used to test the significance of the differences, in terms of p_{50} and MGT detected among the different seed lots and species. Bonferroni post-hoc tests were used to pairwise compare the differences in p_{50} and MTG between seed lots of the species with three seed lots. Data were checked for normality and homogeneity of variance to meet the ANOVA assumption. To this end, MGT data were log-transformed. The F-test was used to determine whether there was a significant increase in residual deviance by constraining seed survival curves for reduced seed number and standard longevity test data to a single slope (σ^{-1}) and intercept (K_i). Seed germination was modelled using a binomial probability distribution and a logit link function by means of a generalized linear model (GLM). The Kruskal–Wallis tests, GLMs and ANOVAs were performed using SPSS for Windows, version 21.0.

Results

Seed heteromorphy

Based on the morphology of the spike and the presence/absence of seed dimorphism within-spikelet, the six species tested in the current experiment were divided into three groups (Figure 1), and the seed morphs identified were considered as different seed lots during the germination experiment:

(1) In *Ae. geniculata*, *Ae. peregrina*, *Ae. neglecta* and *Ae. uniaristata*, a clear seed dimorphism was observed in the seed pair of the basal fertile spikelet. The larger and brighter coloured (here after referred as 1a) and the smaller and darker (hereafter referred to as 1b) seeds of the seed pairs, were considered as different seed lots during the ageing experiment (Table 1, Figure 1). In *Ae. peregrina* and *Ae. neglecta*, an additional seed lot was considered (hereafter referred as 2a), consisting of one seed of the second fertile spikelet (only the larger and brighter in case of a dimorphic pair; Table 1, Figure 1).

(2) *Ae. biuncialis* shows a similar spike morphology to the aforementioned species, although in our accession no clear size and colour dimorphism could be noticed between seeds from the same spikelet. Therefore, three seed lots were considered for *Ae. biuncialis* including seeds from the first (basal) fertile spikelet, seeds from the second fertile spikelet and seeds from the third fertile spikelet (hereafter referred as 1, 2 and 3; Table 1, Figure 1).

(3) In *Ae. cylindrica* the spike disarticulates at maturity into several (6–8) spikelets, therefore the spikelet serves as the dispersal unit (van Slageren, 1994). Three seed lots were considered for this latter species, including the larger (a) and smaller (b) seed of the dimorphic pair inside the spikelet and the solitary seed (s) when there was only one seed inside the spikelet (Table 1, Figure 1). The dimorphic pair in *Ae. cylindrica* showed only size dimorphism but no colour differences.

Larger seeds from the dimorphic pairs are defined as 'primary seeds' (seed lots 1a, a) while the smaller ones are 'secondary seeds' (seed lots 1b, b). Example pictures and X-ray scans of the spikes and pictures of the seed morphs are provided in Figure 2.

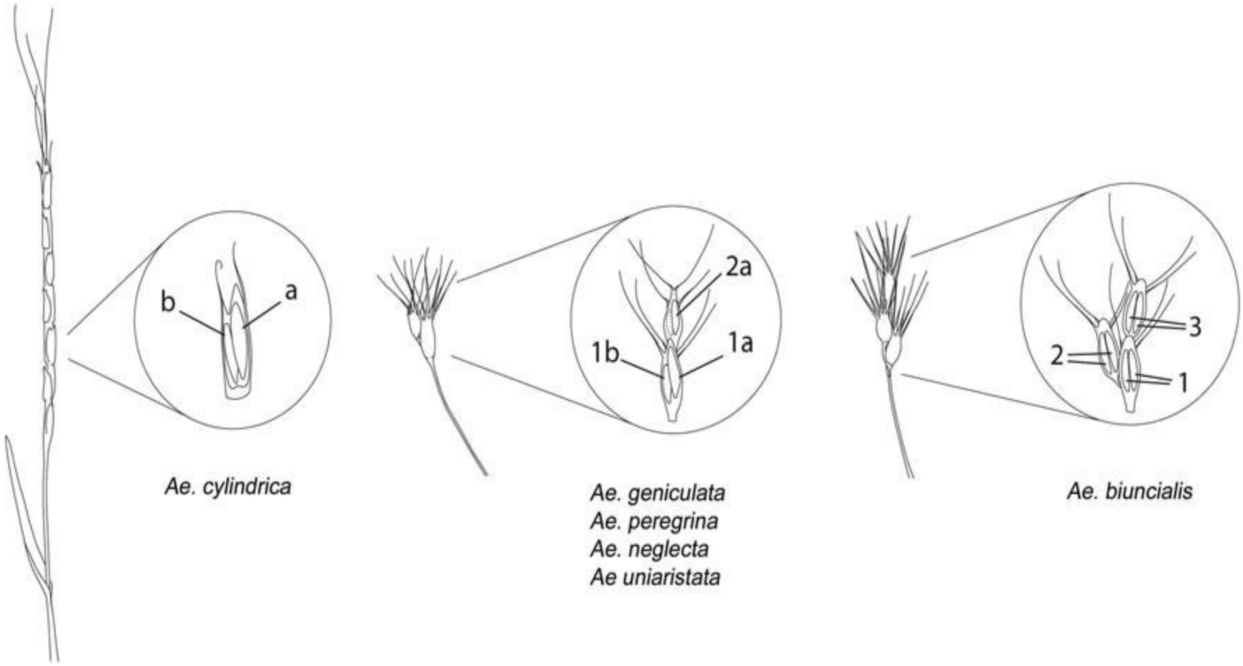

Figure 1. Schemes of the spikes and internal spikelet morphologies (in the insets) encountered in the tested *Aegilops* species. On the left, a spike and a spikelet of *Ae. cylindrica* with the larger (a) and smaller (b) seeds. In the middle, the spike and spikelet morphology of *Ae. geniculata*, *Ae. uniaristata*, *Ae. peregrina* and *Ae. neglecta*. First fertile spikelet had two dimorphic seeds: one larger (1a) and one smaller (1b). In *Ae. peregrina* and *Ae. neglecta* another seed lot was considered (2a) consisting of solitary seeds of the second fertile spikelet or larger seeds in case of the presence of a dimorphic pair also in the second spikelet (smaller seed in dashed line). On the right, spike morphology of *Ae. biuncialis*, a species that does not show within-spikelet seed dimorphism. Three seed lots were considered, seeds of the basal fertile spikelet (1), seed of the second fertile spikelet (2), and seeds of the third fertile spikelet (3).

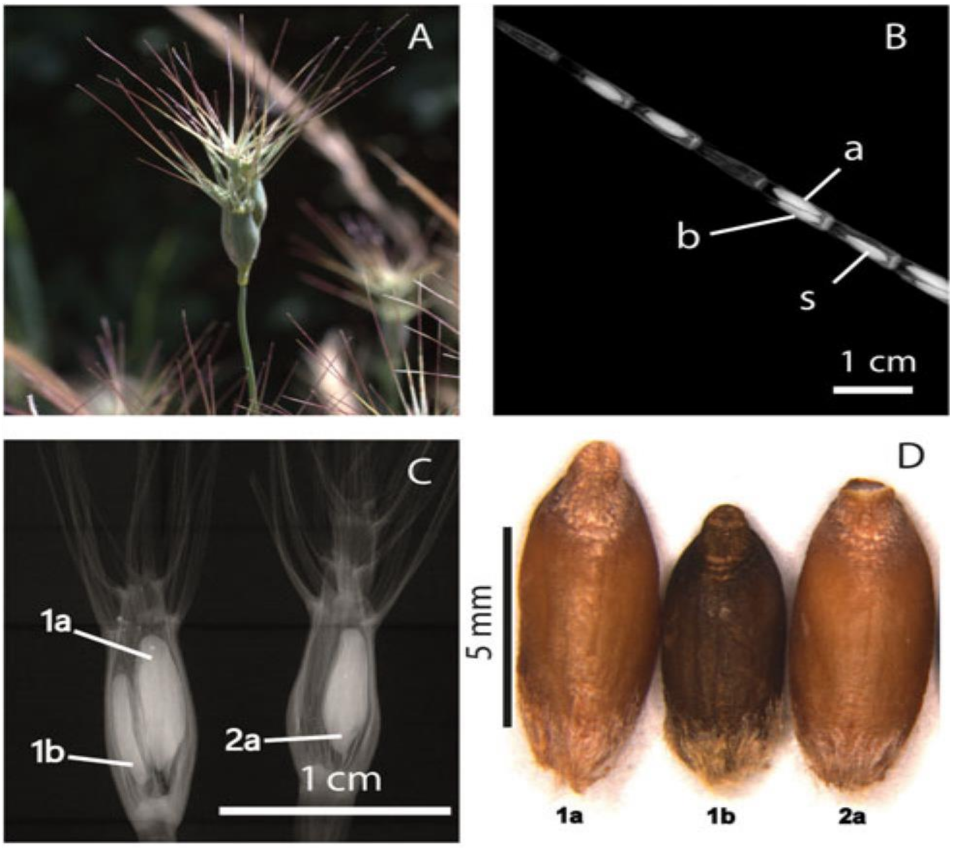

Figure 2. (A) Spike of *Ae. geniculata* in the field. (B) X-ray scan of a spike of *Ae. cylindrica*. Different spikelets can be observed enclosing either one seed (seed lot s) or a dimorphic seed pair with one seed (seed lot a) larger than the other (seed lot b). (C) X-ray scan of a basal fertile spikelet of *Ae. geniculata* (on the left); the bigger (1a) and the smaller seed (1b) of the dimorphic seed pair can be observed. Moreover, the 2a seed of the second fertile spikelet (on the right) can also be observed. 2a seed lot was not considered for *Ae. geniculata* in the experiment, but was tested in *Ae. neglecta* and *Ae. peregrina*, species with a similar spike morphology to *Ae. geniculata*. The X-ray scanning was performed with a Faxitron X-ray scanner at the Millennium Seed Bank of the Royal Botanic Gardens, Kew. (D) Seeds extracted from the same spike and belonging to seed lots 1a, 1b and 2a of *Ae. neglecta*. The picture was taken using a microscope (Zeiss Stemi SV 11) with camera (Zeiss AxioCam). All the pictures were taken by F. Guzzon and edited by M. Canella (University of Padua).

Seed mass

Seed mass varied significantly both among species (Kruskal– Wallis, $P < 0.001$) and within all the species among seed lots ($P < 0.001$). Hence in all the species showing dimorphic seed pairs (e.g. *Ae. cylindrica, Ae. geniculata, Ae. neglecta, Ae peregrina and Ae. uniaristata*; Figure 1) primary seeds (a, 1a) were significantly heavier than secondary (b, 1b; Table 2; $P < 0.05$). In *Ae. neglecta*, seed lot 2a was lighter than both 1a and 1b ($P < 0.001$), while in *Ae. peregrina* 2a was lighter than 1a ($P = 0.020$) and not significantly different from 1b (Table 2; $P = 0.159$). The seed lot s of *Ae. cylindrica* was significantly lighter than a ($P < 0.001$) but not different in seed mass from b ($P = 0.779$). Finally in *Ae. biuncialis*, the only tested species not showing within spikelet dimorphism (Figure 1), seeds 1 were lighter than 2 and 3 ($P < 0.001$) while seed lots 2 and 3 did not different significantly between them (Table 2; $P = 0.799$).

Seed longevity

The estimated longevity of seeds (p_{50}) ranged from 22.74 ± 2.803 days (*Ae. biuncialis* 2) to 85.72 ± 2.203 days (*Ae. neglecta* 1b; Table 2). Species and seed lot had a significant effect on the p_{50} (d.f. = 5; F = 11.413; $P < 0.001$ and d.f. = 8; F = 11.078; $P < 0.001$). Seed longevity was not statistically different among seed lots in *Ae. peregrina* ($P = 0.284$), as well as between seed lot 3 and both 1 ($P = 0.084$) and 2 ($P = 0.186$) in *Ae. biuncialis*. In *Ae. geniculata*, *Ae. neglecta* and *Ae. uniaristata*, the secondary seeds (1b) of the second fertile spikelets were always longer lived than the primary ones (1a) ($P < 0.05$), although to a different extent across species. In particular, estimated secondary seeds (1b) longevity was 1.4- to 1.9-fold greater than primary ones (1a) for *Ae. geniculata*, *Ae. neglecta* and *Ae. uniaristata*. Similarly, in *Ae. cylindrica* the estimated longevity of the secondary seed lot (b) was significantly ($P < 0.001$) 1.5-fold greater than the primary seed lot (a). At the same time, the estimated seed longevity of the solitary seeds (s) was intermediate between that of seed lot a ($P = 0.026$) and b ($P = 0.001$). Similarly, in *Ae. neglecta* p_{50} of seed lot 2a was intermediate between that of 1a and 1b ($P < 0.001$) (Figure 3, Table 2).

Within each species, the slopes (σ^{-1}) of the survival curves across seed lots were not significantly different ($P > 0.05$). On the other hand, differences in the intercept (K_i) of the survival curves were significant ($P < 0.05$) in all the pairwise comparison, except in *Ae neglecta* 1a and 2a ($F_{1,3}$=3.655), *Ae biuncialis* 1 and 3 ($F_{1,3}$=5.988), *Ae biuncialis* 2 and 3 ($F_{1,3}$=2.422) and in all pairwise comparisons between seed lots in *Ae. peregrina* (1a and 1b, $F_{1,3}$=3.693; 1a and 2a, $F_{1,3}$=0.948; 1b and 2a, $F_{1,3}$=1.927).

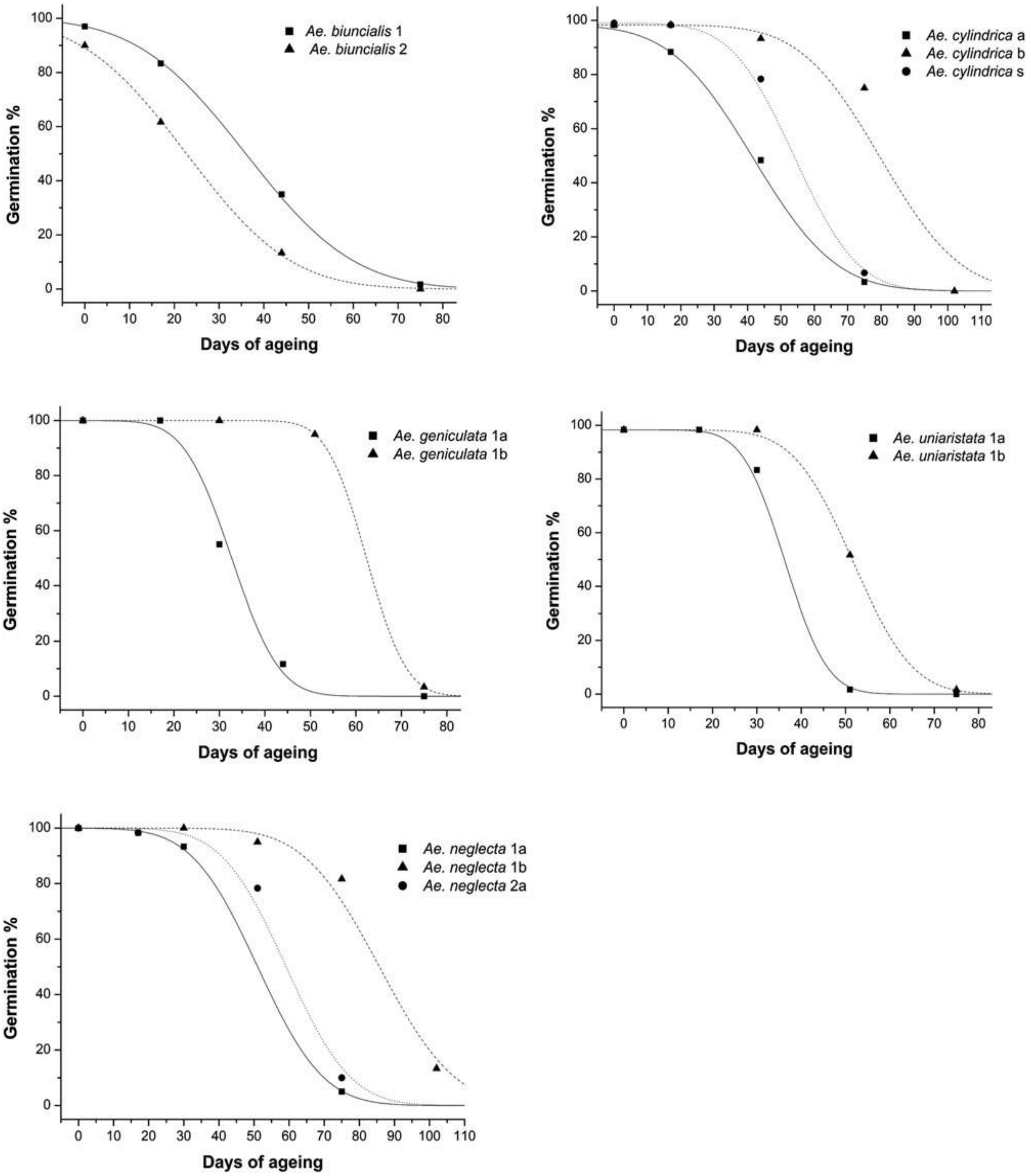

Figure 3. Survival curves fitted by probit analysis of indicated Aegilops species. In *Ae. biuncialis* the curve of the seed lot 3 is not presented as the p_{50} values of this seed lot was not different from seed lots 1 and 2 of the same species (see text). Survival curves of *Ae. peregrina* are not presented as the values of p_{50} for the three seed lots tested for this species (1a, 1b, 2a) were not significantly different (see text).

Species	**Seed lot**	**Mass(mg)**	***p50* (days)**	***Ki* (NEDs)**	**σ^{-1} (days^{-1})**	**MTG (hours)**	**Time points (days)**
Ae. biuncialis	**1**	6.56±0.12	36±2.4	1.882±0.272	0.052±0.006	58±3	0, 17, 44, 75
	2	11.87±0.20	22.7±2.8	1.230±0.277	0.054±0.009	61±3	0, 17, 44, 75
	3	11.51±0.14	28.2±2.4	1.620±0.283	0.057±0.008	58±1	0, 17, 44, 75
Ae. cylindrica	**a**	17.91±0.15	41.7±2.4	2.136±0.280	0.051±0.006	74±2	0, 17, 44, 75
	b	10.67±0.06	79.8±2.4	4.229±0.513	0.053±0.006	64±2	0, 17, 44, 75, 102
	s	11.88±0.24	54.1±2.2	3.639±0.450	0.067±0.008	61±4	0, 17, 44, 75
Ae. geniculata	**1a**	14.76±0.16	32.7±1.1	3.968±0.475	0.121±0.014	47±1	0, 17, 30, 44, 75
	1b	7.54±0.11	62.3±1.5	9.04±1.08	0.145±0.017	75±5	0, 30, 51, 75
Ae. peregrina	**1a**	11.86±0.17	46.8±3.3	2.210±0.277	0.047±0.006	62±1	0, 17, 30, 75
	1b	4.6±0.08	60.2±4.4	1.951±0.334	0.032±0.005	105±8	0, 30, 75, 102
	2a	5.8±0.30	50.2±2.9	2.567±0.394	0.051±0.007	81±3	0, 30,75, 102
Ae. neglecta	**1a**	30.12±0.18	51±2.6	3.458±0.364	0.068±0.007	48±0.4	0, 17, 30, 75
	1b	13.33±0.12	85.7±2.2	5.063±0.595	0.059±0.007	112±7	0, 30, 51, 75, 102
	2a	9.94±0.26	59.26±2.	4.094±0.609	0.069±0.01	65±4	0, 17, 51, 75
Ae. uniaristata	**1a**	14.72±0.14	36.4±1.3	4.847±0.578	0.133±0.016	44±0.1	0, 17, 30, 51, 75
	1b	6.16±0.12	51.7±1.6	4.888±0.652	0.094±0.013	68±1	0, 30, 51, 75

Table 2. Values (mean ± standard error) of seed mass, parameters obtained through fitting of the basic seed viability equation (p50, Ki, σ^{-1}) and the initial MTG (before the accelerated aging treatment) for each seed lot. The experimental time points in which seed viability was tested are also indicated.

Seed germination in the laboratory

Under laboratory conditions, seed morphs of all species showed high germination percentage (on average >90%), without significant differences across both, species (d.f. = 5, Wald chi-square = 5.342, P = 0.376) and seed morphs (d.f. = 8, Wald chi-square = 12.603, P = 0.126). Mean time to germinate (MTG) was significantly different across species and seed lots (d.f. = 5; F = 2.457; P < 0.05 and d.f. = 8; F = 7.330; P < 0.001).). In *Ae. geniculata, Ae. neglecta, Ae. peregrina* and *Ae. uniaristata*, the MGT was significantly greater (1.6, 2.3, 1.7 and 1.5-fold respectively) in the secondary seeds (1b) of the first spikelet then in the primary ones (1a) (P < 0.05). In *Ae. peregrina* the MTG of seed lot 2a was similar to both those showed by seed lots 1a (P = 0.196) and 1b (P = 0.266), while in *Ae. neglecta* seed lot 2a germinated significantly faster than seed lot 1b (P < 0.05), but similarly to seed lot 1a (P = 0.195). Finally, no significant differences were observed in the MTG within seed lots in *Ae. cylindrica* (a,b,s) and *Ae. biuncialis* (1,2,3) (P > 0.05) (Table 2). Supplementary Figure 2 outlines germination progress curves among the different seed lots.

Seed germination in the field

Ten weeks after sowing (and few days after the first rains; end of September 2016) 41.43 ± 14.8% of the primary seeds of the first basal fertile spikelets (seed lot 1a) of *Ae. neglecta* had emerged (Figure 4). Two weeks later, 90 ± 8.5% of the primary seeds emerged. Moreover, 1 year after sowing (July 2017) the secondary seeds of the first basal fertile spikelets (seed lot 1b) were all still ungerminated. However, by that time 100% of a subsample of them, retrieved from the soil and extracted from the spikes, could germinate in 3 days under laboratory conditions. Finally, germination of seeds left in the field within the spikes was complete (100%) in September 2017, 14 months after sowing and 1 year after the emergence of primary seeds (Figure 4).

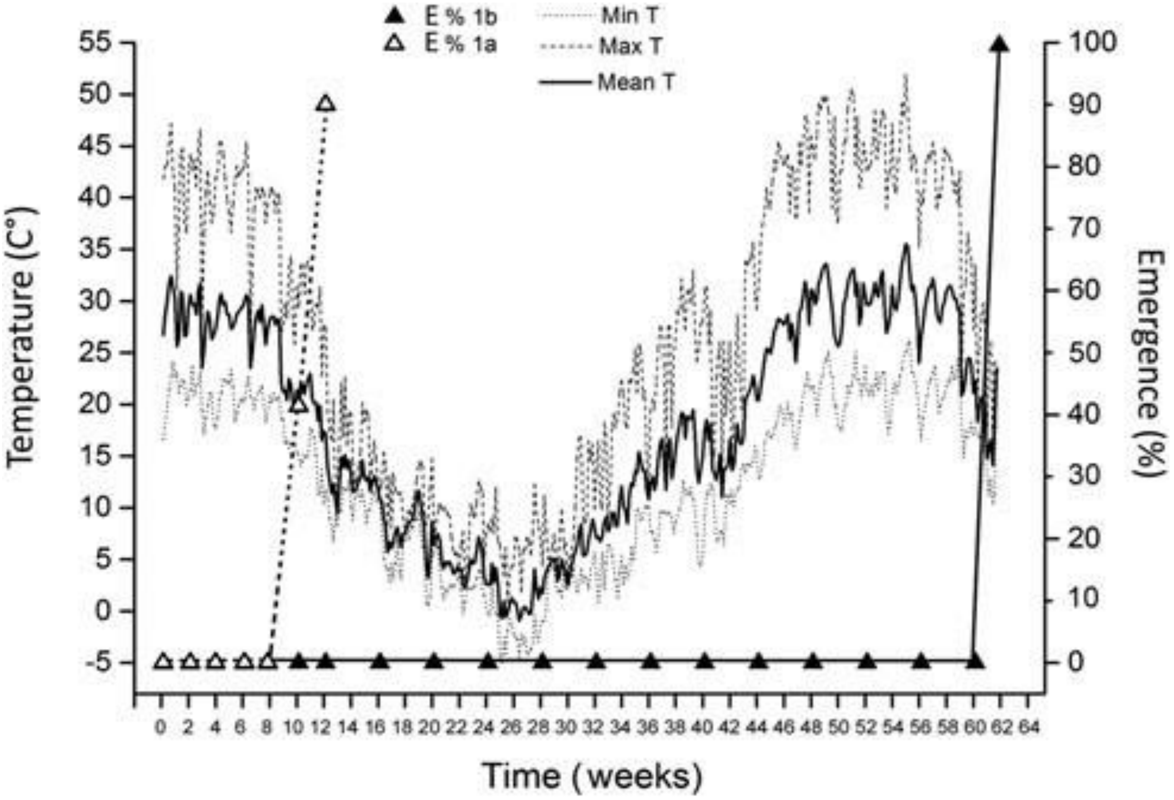

Figure 4. Percentages of the emergence from primary (1a; white triangles) and secondary (1b; black triangles) seeds of *Ae. neglecta* in the field. Also shown is the mean daily soil temperature (continuous black line), mean minimum soil temperature (dotted line) and mean maximum soil temperature (dashed line).

Discussion

In this study we estimated the longevity of heteromorphic seeds in six different species of *Aegilops*. Our aim was to clarify longevity across species and seed morphs of the same dispersal units, thereby providing novel insights into the germination patterns of *Aegilops*. Results from controlled ageing tests (CAT) were implemented with a field germination experiment on *Ae. neglecta* to better understand emergence phenology and its relation to seed lifespan.

All species studied here, except *Ae. biuncialis*, showed within-spikelet seed dimorphism, with primary seeds being always significantly heavier than secondary seeds. At the same time, seeds from different spikelets also differed in their mass. The seed heteromorphy detected in the study accessions is consistent with the findings of Datta et al. (1970), Marañon (1989) and Dyer (2017) in *Ae. geniculata*, *Ae. neglecta* and *Ae. triuncialis*, suggesting that a significant difference in the mass of the different seed morphs produced by the same spike and especially the presence of dimorphic seed pairs is a widespread characteristic in the genus *Aegilops*.

Dimorphic seeds studied here reflected differences in both germination and longevity. In particular, the germination of seeds in the laboratory was high (on average >90%) and quick (i.e. with MGT ranging from 44 to 112 hours; Table 2) indicating the absence of dormancy mechanisms when seeds are extracted from their dispersal units. At the same time, the secondary seeds (1b) of the dimorphic pair showed a delayed germination compared with the primary seeds (1a), i.e. in *Ae. geniculata*, *Ae. neglecta*, *Ae. peregrina* and *Ae. uniaristata*, confirming previous observations (Datta et al. 1970; Marañon 1989). However, in *Ae. cylindrica* the MTG was similar between the dimorphic seed pair, underlining that different germination patterns may exist across species in *Aegilops*. A delayed germination in the smaller seeds of several *Aegilops* has also been observed in natural conditions (i.e. with seeds enclosed in the dispersal unit) and explained by the influences of both maternal and sibling factors, through the presence of an inhibitor in the glumes, internal levels of gibbellerins or competition for essential metabolites among sibling seeds (Lavie et al. 1974; Wurzburger and Leshem 1967; Wurzburger and Leshem 1969; Wurzburger and Koller 1973; Dyer 2004). However, the implications of such constrains on the emergence phenology in nature were not well understood. Here we have shown that differences in MTG between dimorphic pairs of *Ae. neglecta* were exacerbated under field experiments, with primary seeds (1a) emerging soon after dispersal (i.e. 3 months later, in autumn, after the first rains) and secondary seeds (1b) germinating 14 months after dispersal (Figure 1), indicating the possibility of dormancy mechanisms in the secondary seeds imposed by the spikelet (Datta et al. 1970). The dormancy of the secondary seed may be released when the primary, larger seed is not present any more in the dispersal unit and the effects of the water-soluble inhibitor have been weakened by several wet and dry periods (Dyer 2004). Nevertheless, further studies are needed to clarify the dormancy of *Aegilops* secondary seeds when enclosed in the dispersal units, since other explanations of this phenology (e.g. different thermal requirement for the germination of the different seed morphs) cannot be completely ruled out. The delay in the germination of the secondary seeds in the field is an interesting and confirmatory observation, outlining that the shift in the germination timing between seed morphs in nature spreads the seedling emergence over two consecutive years.

Supporting a previous observation that CAT holds a great potential to predict seed persistence in soil seed banks (Long et al. 2008), our results show that seed longevity of the different seed morphs reflected their soil seed bank persistence in *Ae. neglecta*. In this species, primary seeds (1a) were significantly shorter lived than secondary seeds (1b) from the same spikelets and, consistently, the former germinated much sooner after dispersal than the latter (see above). Consequently, it appears that the higher resilience to ageing of the secondary seeds detected in *Ae. neglecta* may be needed to maintain high viability after dispersal to fulfil germination phenology.

Additionally, differences in p_{50} across seed morphs could be linked to differences in their initial seed viabilities (K_i) and not to differences in the deterioration rate (σ^{-1}) (Figure 3). This strategy allows secondary seeds to retain full viability during the initial stages of ageing for a longer time compared with primary ones, both under CAT and in the field. Indeed, results from field experiments in *Ae. neglecta* showed that longer-lived secondary seeds retained the full viability for over 1 year after sowing, with 100% of them emerging 14 months later. The same dimorphism in seed longevity was shown in all other species with within-spikelet dimorphism (except *Ae. peregrina*), indicating that a similar germination phenology should be expected also in these taxa. The delayed germination and high resilience to ageing showed by secondary seeds reinforce the hypothesis of a bet-hedging strategy in the germination ecology of *Aegilops* (Dyer 2004). Supporting this view, seed heteromorphy has been associated with a bet-hedging germination strategy in several plant genera (Silvertown 1984; Imbert 2002).

A major question is whether the mechanisms of seed ageing under CAT resemble those of the cool dry conditions of seed banks (i.e. 15% RH, –20°C), rather than those occurring in the wild (Long et al. 2008). Despite the fact that we could not investigate the biochemical process of ageing and the exact seed water content, the air humidity the spikes of *Ae. neglecta* experienced at the soil surface after dispersal was (on average) similar to that used in CAT (60% RH) for most of the time (i.e. 50–70% for 10 months, December to September), although temperatures were much lower (i.e. <15°C for 6 months, then between 20 and 27°C) (Supplementary Figure 1). In this regard, longevity of seeds at RH <70% is increased at lower temperatures, explaining the high survival 1 year after sowing of the secondary seeds. Temperature enhances the rate of deteriorative reactions occurring in seeds. In particular, above a critical temperature (e.g. estimated in approximately 40°C in *Vigna radiata*; Murthy et al. 2003) the solid-like glassy state may soften into the rubbery or even liquid state (i.e. glass transition temperature), enhancing molecular mobility, thereby the deteriorative reactions proceed rapidly (Walters 1998; Buitink and Leprince 2008). Consequently, our results indicate that differences in the deterioration process that seeds experience in the wild and under CAT might exist mostly due to temperature differences.

Despite our data reinforcing the hypothesis that in the dimorphic seed pairs of *Aegilops*, the primary and shorter-lived seeds germinate soon after the dispersal, while the secondary and longer-lived seeds create a more persistent soil seed bank (Datta et al. 1970; Marañon 1989; Onnis et al. 1995), the similar longevity found among seed lots in *Ae. peregrina* indicates that the regeneration strategy may follow different patterns in this species. Additionally, in seed lot 2a (consisting of seeds of the second fertile spikelet; Figure 1) of *Ae. neglecta* the p_{50} was intermediate to that of primary and secondary seed lots (1a and 1b). Similarly, in *Ae. cylindrica,* seed lots from single-seeded spikelet (s) had an intermediate p_{50} comparable to that of the primary (a) and the secondary (b) seeds from the double-seeded spikelet. Moreover, in *Ae. biuncialis*, even if seed dimorphism was not detected, seeds from different spikelets on the same spikes significantly differed in their p_{50}. Therefore, further studies are needed to understand whether longevity differences across these latter seed morphs may correlate with their germination phenology (and/or vice versa), as shown here for dimorphic pairs of *Ae. neglecta*. Supporting this view, previous studies highlighted some germination differences across a range of seed morphs from different spikelets in *Aegilops* (Datta et al. 1970; Fandrich and Mallory-Smith 2006; Orsenigo et al. 2017).

Seed longevity guarantees the long-term ex situ conservation of plant genetic resources (PGR) within gene banks (Walters et al. 2005). The longevity ranking of seed lots based on data from controlled ageing tests is expected to be similar to that for seed lots in long-term storage, i.e. seed lots that are identified as losing viability quickly in the controlled ageing test will also lose viability quickly during long-term conservation storage (Probert et al. 2009; Hay and Whitehouse 2017). The significant differences in seed longevity among heteromorphic seeds in most of the *Aegilops* species tested here, suggest that different seed morphs are also likely to have different longevities under ex situ seed bank storage, with secondary seeds being longer lived than primary seeds.
The fundamental aim of ex situ seed collections of CWR is to capture all the possible adaptive traits in the seed samples (Warschefsky et al. 2014). Several differences have been detected at the biochemical, genetic and morphological levels, as well as in abiotic stress resistance among different seed morphs in species of Poaceae (Datta 1970; De Gara et al. 1991; Cremonini et al., 1994; Frediani et al. 1994; Orsenigo et al. 2017). It follows that different seed morphs of *Aegilops* should be held separately in storage and their viability, regeneration or recollection intervals (Hay and Probert 2013) should follow different timings, in order to avoid important loss of genetic resources.

Finally, seed longevity in the *Aegilops* species tested here was, on average, longer compared with that of other grasses. For example, across 722 seed collections of Poaceae exposed to CAT, Probert et al. (2009) found an average p_{50} value of 26.7 days. Similarly, Mondoni et al. (2011) could not find any grasses with a p_{50} higher than 38 days. However, in our study overall p_{50} in *Aegilops* was on average higher (i.e. about 50 days), with some seed lots showing a p_{50} of about 80 days (Ae. *cylindrica* b, *Ae. neglecta* 1b). Thus, the seed lots in our study can be described as having a medium longevity in air-dry storage, based on a logarithmic scale to categorize species according to their relative longevity

(Probert et al., 2009; i.e. $p_{50} \leq 1$ day indicates ‘very short’ seed longevity; p_{50} >1 to ≤10, ‘short’; p_{50} >10 to ≤100, ‘medium’; p_{50} >100 to ≤1000, ‘long’; p_{50} >1000, ‘very long’). Such higher seed longevity of *Aegilops* compared with that shown by other Poaceae may be due to specific adaptations of seeds of this genus to warm and dry environments, where seeds need to persist, in a dry state, for long periods between bouts of rainfall sufficient to allow successful establishment (Probert et al. 2009). Supporting this view, seeds produced by species growing in Mediterranean habitats, as the ones in which *Aegilops* thrives, are known to be among the longest lived found worldwide (Merrit et al. 2014).

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References

Bhatt A, Santo A (2016) Germination and recovery of heteromorphic seeds of *Atriplex canescens* (Amaranthaceae) under increasing salinity. Plant Ecology, 217, 1069–1079.

Bernareggi G, Carbognani M, Petraglia A and Mondoni A (2015) Climate warming could increase seed longevity of alpine snowbed plants. Alpine Botany, 125, 69–78.

Bilz M, Kell SP, Maxted N and Lansdown RV (2011) European Red List of Vascular Plants. Luxembourg, Publications Office of the European Union.

Buitink J, Leprince O (2008) Intracellular glasses and seed survival in the dry stat. Comptes Rendus Biologies, 331, 788–795.

Cremonini R, Colonna N, Stefani A, Galasso I, Pignone D (1994) Nuclear DNA content, chromatin organization and chromosome banding in brown and yellow seeds of *Dasypyrum villosum* (L.) Candargy. Heredity, 72, 365–373.

Datta SC, Evenari M, Gutterman Y (1970) Heteroblasty of *Aegilops ovata* L. Israel Journal of Botany, 19, 463–483.

Davies RM, Newton RJ, Hay FR, Probert RJ (2016) 150-seed comparative longevity protocol – a reduced seed number screening method for identifying short-lived seed conservation collections. Seed Science and Technology, 44, 1–16.

De Gara L, Paciolla C, Liso R, Stefani A, Arrigoni O (1991) Correlation between ascorbate peroxidase activity and some anomalies of seedlings from aged caryopses of *Dasypyrum villosum* (L.) Borb. Journal of Plant Physiology, 137, 697–700.

Dempewolf H, Eastwood RJ, Guarino L, Khoury CK, Müller JV, Toll J (2014) Adapting agriculture to climate change: a global initiative to collect, conserve, and use crop wild relatives. Agroecology and Sustainable Food Systems, 38, 369–377.

De Pace C, Vaccino P, Cionini PG, Pasquini M, Bizzarri M, Qualset CR (2011) *Dasypyrum*, pp. 185–292 in Kole C (ed.), Wild Crop Relatives: Genomic and Breeding Resources. Cereals. Berlin Heidelberg, Springer-Verlag.

Duràn JM, Retamal N (1989) Coat structure and regulation of dormancy in *Sinapis arvensis* L. seeds. Journal of Plant Physiology, 135, 218–222.

Dyer AR (2004) Maternal and sibling factors induce dormancy in dimorphic seed pairs of *Aegilops triuncialis*. Plant Ecology, 172, 211–218.

Dyer AR (2017) The seed ecology of *Aegilops triuncialis*: linking trait variation to growing conditions. Seed Science Research, 27, 183–198.

El-Keblawy A, Bhatt A (2015) Aerial seed bank affects germination in two small-seeded halophytes in Arab Gulf desert. Journal of Arid Environments, 117, 10–17.

Ellis RH, Roberts EH (1980) Improved equations for the prediction of seed longevity. Annals of Botany, 45, 13–30.

Fandrich L, Mallory-Smith C (2005) Temperature effects on jointed goatgrass (*Aegilops cylindrica*) seed germination. Weed Science, 53, 594–599.

Fandrich L, Mallory-Smith C (2006) Jointed goatgrass (*Aegilops cylindrica*) seed germination and production varies by spikelet position on the spike. Weed Science, 54, 443–451.

Frediani M, Colonna N, Cremonini R, De Pace C, Delre V, Caccia C, Cionini PG (1994) Redundancy modulation of nuclear DNA sequences in *Dasypyrum villosum*. Theoretical and Applied Genetics, 88, 167–174.

Guzzon F, Müller JV, Abeli T, Cauzzi P, Ardenghi N.M.G., Balestrazzi A, Rossi G, Orsenigo S (2015) Germination requirements of nine European *Aegilops* species in relation to constant and alternating temperatures. Acta Botanica Gallica, Botany Letters, 162, 349–354.

Hay FR, Adams J, Manger K , Probert RJ (2008) The use of nonsaturated lithium chloride solutions for experimental control of seed water content. Seed Science and Technology, 36, 737–746.

Hay FR, Probert RJ (2013) Advances in seed conservation of wild plant species: a review of recent research. Conservation Physiology 1, cot030.

Hay FR, Whitehouse KJ (2017) Rethinking the approach to viability monitoring in seed genebanks. Conservation Physiology 5, cox009.

Imbert E (2002) Ecological consequences and ontogeny of seed heteromorphism. Perspectives in Plant Ecology, Evolution and Systematics 5, 13–36. ISTA (2018) International Rules for Seed Testing. Bassersdorf, Switzerland, International Seed Testing Association.

Kilian B, Mammen K, Millet E, Sharma R, Graner A, Salamini F, Hammer K , Özkan H (2011). *Aegilops*, pp. 1–76 in Kole C (ed.), Wild Crop Relatives: Genomic and Breeding Resources. Cereals. Berlin Heidelberg, Springer-Verlag.

Lavie D, Levy EC, Cohen A, Evenari M, Guttermann Y (1974) New germination inhibitor from *Aegilops ovata* L. Nature, 249, 388.

Long RL, Panetta FD, Steadman KJ, Probert R, Bekker RM, Brooks S, Adkins SW (2008) Seed persistence in the field may be predicted by laboratory-controlled aging. Weed Science, 56, 523–528.

Marañon T (1989) Variation in seed size and germination in three *Aegilops* species. Seed Science and Technology, 17, 583–588.

Matilla A, Gallardo M, Puga-Hermida MI (2005) Structural, physiological and molecular aspects of heterogeneity in seeds: a review. Seed Science Research, 15, 63–76.

Maxted N, White K, Valkoun J, Konopka J, Hargreaves S (2008) Towards a conservation strategy for *Aegilops* species. Plant Genetic Resources Characterization and Utilization, 6, 126–141.

Merritt DJ, Martyn AJ, Ainsley P, Young RE, Seed LU, Thorpe M, Hay FR, Commander LE, Shackelford N, Offord CA, Dixon KW, Probert RJ (2014) A continental-scale study of seed lifespan in experimental storage examining seed, plant, and environmental traits associated with longevity. Biodiversity and Conservation, 23, 1081–1104.

McCouch S, Baute GJ, Bradeen J, Bramel P, Bretting PK, Buckler E, Burke JM, Charest D, Cloutier S, Cole G, Dempewolf H, Dingkuhn M, Feuillet C, Gepts P, Grattapaglia D, Guarino L, Jackson S, Knapp S, Langridge P, Lawton-Rauh A, Lijua Q, Lusty C, Michael T, Myles S, Naito K, Nelson RL, Pontarollo R, Richards CM, Rieseberg L, Ross-Ibarra J, Rounsley S, Hamilton RS, Schurr U, Stein N, Tomooka N, van der Knaap E, van Tassel D, Toll J, Valls J, Varshney RK, Ward J, Waugh R, Wenzl P, Zamir D (2013) Feeding the future. Nature, 499, 23–24.

Mondoni A, Probert RJ, Rossi G, Vegini E, Hay FR (2011). Seeds of alpine plants are short lived: implications for long-term conservation. Annals of Botany, 107, 171–179.

Murthy U.M.N., Kumar PP, Sun WQ (2003) Mechanisms of seed ageing under different storage conditions for *Vigna radiata* (L.) Wilczek: lipid peroxidation, sugar hydrolysis, Maillard reactions and their relationship to glass state transition. Journal of Experimental Botany, 54, 1057–1067.

Nave M, Avni R, Ben-Zevi B, Hale I, Distelfeld A (2016) QTLs for uniform grain dimensions and germination selected during wheat domestication are co located on chromosome4B.Theoretical and AppliedGenetics, 129, 1303–1315.

Newton R, Hay F, Probert R (2009) Protocol for comparative seed longevity testing. Technical Information Sheet_01. London, Royal Botanic Gardens Kew.

Onnis A, Bertacchi A, Lombardi T, Stefani A (1995) Morphology and germination of yellow and brown caryopses of *Aegilops geniculata* Roth (Gramineae) populations from Italy. Plant Biosystems, 129, 813–821.

Orsenigo S, Guzzon F, Abeli T, Rossi G, Vagge I, Balestrazzi A, Mondoni A, Müller JV (2017) Comparative germination responses to water potential across different populations of *Aegilops geniculata* and cultivar varieties of *Triticum durum* and *Triticum aestivum*. Plant Biology, 19, 165–171.

Perrino EV, Wagensommer RP, Medagli P (2014) *Aegilops* (Poaceae) in Italy: taxonomy, geographical distribution, ecology, vulnerability and conservation. Systematics and Biodiversity, 12, 331–349.

Probert RJ, Daws MI, Hay FR (2009) Ecological correlates of ex situ seed longevity: a comparative study on 195 species. Annals of Botany 104, 57–69.

Puga-Hermida MI, Gallardo M, Rodríguez-Gacio MC, Matilla AJ (2003) The heterogeneity of turnip tops (*Brassica rapa*) seeds inside the silique affects germination, the activity of the final step of the ethylene pathway, and abscisic acid and polyamine content. Functional Plant Biology, 30, 767–775.

Rossi G, Montagnani C, Gargano D, Peruzzi L, Abeli T, Ravera S, Cogoni A, Fenu G, Magrini S, Gennai M, Foggi B, Wagensommer RP, Venturella G, Blasi C, Raimondo FM, Orsenigo S (2013) Lista Rossa della Flora Italiana. 1. Policy Species e altre specie minacciate. Rome, Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare.

Silvertown JW (1984) Phenotypic variety in seed germination behavior: the ontogeny and evolution of somatic polymorphism in seeds. American Naturalist, 124, 1–16.

van Slageren MW (1994) Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. and Spach) Eig (Poaceae). Wageningen, Agricultural University Papers.

Venable DL (1985) The evolutionary ecology of seed heteromorphism. American Naturalist, 126, 577–595.

Volis S (2016) Seed heteromorphism in *Triticum dicoccoides*: association between seed positions within a dispersal unit and dormancy. Oecologia, 181, 401–412.

Walters C (2003) Optimising seed banking procedures, pp. 723–743 in Smith RD, Dickie JB, Linington SH, Pritchard HW, Probert RJ (eds), Seed Conservation: Turning Science into Practice. London, Royal Botanic Gardens, Kew.

Walters C (1998) Understanding the mechanisms and kinetics of seed aging. Seed Science Research, 8, 223–244.

Walters C, Wheeler LM, Grotenhuis JM (2005) Longevity of seeds stored in a genebank: species characteristics. Seed Science Research, 15, 1–20.

Warschefsky E, Penmetsa RV, Cook DR, von Wettberg E.J.B. (2014) Back to the wilds: evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. American Journal of Botany 101, 1791–1800.

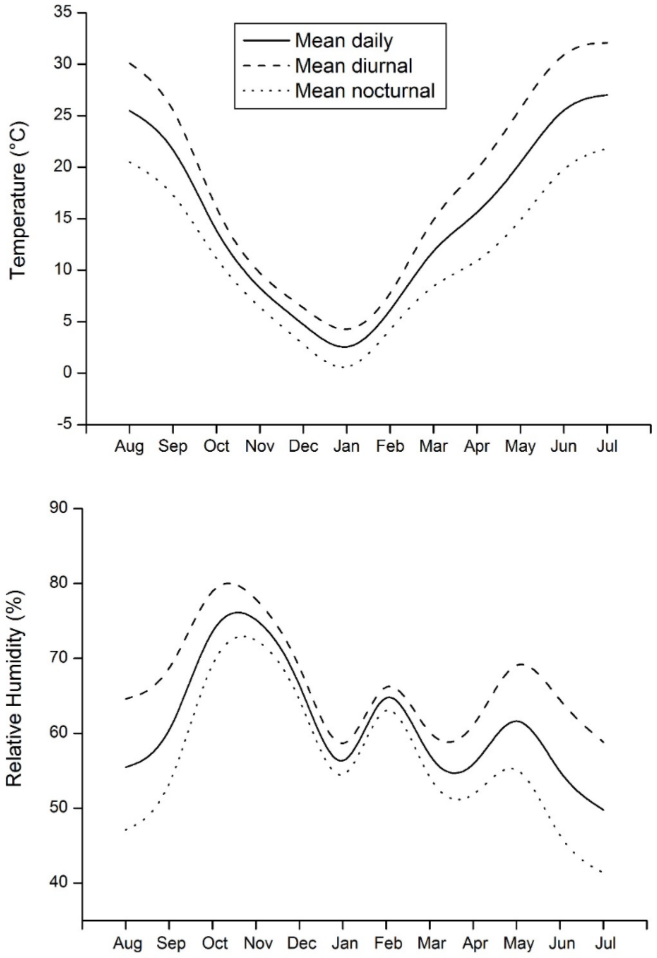
Wurzburger J, Koller D (1973) Onset of seed dormancy in *Aegilops kotschyi* Boiss. and its experimental modification. New Phytologist 5, 1057–1061.

Wurzburger J, Leshem Y (1967) Gibberellin and hull controlled inhibition of germination in *Aegilops kotschyi* Boiss. Israel Journal of Botany, 16, 181–186.

Wurzburger J, Leshem Y (1969) Physiological action of the germination inhibitor in the husk of *Aegilops kotschy* Boiss. New Phytologist, 68, 337–341.

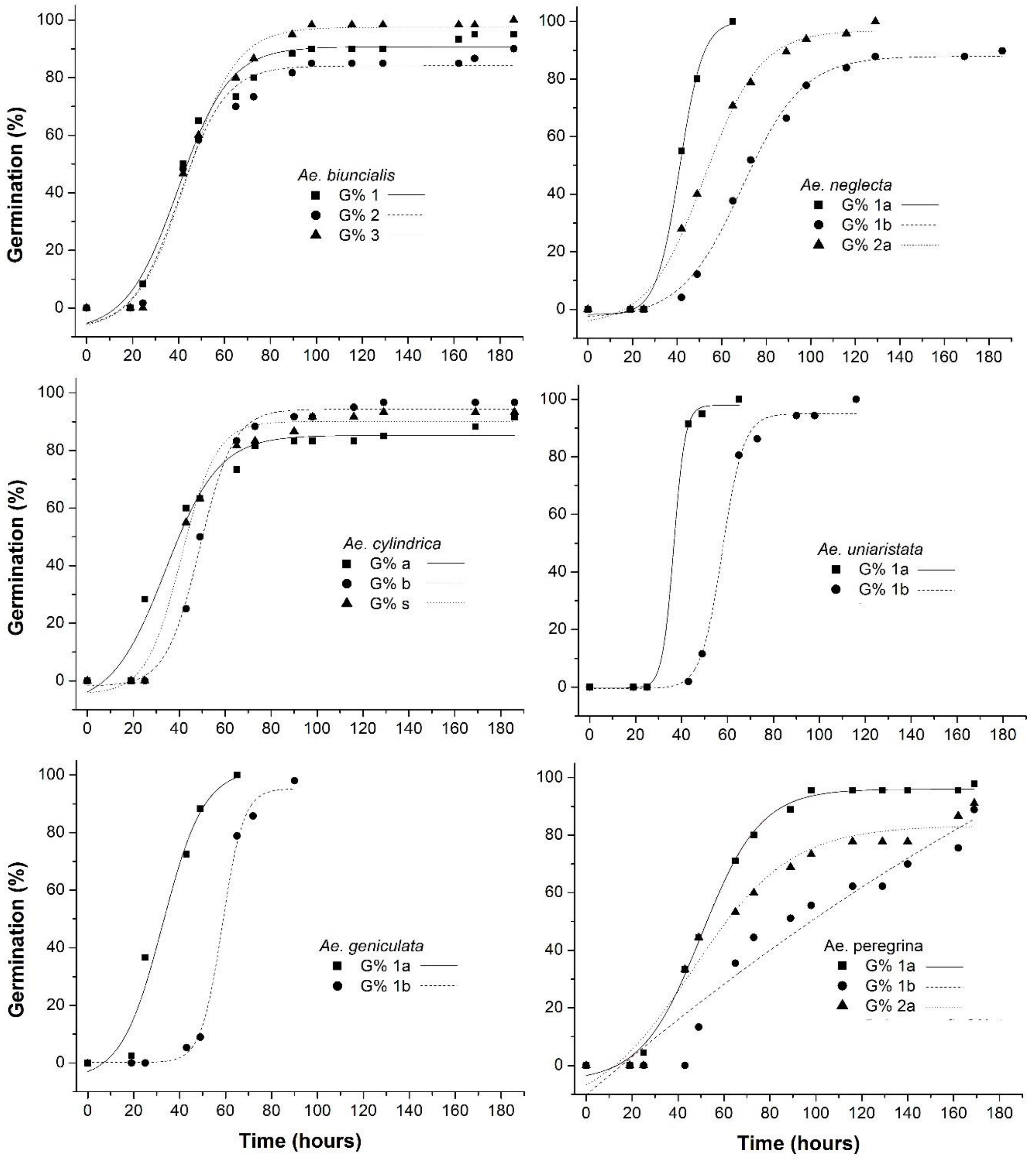
Wurzburger J, Leshem Y, Koller D (1976) Correlative aspects of imposition of dormancy in caryopses of *Aegilops kotschyi*. Plant Phisiology, 57, 670–671.

Supplementary Figure 1



Supplementary Figure 1: Mean daily (straight line), mean diurnal (spotted line; data from 8:00 to 20:00 plotted together) and mean nocturnal (dashed line; data from 20:00 to 8:00 plotted together) relative humidity (upper graph) and temperatures (lower graph) in the field experimental site. Data retrieved from a WatchDog data logger place at soil surface level.

Supplementary Figure 2



Supplementary Figure 2: Germination progress curves of all the species and seed lots at initial germination (germination of unaged seeds before the start of CAT). The germination curves were fitted with Boltzmann equations. The curves are limited at 190 hours for graphic purposes despite a few seeds germinated after this time point.

Article IV

Guzzon F., Ardenghi N.M.G. (2018) Could taxonomic misnaming threaten the ex situ conservation and the usage of plant genetic resources? Biodiversity and Conservation, 27(5), 1157-1172.

Supplementary material is provided after the references section: pp 105-113

Could taxonomic misnaming threaten the ex situ conservation and the usage of plant genetic resources?

Abstract Ex situ conservation of plant germplasm, especially seed banking, is a favourable and widely used method for the conservation of plant genetic resources (PGR). The long-term conservation of these resources is fundamental for food security and plant breeding in order to stem the losses in agrobiodiversity and to meet the global challenges that agriculture is facing. The conservation and accessibility of PGR relies on their correct taxonomic labelling and on the building of a searchable database that links ex situ collections together. In the current study, we analysed the impact of taxonomic misnaming in the two major PGR databases (Genesys PGR, EURISCO), listing accessions conserved worldwide. The aim was to understand if taxonomic misnaming issues prevent PGR conservation. We chose as a case-study seed collections of accessions of the genus *Citrullus* (watermelon genepool), the taxonomy and nomenclature of which have been largely revised in recent times. We observed that taxonomic misnaming greatly limits PGR conservation with only 3% of the accessions listed in the databases correctly named; moreover, 28% were affected by taxonomic errors that prevent the establishment of the accessions' taxonomic identity, with consequences on their conservation and exploitation. The existence of the problem was also confirmed by the experimental propagation of three misnamed accessions. We suggest herein a series of actions that, put in place, could solve the extant misnaming issues in the databases and prevent their reoccurrence, allowing the correct conservation and the usability by the stakeholders of all the accessions.

Keywords: Botanical nomenclature · Database · Genebank · Seed conservation · Taxonomy · Watermelon

Introduction

In the next decades, a growing human population, changing diets, extreme climate conditions, lower availability of natural resources, higher competition for arable soils with non-food crops, soil degradation, and the need to minimize harmful impacts on ecosystems, will pose new challenges to global food security (Godfray et al. 2010; Asseng et al. 2015). To cope with the aforementioned challenges, both agricultural production and sustainability need to increase (Tilman et al. 2002). The selection of new crop cultivars with favourable traits such as increased drought and heat tolerance, and input use efficiency, will be key to this process (Esquinas-Alcazar 2005; McCouch et al. 2013). To meet this goal, the eroded genepool of modern crop plants needs to be broadened and the widest genetic diversity needs to be available and exploitable, in order to select for the improved cultivars of the future (Ford-Llyod et al. 2011; Guarino and Lobell 2011; Vincent et al. 2013; Warschefsky et al. 2014). This is achievable only by conserving and keeping available, for research and breeding programmes, the widest possible genepool especially landraces and crop wild relatives (CWR) (McCouch et al. 2013). In many areas of the world, in the last few decades, genetic erosion (the loss of genetic diversity in the form of alleles and genotypes as well as domestic crop accessions) of higher than the 70% has been observed (Hammer et al. 1996; Veteläinen et al. 2009; van de Wouw et al. 2010). Given this scenario, it is of key-importance to conserve agrobiodiversity in the long-term. More specifically, this is the diversity of crop species used in different agro-ecosystems as well as the genetic diversity within and among crop and CWR accessions (Last et al. 2014). Furthermore, the conservation of plant genetic resources for food and agriculture (PGRFA) is fundamental to achieving target 9 of the 2011–2020 Global Strategy for Plant Conservation as well as target 13 of the Aichi biodiversity targets. Thus, the effective conservation of agrobiodiversity and its sustainable use is considered to be of pivotal importance by the Convention of Biological Diversity (CBD) (Convention of Biological Diversity 2011, 2012).

One of the most effective strategies to ensure the conservation and availability of PGRFA is through ex situ conservation in genebanks (McCouch et al. 2013; Davies and Allender 2017). In particular, seed banking shows several advantages as a long-term ex situ conservation strategy for plant species and therefore is used for the maintenance of most of the PGR (plant genetic resources) collections ex situ. Seed material is relatively easy to collect, can be stored in small spaces, can provide a decent sample of the genetic diversity within the species and often remains viable for long periods. Furthermore, seed collections do not require a high level of maintenance and are also economically viable (Li and Pritchard 2009; Riviere and Muller 2017). Worldwide there are more than 1750 genebanks containing over 7.4 million accessions (FAO 2010; Davies and Allender 2017).

All countries are highly dependent upon PGRFA conserved beyond their borders. This global interdependence, and therefore global flows of PGRFA among conservation facilities, are likely to increase in order to cope with future challenges, especially climatic changes (Galluzzi et al. 2016).

The stakeholders of PGRFA indicated as a major constraint in the usage of conserved germplasm the difficulty in accessing it and to obtaining associated information (Kell et al. 2017). The accessibility of PGR accessions is strictly linked with the existence and updating of global information databases, this involves the gathering of data for accessions from many collections into a centralized source, therefore facilitating the transfer of the germplasm among institutions. Consequently, the building and improvement of information systems that link collections together in order to create a single searchable database of genetic resources is a high priority in PGR conservation and utilization (Khoury et al. 2010).

The correct and clear taxonomic identification and labelling of accessions is of key importance to making accessions of PGR, conserved ex situ, usable (Dempewolf et al. 2017). Taxonomic issues are indeed demonstrated to highly threaten the effectiveness of conservation programmes (Garnett and Christidis 2017). Moreover, the updated and precise taxonomic labelling of PGR accessions in public databases is also fundamental to performing prioritization studies aimed at finding gaps in ex situ conservation (Dempewolf et al. 2014). This is because a correct taxonomic naming of accessions is essential for the identification of PGR taxa that are currently underrepresented in ex situ conservation facilities worldwide and therefore have a high priority for future collecting missions and urgent conservation measures (Maxted et al. 2010; Castañeda-Álvarez et al. 2016).

In order to better understand the extent of taxonomic misnaming in databases of PGR accessions which are conserved ex situ and whether this issue could prevent their exploitation and conservation, we analysed and quantified the occurrence of this problem in seed accessions belonging to the watermelon genepool (*Citrullus*). We decided to focus on this genus as a case study, since it has a relatively small number of taxa (species and subspecies) and, after having been considered for long a critical taxonomic group, its systematics, taxonomy, and nomenclature have been revised and improved in recent years, with the aid of genetic investigation too (see Nesom 2011; Schaefer and Renner 2011; Chomicki and Renner 2014; Renner et al. 2014; Paris 2015). Moreover, *Citrullus* is of significant importance as a vegetable crop (Applequist 2016), with 3.5 million hectares of agricultural land used to cultivate watermelons in 2014 when annual production reached 111 million tons, which was 9.5% of global vegetable production, grown on 6% of the area used globally for the cultivation of vegetables (FAOSTAT 2017). The genus is of great importance in terms of food security in desert and semi-arid areas (Mujaju et al. 2011; Modi and Zulu 2012). *Citrullus* includes eight taxa (seven species, one of which articulated into two subspecies), three are widely cultivated [*C. amarus*, *C. lanatus* subsp. *lanatus, C. mucosospermus*], two are only locally cultivated [*C. colocynthis* and *C. lanatus* subsp. *kordophanus*], and three have only wild populations [*C. ecirrhosus*, *C. naudinianus*, *C. rehmii*] (Chomicki and Renner 2014; Paris 2015).

The aims of the current research, adopting *Citrullus* as a case study, are: (a) to understand and define which are the most frequently nomenclature-related issues in databases of PGR accessions, (b) to clarify if these issues could prevent the usage of these accessions, and (c) to identify if and how the discovered issues may be resolved.

Materials and methods

We checked for *Citrullus* accessions in the two major databases of plant genetic resources worldwide: Genesys PGR and EURISCO. Genesys is a global portal that lists more than 3.6 million accessions of plant genetic resources from 482 institutes worldwide. It is a gateway from which germplasm accessions from genebanks can be found and ordered (Genesys 2017). EURISCO is a search catalogue providing information about ex situ plant collections maintained by institutions located in Europe, and includes data for 1.9 million accessions (EURISCO 2017). Individual databases contributing to EURISCO simultaneously upload data into Genesys (Genesys 2017), but, not knowing the details of updating between the two databases, we decided to consider both separately in our analysis.

We searched as keywords, the existing nomenclatural combinations acquired from literature (e.g., Pangalo 1930; Mansfeld 1959; Fursa 1972; Nesom 2011; Chomicki and Renner 2014; Paris 2015). In order to increase the possibilities of finding accessions, we also searched for the most widespread vernacular names (e.g., colocynth, tsamma, citron, egusi), with reference to geographic provenance and biological status (wild, landrace, modern cultivar) too. The resulting accession names were examined on nomenclatural grounds, by comparing them with the most updated taxonomic and nomenclatural treatments of *Citrullus* (see Nesom 2011; Chomicki and Renner 2014; Renner et al. 2014; Paris 2015); names were further checked in compliance with the rules of the International Code of Nomenclature for algae, fungi, and plants (ICN) (McNeill et al. 2012). A complete list of the accepted names and synonyms we came across in the consulted databases are provided in Supplementary Material 1.

The encountered misnaming issues were classified into "issue types" and then grouped into two main "issue categories" (Table 1). If an accession was indeed wrongly named, we attributed the misnaming to one or more issue types and then to one or more issue categories. Eventually, when practicable, we proposed, for each accession, the most updated scientific name (Table 1), based on the updated taxonomic and nomenclatural treatment of *Citrullus* reported in Supplementary Material 1. We then provided a numerical estimation of each misnaming issue type and category found in the two databases, this was intended to evaluate the extent of taxonomic misnaming in PGR databases (Supplementary Material 2, 3).

Additionally, by analysing the different databases, we found that the name of the same taxon changes among institutions, we referred to this as “variability”. When more scientific names are applied to the same taxon within the same institution we referred to “intra-institution variability”, among different institutions was “inter-institution variability” (Table 2), within the same database “intra-database variability” or between different databases “interdatabase variability” (Table 3). We chose *C. amarus* as a target taxon to illustrate this point. In each database, we searched for the current accepted name and its synonyms.

Eventually, in order to verify in practical terms the validity of our doubts, three accessions that appeared to be misnamed in the Genesys and EURISCO databases were propagated at the CREA-ORL institute of Montanaso Lombardo (northern Italy, province of Lodi). The accession names were first revised on nomenclatural grounds and then, after propagation, their taxonomic identity was checked (Table 4; Figure 1). Cultivation was performed in purity to avoid crossbreeding among the different accessions: female flowers were isolated with paper bags and hand pollination was performed. Herbarium vouchers are stored at the Herbarium of the University of Pavia (PAV).

Results

Misnaming issues: categorization

We identified six types of misnaming issues (Table 1), along with several misprints regarding both the names of the taxa and their authors. Each issue has been classified into two “issue categories”:

1. “Nomenclatural inaccuracy” (N): the scientific name associated with the accession does not correspond to the most updated scientific name, but it represents a synonym (homotypic or heterotypic; see Glossary and Art. 14.4 of the ICN) or a spelling variant of the latter; it does not prevent the establishment of the real taxonomic identity of the accession;

2. “Taxonomic error” (T): the scientific name associated with the accession prevents the establishment of the real taxonomic identity of the accession.

Table 1 Misnaming issue types (“Issue types”), with definitions and examples (“Issue types definition”) acquired from the results of our database search. We also listed the accession name (“Accession scientific name”); the issue categories (“Issue category”); and the updated scientific name we proposed (“Current scientific name”)

Accession scientific name	**Issue types**	**Issue category**	**Current scientific name**	**Issue types definition**
Citrullus aedulis Pangalo	synonym	N	*C. lanatus* (Thunb.) Matsum. & Nakai subsp. *lanatus*	The accession name is a synonym of the currently accepted scientific name (see Supplementary Material 1).
Citrullus lanatus (Thunb.) Matsum. et Nakai subsp. *vulgaris* var. *caffer* (Schrad.) Mansf.	non-existent name	N	*C. amarus* Schrad.	The accession name, as written, does not exist. In this case, the combination published by Mansfeld (1959) is *C. lanatus* var. *caffer* (Schrad.) Mansf. (see Tab. 1), without indication of the subspecies.
Citrullus lanatus Mansf.	authors	N	*C. amarus* Schrad., *C. lanatus* (Thunb.) Matsum. & Nakai s.l. or *C. mucosospermus* (Fursa) Fursa	The authors associated with the accession name are incorrect. In this case, the accession name refers to *C. lanatus* (Thunb.) Mansf., but the combination by Mansfeld (1959) is an isonym (see Art. 6.2, Note 2 of the *ICN* and Supplementary Material 1), having been published later than that by Matsumura and Nakai (1916), who are the correct authors of the combination; in addition, the accession name lacks of the basionym’s author, “(Thunb.)”. Since no subspecies is mentioned, this accession name affects the establishment of the taxonomic identity of the accession, thus it represents a “taxonomic error” too, which is properly explained below.

Citrullus sp.	no species	T	?	The accession name is represented only by the name of the genus.
Citrullus lanatus (Thunb.) Matsum. & Nakai	no subspecies	T	*C. lanatus* (Thunb.) Matsum. & Nakai s.l., *C. amarus* Schrad. or *C. mucosospermus* (Fursa) Fursa	The subspecies is not indicated. As a consequence, in this example, it is impossible to establish whether the accession belong to the nominal subspecies or subsp. *kordophanus* Ter-Avan. Moreover, since *C. amarus* Schrad. and *C. mucosospermus* (Fursa) Fursa have both been treated as subspecies or varieties of *C. lanatus* until recent times (see: Supplementary Material 1), it cannot be excluded that this name may also refer to one of these two species.
Citrullus lanatus (Thunb.) Matsum. & Nakai subsp. *lanatus* (citroides group)	conflicting scientific and cultivar names	T	*C. amarus* Schrad.	The cultivar name is not applied to the appropriate taxon. In this case, taking the cultivar name as correctly indicated ("citroides group"), the correct accession name should be *C. amarus* Schrad. 'Citroides' group.

Table 2 Intra- and inter-institution variability of taxon names

Institution	Scientific name	Database(s)	No. of accessions
RUS001	*Citrullus lanatus* var. *citroides* (L. H. Bailey) Mansf.	Genesys	41
RUS001	*Citrullus lanatus* var. *capensis* (Alef.) Fursa	Genesys	28
RUS001	***Citrullus amarus* Schrad.**	Genesys	0
HUN003	*Citrullus lanatus* var. *caffer* (Schrad.) Mansf. [“subsp. *vulgaris*”]	Genesys EURISCO	25
HUN003	*Citrullus lanatus* var. *citroides* (L.H.Bailey) Mansf.	Genesys EURISCO	1
HUN003	***Citrullus amarus* Schrad.**	Genesys EURISCO	0

The scientific names have been transcribed without misprints; although occurring in both Genesys and EURISCO databases, the indication of “subsp. *vulgaris*” within the combination *C. lanatus* var. *caffer* (Schrad.) Mansf. is wrong (see Table 1), thus we inserted it between square brackets. The current accepted taxon name is in bold

Table 3. Intra- and iter-database variability of taxon names

Scientific name	No. of accessions in:	
	Genesys	EURISCO
***Citrullus amarus* Schrad.**	0	0
Citrullus lanatus var. *citroides* (L. H. Bailey) Mansf.	183	5
Citrullus lanatus var. *caffer* (Schrad.) Mansf. [“subsp. *vulgaris*”]	25	26
Citrullus lanatus (Thunb.) Matsum. & Nakai subsp. *lanatus* (‘Citroides’ group)	15	18
Citrullus lanatus var. *capensis* (Alef.) Fursa	28	0

The scientific names have been transcribed without misprints; although occurring in both Genesys and EURISCO databases, the indication of “subsp. *vulgaris*” within the combination *C. lanatus* var. *caffer* (Schrad.) Mansf. is wrong (see Table 1), thus we inserted it between square brackets. The current accepted taxon name is in bold

Misnaming issues: quantification

By querying the two PGR databases: 8494 single entries of *Citrullus* accessions were obtained, 6631 entries from Genesys and 1863 from EURISCO. The scientific names of 5864 accessions (69.03% of the total) showed nomenclature inaccuracies (N), while the scientific names of 2355 accessions (27.72% of the total) showed taxonomic errors (T) (Supplementary Material 2, 3; Figure 2).

Table 4. Propagated *Citrullus* accessions. We listed: code of the institution where they are stored ("Institution"); accession number ("Accession number"); country where the accessions were originally collected ("Origin"); common English name ("Common English name(s)"); searched database ("Database"); accession scientific name as appearing in the databases ("Accession scientific name"); biological status ("Biological status"); scientific name obtained by revising the original accession name on nomenclatural grounds only ("Accession revised scientific name"); identity of the accession revaluated after the propagation ("Accession revised identity"); misnaming issue category obtained from the comparison between the "Accession scientific name" and the "Accession revised identity"

Institution	**Accession number**	**Origin**	**Common English name(s)**	**Database**	**Accession scientific name**	**Biological status**	**Accession revised scientific name**	**Accession revised identity**	**Issue category**
GBR004	83216	Botswana	-	Genesys, EURISCO	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	Natural	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*	***C. amarus***	T
RUS001	4679	Republic of South Africa	-	Genesys	*Citrullus lanatus* (Thunb.)Matsum. et Nakai var. *citroides* (Bailley) Mansf.	Founder stock/base population	*C. amarus*	***C. amarus***	N
USA016	PI490380	Mali	Egusi	Genesys	*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*	Traditional cultivar/landrace	*C. lanatus* subsp. *lanatus*	***C. mucosospermus***	T

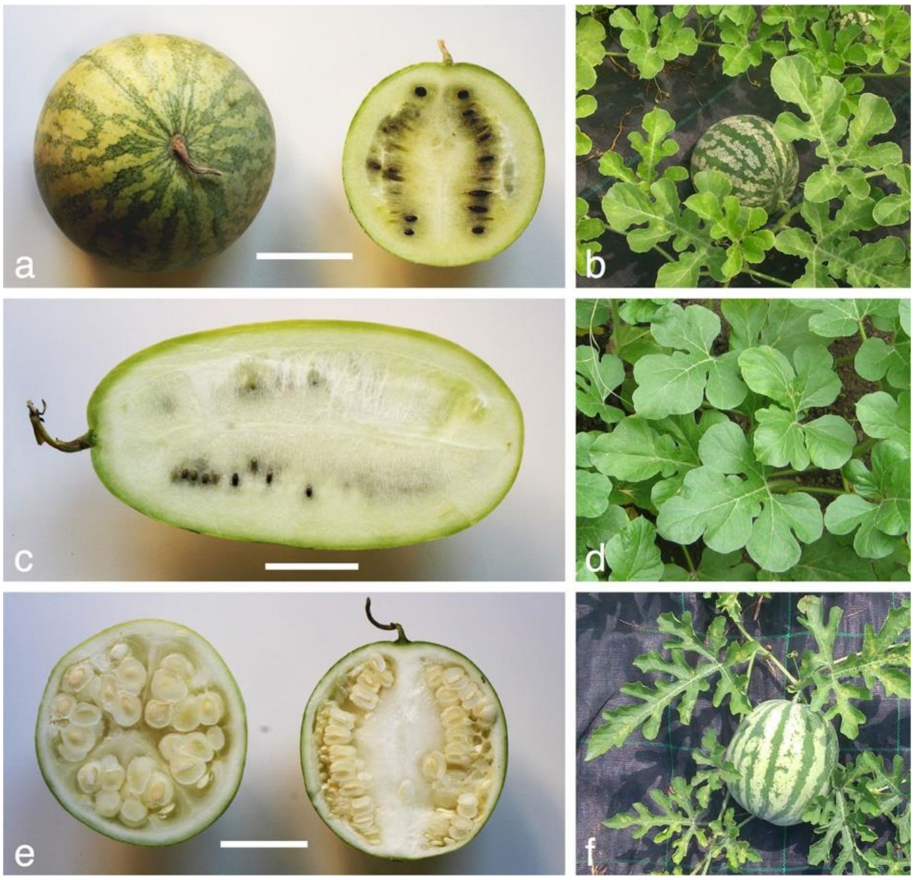
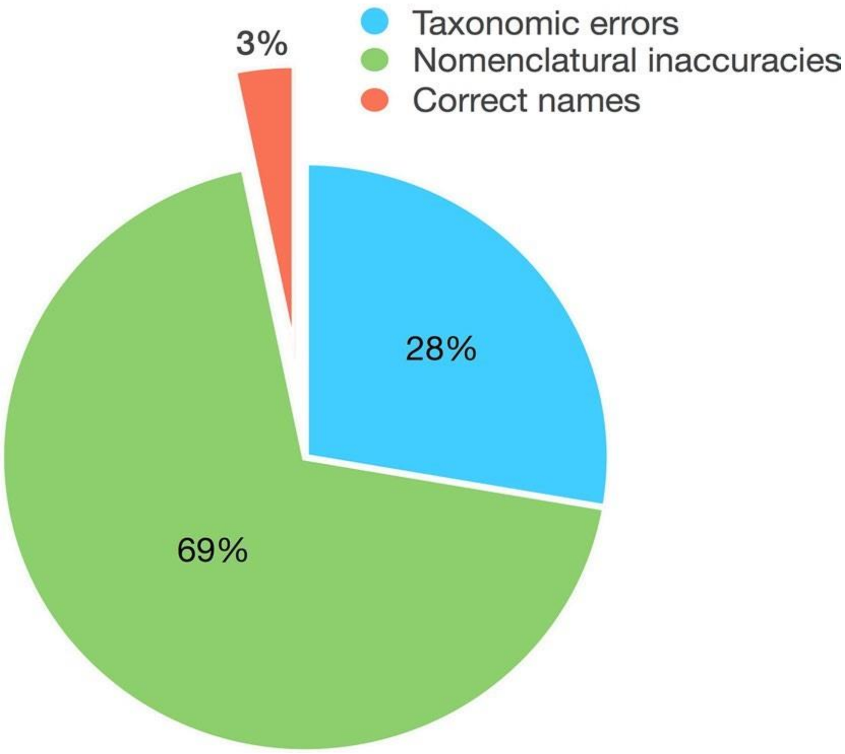


Figure 1. Propagated *Citrullus* accessions (see Table 1): a, c, e fruits, scale bar = 5 cm; b, d, f foliage. a, b GBR004-83216: *C. amarus*; c, d RUS001-4679: *C. amarus*; e, f USA016- PI490380: *C. mucosospermus*. Photos by T. Abeli (a, c, e), V. Ottobrino (b, f), and N. M. G. Ardenghi (d)

In detail, 9.85% of the misnaming issues encountered in both the databases fell within the issue type “authors” (N); 66.79% fell within “synonym” (N); 1.40% fell within “nonexistent name” (N); 0.94% fell within “no species” (T); 26.60% fell within “no subspecies” (T); and 0.38% fell within “conflicting scientific and cultivar names” (T) (the sum of the aforementioned percentages exceeds 100% because some of the accessions showed more than one issue type). Misprints regarding the taxon names or their authors affected 12.54% of the accessions (Supplementary Material 2, 3).

Only 275 accessions (3.23% of the total) were correctly and unambiguously named according the most recent and updated taxonomic and nomenclatural treatment of *Citrullus* (Supplementary Material 1, 2, 3). Considering each database individually the percentages are similar: 2.88% (190 accessions) in Genesys and 4.59% (85 accessions) in EURISCO (Supplementary Material 2, 3).

Figure 2. Percentages of the nomenclature inaccuracies (green), taxonomic errors (light blue), and correct names (red) pooling together the data of *Citrullus* accessions extracted from both Genesys and EURISCO databases



Variability

Taking the name *Citrullus amarus* as an example, we discovered the following variability concerning the accessions' names:

1. "Intra- and inter-istitution variability": in Table 2, it can be seen that within two institutions (RUS001 and HUN003) different accessions belonging to the same species are named using different nomenclatural combinations, which are synonyms of the current accepted name. Moreover, each of the two institutions uses a name not employed by the other institution (*C. lanatus* var. *capensis* for RUS001, *C. lanatus* var. *caffer* for HUN003). None of the accessions have been updated with the currently accepted scientific name, *C. amarus*.
2. "Intra- and inter-database variability": Table 3 reveals that both databases employ more nomenclatural combinations (four in Genesys and three in EURISCO) to name accessions belonging to the same species. Moreover, one database (Genesys) uses a name (*C. lanatus* var. *capensis*) not adopted by the other (EURISCO). Similarly to what found in "intra- and inter-istitution variability", there are no accessions bearing the name *C. amarus* in the two databases.

Cultivation

The cultivation in purity confirmed that the names of two out of the three misnamed propagated accessions did not correspond to their actual taxonomic identity (taxonomic error). While the identity of RUS001-4679, although stored with a misprinted heterotypic synonym [“*Citrullus lanatus* (Thunb.) Matsum. et Nakai var. *citroides* (Bailley) Mansf.”, thus a nomenclatural inaccuracy; see Supplementary Material 1 and Table 1], was confirmed (*C. amarus*; Table 4; Figure 1), the identity of GBR004-83216 and USA016-PI490380 turned out to be incorrect or unclear. Specifically, the identity of GBR004-83216 appeared to be doubtful already at the stage of the nomenclatural revision, since the institution did not provide any infraspecific rank for *C. lanatus*, preventing any safe choice among *C. amarus*, the subspecies of *C. lanatus*, and *C. mucosospermus* (see Table 1, third example). The cultivation allowed us to resolve this issue, revealing that the correct identity of the accession is *C. amarus* (Table 4; Figure 1). In USA016-PI490380, the scientific name of the accession (*C. lanatus* var. *lanatus*, a homotypic synonym of *C. lanatus* spp. *lanatus*, see Supplementary Material 1) appears to be in conflict with the vernacular name (Egusi), which applies to another species (*C. mucosospermus*, see Supplementary Material 1). In this case, propagation also proved to be decisive in resolving the issue, being *C. mucosospermus* the plant having emerged from the seeds constituting the accession (Table 4; Figure 1).

Discussion

The results of our database search and subsequent revision of the accessions’ scientific names, highlight the fact that taxonomic misnaming issues actually greatly limit the conservation and usage of *Citrullus* seed material conserved ex situ; only 3% of the material is correctly and unambiguously named, in conformity to the most updated taxonomic and nomenclatural treatment. Some sort of nomenclatural inaccuracies has been found for 69% percent of the material (Figure 2; Supplementary Material 2, 3), which could be merely solved by updating the databases through the application of the current taxonomic and nomenclatural treatment. On the other hand, 28% of the accessions showed taxonomic errors (Figure 2, Supplementary Material 2, 3) and therefore cannot be unequivocally attributed to any existing taxon; as a consequence, they are prevented from being employed in any research, breeding, cultivation, reintroduction or conservation project. Their usage can be recovered only by means of re-determination (if a herbarium voucher of the seed accession is available) or re-propagation, followed by re-determination, which enables the revision of the accession's taxonomic identity and its re-accessioning under the correct name. If this re-propagation and re-determination process is not undertaken, research and breeding activities on 28% of the *Citrullus* accessions currently stored worldwide will be corrupted by an erroneous taxonomic identification, causing their priceless genetic variability and the potential of their useful traits to be improperly exploited. Moreover, it is possible, considering that species barriers to crossing in *Citrullus* are weak (Assis et al. 2000), that, during the re-propagation phase, introgressants or other intermediates might be found. Specifically, introduction and cropping of dessert watermelons, *Citrullus lanatus*, in parts of Africa in which other *Citrullus* species are indigenous, might have resulted in introgression of dessert watermelon genes into indigenous germplasm, thus complicating taxonomic identification. We suggest classifying these introgressants as *Citrullus* sp. in the databases, with their possible intermediate origin in their passport data. The results of our propagation experiment (Figure 1, Table 4) show unequivocally in practical terms the existence of the problem of taxonomic identity, since the revised taxonomic identity of two out of the three propagated accessions did not correspond to the originally adopted accession name. On the other hand, the propagation experiment demonstrates the feasibility and effectiveness of a growth and redetermination phase in recovering the usability of accessions affected by taxonomic issues.

Our results clearly show great variability in the taxonomic and nomenclatural treatments adopted by the different institutions and even by the same institution (Tables 2, 3). As shown by Table 2, different accessions belonging to the same taxon are named using different synonym combinations, leading to an apparent overestimation of the taxa conserved ex situ by each institution. Specifically, in our example, two taxa (*C. lanatus* var. *citroides* and *C. lanatus* var. *capensis* in RUS001, and *C. lanatus* var. *caffer* and *C. lanatus* var. *citroides* in HUN003) appear to be conserved rather than one (*C. amarus*) (Table 2). On the other hand, it is even worse to notice that the variability in accession naming, along with the aforementioned misnaming issues, can lead to a great underestimation of the overall number of accessions conserved per taxon. As shown in Table 3, for instance, it appears that no seed accession of *C. amarus* is conserved worldwide. This would make this species, which is widespread in southern Africa (Paris 2015) and frequently cultivated in all the tropical and sub-tropical areas of the world (Laghetti and Hammer 2007), of extremely high priority for ex situ seed conservation measures, when in fact accessions stored under four different synonyms actually exist (49 in EURISCO and 251 in Genesys). The apparent and erroneous underestimation of the number of stored accessions of a certain taxon is of particular relevance since it could diminish the importance of prioritization studies intended to unveil which taxa are currently underrepresented in long-term conservation facilities and therefore which of them should be the target of collecting missions. Prioritization studies are indeed based on the quantification of the accessions conserved ex situ of a particular target species or group of species (Maxted et al. 2010; Castañeda-Álvarez et al. 2016).

Achieving the goal of having the biggest number of accessions correctly named is fundamental to standardizing the naming process of accessions in the PGR databases, using as a reference the most updated taxonomic and nomenclatural treatments. Special attention should be paid to the choice of taxonomic ranks, without neglecting the infraspecific ones (e.g., subspecies), both for their employment in distinguishing between closely related wild and domesticated taxa (see e.g., Galasso et al. 2017), and for their possible raising to higher ranks (e.g., species) as a result of the advances in taxonomic knowledge. This latter case is illustrated by *C. lanatus*, which until recent times has been divided into various subspecies and varieties, that have been subsequently regarded as independent species (Supplementary Material 1). Thus, when accessions are stored under names without mention of subspecies or variety, they are almost impossible to interpret on taxonomic grounds. In our case, a simple "*C. lanatus*" entry may refer to *C. amarus*, *C. mucosospermus* or one of the two subspecies of *C. lanatus* (Tables 1, 2; Supplementary Material 2, 3).

To avoid the re-occurrence of all the aforementioned problems, a process of taxonomic and nomenclatural peer review is urgently needed before making each new accession public, in order to guarantee that each single accession is usable by the stakeholders. This great number of misnaming issues and variabilities in the application of taxon names also compromises the database analyses that, nowadays, are fundamental to planning and performing ex situ conservation programmes intended to find out taxa and locations underrepresented in current seed collections (Castañeda-Álvarez et al. 2016; Guzzon and Müller 2016).

It is known that the efficiency of species conservation measures greatly depends on the reliability of the taxonomic information available (Bortolus 2008). The conservation and utilization of PGR depends on the building and update of global searchable databases (Khoury et al. 2010). Our investigation demonstrates that taxonomic misnaming threatens the ex situ conservation efforts of the genus *Citrullus*. Our results confirm that taxonomic issues are a major problem in aggregator databases and that those issues have serious implications for the uncritical use of specimen data from botanical collections (Goodwin et al. 2015). On the basis of our experience, we here propose a series of actions useful for understanding the extent of the phenomenon and for solving its detrimental effects across the genepools of plant genetic resources for food and agriculture (PGRFA):

1. Perform studies similar to the present one on further PGRFA genepools to unveil the extent of similar problems also in other taxa;

2. Establish taxonomic authorities in order to provide an updated and standardized taxonomic and nomenclatural treatment that should be followed in the application of the accessions' scientific names for a certain genepool within and the among the PGRFA databases;
3. Update the nomenclature inaccuracies following the most recent nomenclatural treatments;
4. Propagate, identify, and correct the scientific names of accessions affected by taxonomic errors;
5. Always link a herbarium voucher to each seed accession, in order to allow a quick taxon re-determination and to avoid the lengthy and costly process of re-propagation. The collection and preservation of herbarium specimens is often not considered by institutions involved in germplasm conservations;
6. Perform a peer review of the accessions' scientific names before their publication in databases.

This series of actions appears to us to be the only way to solve and prevent the occurrence of large numbers of taxonomic and nomenclatural issues with detrimental effects on PGR conservation and usage. Such procedures require investment in personnel and resources, and therefore would require adequate recognition and funding from government supported sources.

The current study has been undertaken on the genus *Citrullus*, a relatively small genus whose taxonomy has been resolved in recent times. Nevertheless, a remarkable number of issues emerged, with consequences for the interpretation of the data stored in the databases and the practical usage of some accessions. In light of this observation, how serious may be the situation for accessions belonging to critical taxonomic groups with a great importance for food security (e.g., *Triticum*, *Musa*; see Hammer et al. 2011; Čížková et al. 2015) as well as other agriculture-related activities (e.g., *Festuca* s.l.; see Ardenghi et al. 2017), still characterized by controversial and unsolved taxonomy? This highlights once again the key-importance of taxonomic studies, often neglected in modern biology, for species conservation (Garnett and Christidis 2017). Moreover, we suggest that studies similar to the current one should be performed on more crop genepools that significantly contribute to global food security in order to get an idea of the scale of the issues highlighted in this study and for them to be corrected where possible. Genepools of different sizes will have to be considered. Of particular importance are genepools that had a recent taxonomic revision, for instance the pea (*Lathyrus oleraceus* subsp. *oleraceus*), lentil (*Vicia lens* subsp. *lens*), broad bean (*Vicia faba*) and wheat, which are considered some of the founder crops of Neolithic agriculture in the Fertile Crescent and hold a fundamental importance in food security (van Slageren 1994; Kilian et al. 2011; Schaefer et al. 2012).

While the current paper focuses more on PGRFA for their role in food security, the extent of taxonomic misnaming could also affect the conservation of seed accessions of endangered plants and of wild species of interest for reintroduction and translocation programmes.

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References

Applequist WL (2016) Report of the nomenclature committee for vascular plants: 67. Taxon, 75, 169–182. https://doi.org/10.12705/651.15

Ardenghi NMG, Foggi B, Orsenigo S, Maggioni L, Cauzzi P, Rossi G (2017) Festuca and allied genera (Poaceae) as crop wild relatives: checklists and red lists are urgently required. Plant Biosystems, 151, 6–8. https://doi.org/10.1080/11263504.2016.1165753

Asseng S, Ewert F, Martre P, Rötter RP, Lobell DB, Cammarano D, Kimball BA, Ottman MJ, Wall GW, White JW, Reynolds MP, Alderman PD, Prasad PVV, Aggarwal PK, Anothai J, Basso B, Biernath C, Challinor AJ, De Sanctis G, Doltra J, Fereres E, Garcia-Vila M, Gayler S, Hoogenboom G, Hunt LA, Izaurralde RC, Jabloun M, Jones CD, Kersebaum KC, Koehler A-K, Müller C, Naresh Kumar S, Nendel C, O'Leary G, Olesen JE, Palosuo T, Priesack E, Eyshi Rezaei E, Ruane AC, Semenov MA, Shcherbak I, Stöckle C, Stratonovitch P, Streck T, Supit I, Tao F, ThorburnPJ Waha K, Wang E, Wallach D, Wolf J, Zhao Z, Zhu Y (2015) Rising temperatures reduce global wheat production. Nature Climare Change, 5, 143–147.

Bortolus A (2008) Error cascades in the biological sciences: the unwanted consequences of using bad taxonomy in ecology. Ambio, 37, 114–118.

Castañeda-Álvarez NP, Khoury CK, Achicanoy HA, Bernau V, Dempewolf H, Eastwood RJ, Guarino L, Harker RH, Jarvis A, Maxted N, Müller JV, Ramirez-Villegas J, Sosa CC, Struik PC, Vincent H, Toll J (2016) Global conservation priorities for crop wild relatives. Nature Plants, 2:16022.

Chomicki G, Renner SS (2014) Watermelon origin solved with molecular phylogenetics including Linnaean material: another example of museomics. New Phytologist, 205, 526–532.

Čížková J, Hřibová E, Christelová P, Van den Houwe I, Häkkinen M, Roux N et al (2015) Molecular and cytogenetic characterization of wild *Musa* species. PLoS ONE. 10(8):e0134096.

Convention on Biological Diversity (2011) Aichi BiodiversityTargets. https://www.cbd.int/sp/targets. Accessed 2 Sep 2017.

Convention on Biological Diversity (2012) 2011–2020 Global Strategy for Plant Conservation Botanic Gardens Conservation International. Richmond, UK

Davies LR, Allender CJ (2017) Who is sowing our seeds? A systematic review of the use of plant genetic resources in research. Genetic Resources and Crop Evolution, 6, 1–10.

de Assis JGA, Queiroz M, de Araujo SMC, Bandel G, Martins PS (2000) Implications of the introgression between *Citrullus colocynthis* and *C. lanatus* characters in the taxonomy, evolutionary dynamics and breeding of watermelon. Plant Genetic Resources Newslett, 121, 15–19.

Dempewolf H, Eastwood RJ, Guarino L, Khoury CK, Müller JV, Toll J (2014) Adapting agriculture to climate change: a global initiative to collect, conserve, and use crop wild relatives. Agroecology and Sustainable Food System 38, 369–377.

Dempewolf H, Baute G, Anderson J, Kilian B, Smith C, Guarino L (2017) Past and future use of wild relatives in crop breeding. Crop Science, 57, 1070–1082.

Esquinas-Alcázar J (2005) Protecting crop genetic diversity for food security: political, ethical and technical challenges. Nature, 6, 946–953.

EURISCO (2017) EURISCO: finding seeds for the future. http://eurisco.ipk-gatersleben.de. Accessed 29 June 2017

FAO (2010) The second report on the state of the world's plant genetic resources for food and agriculture. Rome. http://www.fao.org/docrep/013/i1500e/i1500e.pdf. Accessed 20 July 2017

FAOSTAT (2017) Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/ en/#data. Accessed 20 July 2017

Ford-Lloyd BV, Schmidt M, Armstrong SJ, Barazani O, Engels J, Hadas R, Hammer K, Kell SP, Kang D, Khoshbakht K, Li Y, Long C, Lu B-R, Ma K, Nguyen VT, Qiu L, Ge S, Wei W, Zhang Z, Maxted N (2011) crop wild relatives—undervalued, underutilized and under threat? Bioscience, 61, 559–565.

Fursa TB (1972) К систематике рода *Citrullus* Schrad. [On the taxonomy of genus *Citrullus* Schrad.]. Bot Zhurn (Moscow & Leningrad), 57, 31–41.

Galasso G, Banfi E, Bartolucci F, Ardenghi NMG (2017) Notulae to the Italian native vascular flora: 3. Italian Botanist, 3, 35–36.

Galluzzi G, Halewood M, Noriega IL, Vernooy R (2016) Twenty-five years of international exchanges of plant genetic resources facilitated by the CGIAR genebanks: a case study on global interdependence. Biodiversity and Conservation, 25, 1421–1446.

Garnett ST, Christidis L (2017) Taxonomy anarchy hampers conservation. Nature, 546, 25–27.

Genesys (2017) Gateway to genetic resources. https://www.genesys-pgr.org/welcome. Accessed 20 May 2017

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food Security: the challenge of feeding 9 billion people. Science, 327, 812–818.

Goodwin ZA, Harris DJ, Filer D, Wood JRI, Scotland RW (2015) Widespread mistaken identity in tropical plant collections. Current Biology, 25, 1057–1069.

Guarino L, Lobell DB (2011) A walk on the wild side. Nature Climate Change, 1, 374–375.

Guzzon F, Müller JV (2016) Current availability of seed material of enset (*Ensete ventricosum*, Musaceae) and its Sub-Saharan wild relatives. Genetic Respurces and Crop Evolution, 63, 185–191.

Hammer K, Knupffer H, Xhuveli L, Perrino P (1996) Estimating genetic erosion in landraces—two case studies. Genetic Resources and Crop Evolution, 43, 329–336.

Hammer K, Filatenko AA, Pistrick K (2011) Taxonomic remarks on *Triticum* L. and x Triticosecale Wittm. Genetic Resources and Crop Evolution, 58, 3–10.

Kell S, Marino M, Maxted N (2017) Bottlenecks in the PGRFA use system: stakeholders' perspectives. Euphytica, 230, 170.

Khoury C, Laliberté B, Guarino L (2010) Trends in ex situ conservation of plant genetic resources: a review of global crop and regional conservation strategies. Genetic Resourcea and Crop Evolution, 57, 625–639.

Kilian B, Mammen K, Millet E, Sharma R, Graner A, Salamini F, Özkan H (2011) *Aegilops*. In: Kole C (ed) Wild crop relatives genomic and breeding resources, cereals. Springer, Berlin, pp 1–76

Laghetti G, Hammer K (2007) The Corsican citron melon (*Citrullus lanatus* (Thunb.) Matsum. et Nakai subsp. *lanatus* var. *citroides* (Bailey) Mansf. ex Greb.) a traditional and neglected crop. Genetic Resources and Crop Evolution, 54, 913–916.

Last L, Arndorfer M, Balázs K, Dennis P, Dyman T, Fjellstad W, Friedel JK, Herzog F, Jeanneret P, Lüscher G, Moreno G, Kwikiriza N, Gomiero T, Paoletti MG, Pointereau MG, Sarthou J-P, Stoyanova S, Wolfrum S, Kölliker R (2014) Indicators for the on-farm assessment of crop cultivar and livestock breed diversity: a survey-based participatory approach. Biodiversity and Conservation, 23, 3051–3071.

Li DZ, Pritchard HW (2009) The science and economics of ex situ plant conservation. Trends in Plant Science, 14, 614–621.

Mansfeld R (1959) Vorläufiges Verzeichnis landwirtschaftlich oder gärtnerisch kultivierter pflanzenarten. Kulturpflanze, 2, 1–659.

Matsumura J, Nakai T (1916) Catalogus seminum et sporarum in Horto Botanico Universitatis Imperialis Tokyoensis per annos 1915 et 1916 lectorum. Tokyo.

Maxted N, Kell SP, Toledo Á, Dulloo E, Heywood V, Hodgkin T, Hunter D, Guarino L, Jarvis A, FordLloyd B (2010) A global approach to crop wild relative conservation: securing the gene pool for food and agriculture. Kew Bull 65:561–576. https://doi.org/10.1007/s12225-011-9253-4

McCouch S, Baute GJ, Bradeen J, Bramel P, Bretting PK, Buckler E, Burke JM, Charest D, Cloutier S, Cole G, Dempewolf H, Dingkuhn M, Feuillet C, Gepts P, Grattapaglia D, Guarino L, Jackson S, Knapp S, Langridge P, Lawton-Rauh A, Lijua Q, Lusty C, Michael T, Myles S, Naito K, Nelson RL, Pontarollo R, Richards CM, Rieseberg L, Ross-Ibarra J, Rounsley S, Hamilton RS, Schurr U, Stein N, Tomooka N, van der Knaap E, van Tassel D, Toll J, Valls J, Varshney RK, Ward J, Waugh R, Wenzl P, Zamir D (2013) Feeding the future, Nature. 499:23–24.

McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prud'homme Van Reine WF, Smith GF, Wiersema JH, Turland NJ (eds) (2012) International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). Regnum Vegetabile 154. Königstein: Koeltz Scientific Books. [online ed., http://www.iapt-taxon.org/nomen/main.php]

Modi AT, Zulu NS (2012) Watermelon landrace seedling establishment and field performance in response to differing water regimes. African Journal of Agricultural Research, 7, 6016–6021.

Mujaju C, Zborowska A, Werlemark G, Garkava-Gustavsson L, Andersen SB, Nybom H (2011) Genetic diversity among and within watermelon (*Citrullus lanatus*) landraces in Southern Africa. Journal of Horticultural Science and Biotechnology, 86, 353–358.

Nesom GL (2011) Toward consistency of taxonomic rank in wild/domesticated Cucurbitaceae. Phytoneuron, 13, 1–33.

Pangalo KI (1930) Арбузы Северного полушария. [Watermelons of the northern hemisphere]. Trudy po prikladnoi botanike, genetike i selektsii, 23, 41–84.

Paris HS (2015) Origin and emergence of the sweet dessert watermelon, *Citrullus lanatus*. Annals of Botany, 116, 133–148.

Renner SS, Chomicki G, Greuter W (2014) (2313) Proposal to conserve the name *Momordica lanata* (*Citrullus lanatus*) (watermelon, Cucurbitaceae), with a conserved type, against *Citrullus battich*. Taxon, 63, 941–942.

Rivière S, Müller JV (2017) Contribution of seed banks across Europe towards the 2020 Global Strategy for plant conservation targets, assessed through the ENSCONET database. Oryx, https://doi.org/10.1017/ S0030605316001496

Schaefer H, Renner SS (2011) Phylogenetic relationships in the order Cucurbitales and a new classification of the gourd family (Cucurbitaceae). Taxon, 60, 122–138.

Schaefer H, Hechenleitner P, Santos-Guerra A, Menezes de Sequeira M, Pennington RT, Kenicer G, Carine MA (2012) Systematics, biogeography, and character evolution of the legume tribe Fabeae with special focus on the middle-Atlantic island lineages. BMC Evolutionart Biology, 12, 250.

Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. Nature, 418, 671–677.

van de Wouw M, Kik C, van Hintum T, van Treuren R, Visser B (2010) Genetic erosion in crops: concept, research results and challenges. Plant Genetic Resources, 8, 1–15.

van Slageren MW (1994) Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (*Poaceae*). Agricultural University Papers, Wageningen

Veteläinen M, Negri V, Maxted N (2009) European landraces: on-farm conservation managment and use. Bioversity Technical Bulletin No. 15. Bioversity International, Rome.

Vincent H, Wiersema J, Kell SP, Dobbie S, Fielder H, Castañeda-Álvarez NP, Guarino L, Eastwood R, Leon B, Maxted N (2013) A prioritised crop wild relative inventory as a first step to help underpin global food security. Biological Conservation, 167, 265–275.

Warschefsky E, Varma Penmetsa R, Cook DR, von Wettemberg EJB (2014) Back to the wild: tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. American Journal of Botany, 101, 1791–1800.

Supplementary materal 1 Updated taxonomic and nomenclatural treatment of *Citrullus*. In “Nomenclature”, besides the accepted scientific names (in bold), we listed the synonyms encountered in the consulted databases, along with the basionyms (“≡” is used to indicate basionyms and homotypic synonyms, while “=” heterotypic synonyms, see *ICN*’s Glossary); cultivar groups (“Cultivar-group”), adapted to the current taxonomic treatment, and common English names (“Common English name(s)”) were acquired mostly from Quattrocchi (1999), Jeffrey (2001), Nesom (2011), and Paris (2015)

Nomenclature	**Cultivar-group**	**Common English name(s)**
Citrullus amarus Schrad. in Eckl. & Zeyh., Enum. Pl. Afric. Austral. 2: 279. 1836 ≡ *Citrullus colocynthis* var. *capensis* Alef., Landw. Fl.: 211. 1866 ≡ *Citrullus lanatus* var. *capensis* (Alef.) Fursa in Bot. Zhurn. (Moscow & Leningrad) 57(1): 37. 1972. = *Citrullus caffer* Schrad. in Linnaea 12: 413. 1838 ≡ *Citrullus lanatus* var. *caffer* (Schrad.) Mansf. in Kulturpflanze, Beih. 2: 422. 1959, nom. illeg. (Arts. 52.1−2 of the *ICN*), nom. inval. (Art. 41.5 of the *ICN*). = *Citrullus vulgaris* var. *citroides* L.H.Bailey in Gentes Herbarum 2(4): 186. 1930 ≡ *Citrullus lanatus* var. *citroides* (L.H.Bailey) Mansf. in Kulturpflanze, Beih. 2: 422. 1959.	Citroides	Citron watermelon; citron; tsamma; fodder melon; preserving melon
Citrullus colocynthis (L.) Schrad. in Linnaea 12: 414. 1838 ≡ *Cucumis colocynthis* L., Sp. Pl. 2: 1011. 1753.	-	Colocynth; bitter apple
Citrullus ecirrhosus Cogn. in Verh. Bot. Vereins Prov. Brandenburg 30(2): 151. 1888.	-	Namib tsamma
Citrullus lanatus (Thunb.) Matsum. & Nakai in Cat. Sem. Spor. [1915 & 1916 Lect.]: 30. 1916 subsp. ***lanatus*** ≡ *Momordica lanata* Thunb., Prodr. Pl. Cap.: 13. 1794, nom. cons. rec. [Taxon 65(1): 173. 2016] ≡ *Citrullus lanatus* (Thunb.) Mansf. in Kulturpflanze, Beih. 2: 421(−422). 1959, isonym (Art 6.3, Note 2 of the *ICN*) = *Cucurbita citrullus* L., Sp. Pl. 2: 1010. 1753 ≡ *Citrullus vulgaris* Schrad. in Eckl. & Zeyh., Enum. Pl. Afric. Austral. 2: 279. 1836, nom. nud. ≡ *Citrullus edulis* Spach, Hist. Nat. Vég. 6: 214. 1838 [10 Mar 1838], nom. illeg. (Arts. 52.1−2 of the *ICN*) ≡ *Citrullus vulgaris* Schrad. in Linnaea 12: 412. 1838 [Apr−Sep 1838] ≡ *Citrullus lanatus* subsp. *vulgaris* (Schrad.) Fursa in Bot. Zhurn. (Moscow & Leningrad) 57(1): 37. 1972 ≡ *Citrullus lanatus* subsp. *vulgaris* var. *vulgaris* (Schrad.) Fursa in Bot. Zhurn. (Moscow & Leningrad) 57(1): 38. 1972. = *Citrullus aedulis* Pangalo in Trudy Prikl. Bot. 23(3): 44. 1930.	Dessert	Dessert watermelon

Citrullus lanatus subsp. ***kordophanus*** Ter-Avan. in Bot. Zhurn. (Kiev) 51(3): 425. 1966 ("*cordophanus*") ≡ *Citrullus lanatus* subsp. *vulgaris* var. *kordophanus* (Ter-Avan.) Fursa in Bot. Zhurn. (Moscow & Leningrad) 57(1): 38. 1972. Note: Ter-Avasyn (1966) employs two orthographical variants of the subspecific epithet: "*kordophanus*", reported two times throughout the text, and "*cordophanus*", used only once, in the title of the work. According to Art. 61.3 of the *ICN*, the variant "*kordophanus*" is to be retained since it best suits the rules and recommendations of Art. 60. Specifically, the letter "c" is likely a typographical error (Art. 60.1), having been used only in one occasion by the author, who probably preferred the variant "*kordophanus*", whose spelling is nearer to that of the latinized Arabic word "كردفان" (Kurdufan/Kordofan, the name of a former Sudan province), whose initial letter "كـ/ك" is transliterated with the letter "k" (Pedersen 2008).	Cordophanus	Gurum; gurma watermelon; gurma melon
Citrullus mucosospermus (Fursa) Fursa in Trudy Prikl. Bot. Genet. Selek. 81: 111. 1983 ≡ *Citrullus lanatus* subsp. *mucosospermus* Fursa in Bot. Zhurn. (Moscow & Leningrad) 57(1): 38. 1972.	Mucosospermus	Egusi watermelon; egusi melon
Citrullus naudinianus (Sond.) Hook.f. in Oliv., Fl. Trop. Afr. 2: 549. 1871 ≡ *Cucumis naudinianus* Sond. in Harv., Fl. Cap. 2: 496. 1862 ≡ *Acanthosicyos naudinianus* (Sond.) C.Jeffrey in Kew Bull. 15(3): 346. 1962.	-	-
Citrullus rehmii De Winter in Bothalia 20(2): 209. 1990.	-	-

Supplementary material 2 *Citrullus* accession names acquired from the Genesys PGR database (accessed 20 May 2017). For each name found ("Accession scientific name"; orthographic errors and misprints have been fully transcribed as they originally appeared in the databases), we provided the number of accessions ("N. of accessions"), the misnaming issues ("Misnaming issue(s)"; their definition is reported in Tab. 1, with the exception of "misprint"= authors of the names are not reported, wrongly abbreviated or contain orthographic errors), the issue categories ("Issue category"), and the updated scientific name we proposed ("Current scientific name"; when an accession name can be referred to multiple taxa, these are separated by a "/")

Accession scientific name	**N. of accessions**	**Misnaming issue(s)**	**Issue category**	**Current scientific name**
Acanthosycios naudinanus	1	synonym; misprint	N	*C. naudinianus*
Acanthosycios naudinanus (Sond.) C. Jeffrey	28	synonym	N	*C. naudinianus*
Acanthosycios naudinanus (Sond.) C.Jeffrey	12	synonym	N	*C. naudinianus*
Citrullus aedulis Pangalo	6	synonym	N	*C. lanatus* subsp. *lanatus*
Citrullus aedulus Pangalo	115	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus colocynthis	26	misprint	-	*C. colocynthis*
Citrullus colocynthis (L.) Schrad.	70	-	-	*C. colocynthis*
Citrullus colocynthis (L.) Schrad. (L.) Schrad.	24	misprint	-	*C. colocynthis*
Citrullus colocynthis (L.) Schrad. (L.) Schrad.	5	misprint	-	*C. colocynthis*
Citrullus colocynthis (L.)Schrad.	8	misprint	-	*C. colocynthis*
Citrullus colocynthis Schrad.ex Eckl.& Zeyh.	1	misprint	N	*C. colocynthis*
Citrullus ecirrhosus Cogn.	6	-	-	*C. ecirrhosus*
Citrullus edulis	1	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus edulis Pangalo	11	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus edulis Pangalo var. *lanatus*	6	non-existent name; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus	387	no subspecies; misprint	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb) Matsumura & Nakai	8	no subspecies; misprint	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.) Mansf.	282	no subspecies; authors	N; T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.) Mastsum. et Nakai	2	no subspecies	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.) Mastsum. et Nakai var. lanatus	346	synonym	N	*C. lanatus* subsp. *lanatus*

Citrullus lanatus (Thunb.) Mastsum. et Nakai var. *vulgaris*	6	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Mastsum. et Nakai var. *vulgaris* (Schrad) Fursa.	16	synonym	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Mastsum. et Nakai var. *vulgaris* Schrad	1	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum.	1	no subspecies; misprint	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.) Matsum. &	7	no subspecies; misprint	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.) Matsum. & Nakai	480	no subspecies	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.) Matsum. & Nakai subsp. *lanatus* (citroides group)	15	conflicting scientific and cultivar names	T	*C. amarus*
Citrullus lanatus (Thunb.) Matsum. & Nakai subsp. *lanatus* (lanatus group)	3	-	-	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum. & Nakai subsp. *vulgaris* (dessert group) (Schrad.) Fursa	145	synonym	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum. & Nakai subsp. *vulgaris* (Schrad.) Fursa	6	synonym	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum. & Nakai var. *citroides* (L. H. Bailey) Mansf.	138	synonym	N	*C. amarus*
Citrullus lanatus (Thunb.) Matsum. & Nakai var. *citroides* (L.H. Bailey) Mansf.	3	synonym	N	*C. amarus*
Citrullus lanatus (Thunb.) Matsum. & Nakai var. *lanatus*	1455	synonym	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum. & var. *lanatus*	1	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum. et Nakai	210	no subspecies	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.) Matsum. et Nakai convar. *lanatus*	1	non-existent name	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum. et Nakai convar. *vulgaris*	14	non-existent name	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum. et Nakai subsp. *vulgaris* (Schrad.) Fursa	1	synonym	N	*C. lanatus* subsp. *lanatus*

Citrullus lanatus (Thunb.) Matsum. et Nakai subsp. *vulgaris* var. *caffer* (Schrad.) Mansf.	25	non-existent name	N	*C. amarus*
Citrullus lanatus (Thunb.) Matsum. et Nakai var. *citroides*	1	synonym; misprint	N	*C. amarus*
Citrullus lanatus (Thunb.) Matsum. et Nakai(Thunb.) Matsum	2	no subspecies; misprint	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.) Matsum. et Nakai(Thunb.) Matsum convar. *vulgaris*	2	non-existent name; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.)Mansf.	1	no subspecies; authors	N; T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.)Matsum. et Nakai var. *capensis* (Alef.) Fursa	28	synonym	N	*C. amarus*
Citrullus lanatus (Thunb.)Matsum. et Nakai var. *citroides* (Bailley) Mansf.	41	synonym; misprint	N	*C. amarus*
Citrullus lanatus (Thunb.)Matsum. et Nakai var. *cordophanus* (Ter.-Avan.) Fursa	27	synonym	N	*C. lanatus* subsp. *kordophanus*
Citrullus lanatus (Thunb.)Matsum. et Nakai var. *lanatus* (Thunb.) Matsum. et Nakai	51	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.)Matsum. et Nakai var. *vulgaris* (Shrad.) Fursa	2228	synonym; misptint	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.)Matsum.& Nakai	1	no subspecies	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.)Matsum.et Nakai	1	no subspecies	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus Mansf.	49	no subspecies; authors	N; T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus Matsum. & Nakai (Thunb.)	4	no subspecies; misprint	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus Thunb.	2	no subspecies; misprint	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus Thunb.) Matsum. et Nakai convar. *vulgaris*	12	non-existent name	N	*C. lanatus* subsp. *lanatus*
Citrullus mucosospermus (Fursa) Fursa	36	-	-	*C. mucosospermus*
Citrullus mucosospermus (Fursa) Fursa Fursa	7	misprint	-	*C. mucosospermus*
Citrullus rehmii De Winter	5	-	-	*C. rehmii*
Citrullus sp.	21	no species	T	?

Citrullus sp. Schrad.	45	no species	T	?
Citrullus spp.	4	no species	T	?
Citrullus vulgairs Schrad	2	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus vulgaris L	1	synonym; authors	N	*C. lanatus* subsp. *lanatus*
Citrullus vulgaris L.	59	synonym; authors	N	*C. lanatus* subsp. *lanatus*
Citrullus vulgaris Schrad.	36	synonym	N	*C. lanatus* subsp. *lanatus*
Citrullus vulgaris Schrad. ex Eckl.	2	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus vulgaris Schrad. ex Eckl. & Zeyh.	48	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus vulgaris Schrad.ex Eckl.& Zeyh.	21	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus vulgaris Schrad.exEckl.&Zeyh.	21	synonym; misprint	N	*C. lanatus* subsp. *lanatus*

Supplementary material 3 *Citrullus* accession names acquired from the EURISCO database (accessed 29 June 2017). See the previous table caption for the legend

Accession scientific name	**N. of accessions**	**Misnaming issue(s)**	**Issue category**	**Current scientific name**
Acanthosycios naudinanus (Sond.) C.Jeffrey	12	synonym	N	*C. naudinianus*
Citrullus aedulis Pangalo	6	synonym	N	*C. lanatus* subsp. *lanatus*
Citrullus aedulus Pangalo	117	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus aedulus	10	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus colocynthis	25	misprint	-	*C. colocynthis*
Citrullus colocynthis (L.)Schrad.	54	-	-	*C. colocynthis*
Citrullus ecirrhosus Cogn.	2	-	-	*C. ecirrhosus*
Citrullus edulis Pangalo	10	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus edulis Pangalo var. lanatus	6	non-existent name; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus	11	no subspecies; misprint	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus Thunb.) Matsum. et Nakai convar. *vulgaris*	10	non-existent name	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus Thunb.	2	no subspecies; misprint	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus Pangalo	1	no subspecies; authors	N; T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus Mansf.	90	no subspecies; authors	N; T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.)Matsum.et Nakai	2	no subspecies	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.) Matsum. et Nakai(Thunb.) Matsum	2	no subspecies; misprint	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.) Matsum. et Nakai(Thunb.) Matsum convar. *vulgaris*	2	non-existent name; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum. et Nakai	210	no subspecies	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.) Matsum. et Nakai var. *citroides*	1	synonym; misprint	N	*C. amarus*

Citrullus lanatus (Thunb.) Matsum. et Nakai subsp. *vulgaris* var. *caffer* (Schrad.) Mansf.	25	non-existent name	N	*C. amarus*
Citrullus lanatus (Thunb.) Matsum. et Nakai subsp. *vulgaris* var. *caffer* (Schrad.) Fursa	1	non-existent name	N	*C. amarus*
Citrullus lanatus (Thunb.) Matsum. et Nakai convar. *lanatus*	1	non-existent name	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum. et Nakai convar. *vulgaris*	14	non-existent name	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum. et Nakai subsp. *vulgaris* (Schrad.) Fursa	1	synonym	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum. & Nakai	201	no subspecies	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.) Matsum. & Nakai var. *lanatus*	3	synonym	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum. & Nakai var. *citroides* (L.H. Bailey) Mansf.	4	synonym	N	*C. amarus*
Citrullus lanatus (Thunb.) Matsum. & Nakai subsp. *vulgaris* (Dessert Group) (Schrad.) Fursa	185	synonym	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum. & Nakai subsp. *Vulgaris* (Schrad.) Fursa	4	synonym	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum. & Nakai subsp. *lanatus* (Lanatus Group)	4	-	-	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Matsum. & Nakai subsp. *lanatus* (Citroides Group)	18	conflicting scientific and cultivar names	N	*C. amarus*
Citrullus lanatus (Thunb.) Mastsum. et Nakai	2	no subspecies	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus lanatus (Thunb.) Mastsum. et Nakai var. *vulgaris*	6	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Mastsum. et Nakai var. *vulgaris* Schrad	1	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Mastsum. et Nakai var. *vulgaris* (Schrad) Fursa	16	synonym	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Mastsum. et Nakai var. *lanatus*	346	synonym	N	*C. lanatus* subsp. *lanatus*
Citrullus lanatus (Thunb.) Mansf.	294	no subspecies; authors	N; T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*

Citrullus lanatus (Thunb) Matsumura & Nakai	8	no subspecies; misprint	T	*C. amarus*/ *C. lanatus* s.l./ *C. mucosospermus*
Citrullus sp.	10	no species	T	?
Citrullus vulgairs Schrad	2	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus vulgaris Schrad. ex Eckl. & Zeyh.	48	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus vulgaris Schrad. ex Eckl.	1	synonym; misprint	N	*C. lanatus* subsp. *lanatus*
Citrullus vulgaris Schrad.	35	synonym	N	*C. lanatus* subsp. *lanatus*
Citrullus vulgaris L.	60	synonym; authors	N	*C. lanatus* subsp. *lanatus*

References not present in the “References” section of the main text:

Pedersen TT (2008) Transliteration of Arabic. http://transliteration.eki.ee/pdf/Arabic_2.2.pdf. Accessed 25 July 2017

Quattrocchi U (1999) CRC World Dictionary of Plant Names. Volume I A—C. CRC Press, Boca Raton, London, New York, Washington, D.C.

Ter-Avasyn DV (1966) Арбу кордофанский *Citrullus lanatus* Mansf. ssp. *cordophanus* Ter-Avan. [Kordofan Watermelon *Citrullus lanatus* Mansf. ssp. *cordophanus* Ter-Avan.] Bot Zhurn (Moscow & Leningrad) 51:423−42.

6. Discussion and conclusions

In the comparative germination studies under osmotic stress, the crop cultivars always germinated quicker and with higher germination percentages, this was to be expected since crops had been artificially bred to achieve a prompt, uniform germination after sowing even under non-optimal environmental conditions (Fuller and Allaby 2009).
Despite that, several ecological mechanisms to cope with marginal environments and drought stress were observed in the study taxa. In particular, *Ae. geniculata* Roth proved to be a drought tolerant species at germination stage, with one population that behaved similarly to the domesticated *Triticum* accessions. The differences in germination behaviour under drought stress among *Ae. geniculata* underlines that the characterisation of CWR for useful traits assessment should take into account the population-specific features of a CWR species. More in detail, the germination of the *Ae. geniculata* populations under the lowest water potentials inversely correlated with the rainfall during the period of seed maturation in the collecting sites. Therefore, it can be hypothesize that, in *Ae. geniculata*, plastic responses to the local environment mediated by parental effects could confer different germination responses to drought in the offspring.

While *Ae. geniculata* proved to be a drought tolerant species, the higher water requirement for germination of *Citrullus amarus* landraces could be due to a drought avoidance strategy to prevent seedlings emergence during dry conditions, unfavourable for establishment (Evans and Etherington, 1990; Gutterman 2000). This pattern, a common behaviour of wild dryland plants, could be related to patterns of wild species, which tend to germinate only under optimal conditions. Indeed, seed germination of the wild *C. amarus*, growing in desert areas, occurs during the short rainy season (McGregor 2012) and seedling emergence does not occur at the soil surface (Ramirez et al. 2014), a typical response of desert plants that prevent seedlings from drying out (Ren et al. 2002). Therefore, it is interesting to notice how the germination behaviour of *C. amarus* landraces is more similar to that of wild populations than that of crop cultivars. Moreover, *C. amarus* landraces showed a higher biomass growth under wet conditions when compared to the dry treatment. These plastic responses to different water treatments can be considered as adaptive traits allowing the *C. amarus* seedlings to maximise their growth in periods of good water availability. Several characteristics detected in *C. amarus*, such as the high vigour of C. amarus seedlings and their strong root systems, are all of potential interest in a rootstock line selection. Very recent researches confirmed the promising value of *C. amarus* as rootstock for *C. lanatus* cultivars (Fredes et al. 2017; Keinath et al. 2018).

Those observations on *Aegilops* and *Citrullus* confirm that CWR and landraces hold interesting adaptive traits towards climate change-driven abiotic stresses, in this case drought, some of which could be used to breed for drought resistant cultivars and water-use efficient rootstock lines (Cohen et al. 2007; Hajjar and Hodgking 2017).

The comparative germination experiment showed differences in the germination of seeds from different spikelets in *Aegilops*. Additionally, I discovered that almost all the species tested showed within-spikelet seed dimorphism, with one seed being always significantly heavier than the other, demonstrating that the presence of dimorphic seed pairs is a widespread characteristic in the genus *Aegilops*. Based on the results of controlled ageing tests (CAT) large seeds were shorter-lived and germinated faster than small seeds in most of the species. Consistently, field experiments with *Ae. neglecta* showed that large seeds of the dimorphic pairs germinated 3 months after dispersal in contrast to 14 months for small seeds. The delayed germination and high resilience to ageing showed by small seeds in *Aegilops* reinforce the hypothesis of a bet-hedging strategy to cope with environments with unpredictable resource availability and to increase the chances that at least some offspring survive, reducing also the competition among sibling seedlings (Dyer 2004). I am carrying out further researches to understand the biological basis of the dimorphism in seed longevity, correlating longevity estimates with antioxidant profiling and expression of antioxidant genes in the different seed morphs. CAT results are expected to be similar to those for seed lots under long-term storage, i.e. large seeds, that are identified as losing viability quicker than small seeds in the CAT, will also lose viability quicker during long-term conservation storage (Probert et al. 2009; Hay and Whitehouse 2017). This could have important implications for the correct conservation of *Aegilops* seed collections e.g. different seed morphs of *Aegilops* should be held separately in storage and their viability, regeneration or recollection intervals (Hay and Probert 2013) should follow different timings, in order to avoid important loss of genetic resources. Further researches, in collaboration with the genebank department of IPK Gatersleben (Germany), are ongoing, to clarify if and how the seed heteromorphy detected in *Aegilops* influences seed longevity under genebank storage conditions and therefore the long-term conservation of the different morphs.

Another limiting factor for the correct conservation and use of PGR proved to be taxonomic misnaming in PGR databases, with only 3% of *Citrullus* material correctly named. Additionally, 28% of the analysed accessions showed taxonomic errors (scientific names associated with the accessions that prevented the establishment of the real taxonomic identity of the accessions) that cannot be unequivocally attributed to any existing taxon. Consequently, the employment of those accessions in any research, breeding, cultivation, reintroduction or conservation project is corrupted by an erroneous taxonomic identification, causing their priceless genetic variability and the potential of their useful traits to be improperly exploited. The usage of those accessions can be recovered only by

means of re-propagation and re-determination, which enable the revision of the accession's taxonomic identity and its re-accessioning under the correct name. Moreover, the results of my propagation experiment showed unequivocally and in practical terms the existence of the problem of taxonomic identity. This great amount of taxonomic misnaming generates a great variability in the nomenclatural treatments adopted by the different genebanks and even by the same genebank, leading often to overestimations but also to underestimations of the taxa conserved in the conservation facilities. This aspect is particular relevant because it could diminish the importance of prioritization studies intended to unveil taxa that are currently underrepresented in genebanks and therefore which of them should be the target of collecting efforts (Maxted et al. 2010; Castañeda-Álvarez et al. 2016). I have proposed a series of actions to prevent the occurrence of large numbers of taxonomic issues with detrimental effects on PGR conservation and usage, in particular: I) perform similar studies on further crop genepools to better clarify the extent of the problem; II) establish taxonomic authorities in order to provide an updated and standardized taxonomic treatment that should be followed in the application of the accessions' scientific names within the databases; III) perform a peer review of the accessions' scientific names before their publication in databases; IV) link a herbarium voucher to each seed accession, in order to allow a quick taxon re-determination.
Finally, considering this great amount of issues in the taxonomic identities of seed accessions, the genetic fingerprinting of the conserved collections could be an important tool to quickly verify the identity of an accession (Simon et al. 2012).

Concluding remarks:

1) This study confirmed the assumption that landraces and CWR often show adaptive traits towards marginal conditions and abiotic stresses; in particular, drought avoidance was detected in *Citrullus* and drought tolerance and seed hetermorphy, connected to a bet-hedging phenology, in *Aegilops*.

2) The suitability of CWR and landraces for crop improvement must take into account ecological and population-specific approaches.

3) Seed heteromorphy, a characteristic found in several cereal genetic resources (e.g. in *Aegilops*, *Dasypyrum* (Coss. & Durieu) T. Durand, *Secale* L., *Triticum*,) can influence seed longevity. The impact of seed heteromorphy on the ex situ conservation of those genera still needs to be clarified.

4) A great amount of taxonomic misnaming is found in seed accessions conserved worldwide. This can have detrimental effects on the effective conservation and use of this material. Urgent actions are needed to solve the problem and prevents its reoccurrence.

References

Castañeda-Álvarez NP, Khoury CK, Achicanoy HA, Bernau V, Dempewolf H, Eastwood RJ,Guarino L, Harker RH, Jarvis A, Maxted N, Müller JV, Ramirez-Villegas J, Sosa CC, Struik PC, Vincent H, Toll J (2016) Global conservation priorities for crop wild relatives. Nature Plants 2:16022.

Cohen R, Burger Y, Horev C, Koren A, Edelstein M (2007) Introduced grafted cucurbit to modern agriculture. The Israeli experience. Plant Disease, 91, 916–923.

Dyer AR (2004) Maternal and sibling factors induce dormancy in dimorphic seed pairs of *Aegilops triuncialis*. Plant Ecology, 172, 211–218.

Evans CE, Etherington JR (1990) The effect of soil water potential on seed germination of some British plants. New Phytologist, 115, 539–548.

Fredes A, Rosellò S, Beltrán J, Cebolla-Cornejo J, Pérez-de-Castro A, Gisbert C, Picó MB (2017) Fruit quality assessment of watermelons grafted onto citron melon rootstock. Journal of the Science of Food and Agriculture, 97, 1646-1655.

Fuller DQ, Allaby R (2009) Seed dispersal and crop domestication: shattering, germination and seasonality in evolution under cultivation. Annual Plant Reviews, 38, 238–295.

Gutterman Y (2000) Environmental factors and survival strategies of annual plant species in the Negev Desert, Israel. Plant Species Biology, 15, 113–125.

Hajjar R, Hodgkin T (2007) The use of Wild Relatives in crop improvement: A survey of developments over the last 20 years. Euphytica, 156, 1-13.

Hay FR, Probert RJ (2013) Advances in seed conservation of wild plant species: a review of recent research. Conservation Physiology, 1, cot030.

Hay FR, Whitehouse KJ (2017) Rethinking the approach to viability monitoring in seed genebanks. Conservation Physiology, 5, cox009.

Keinath AP, Wetcher P, Rutther W, Agudelo P (2018) Cucurbit rootstocks resistant to *Fusarium oxysporum f.* sp. *niveum* remain resistant when co-infected by *Meloidogyne incognita* in the field. Plant Disease. https://doi.org/10.1094/PDIS-10-18-1869-RE.

Maxted N, Kell SP, Toledo Á, Dulloo E, Heywood V, Hodgkin T, Hunter D, Guarino L, Jarvis A, Ford- Lloyd B (2010) A global approach to crop wild relative conservation: securing the gene pool for food and agriculture. Kew Bullettin, 65, 561–576.

McGregor C (2012) *Citrullus lanatus* germplasm of southern Africa. Israel Journal of Plant Sciences 60, 403–413.

Probert RJ, Daws MI and Hay FR (2009) Ecological correlates of ex situ seed longevity: a comparative study on 195 species. Annals of Botany 104, 57–69.

Ramirez AHM, Jhala AJ, Singh, M (2014) Factors affecting germination of citronmelon (*Citrullus lanatus* var. *citroides*). Weed Science, 62, 45–50.

Ren J, Tao L, Liu XM (2002) Effect of sand burial depth on seed germination and seedling emergence of *Calligonum* L. species. Journal of Arid Environments, 51, 603–611.

Simon M, Simon A, Martins F, Boltran L, Tisnè S, Granier F, Loudet O, Camilleri C (2012) The Plant Journal, 69, 1094-1101.

7. Additional scientific production

Beside the aforementioned papers, during my PhD I published other four articles, dealing with germination ecology, ethnobotany and\or plant genetic resources conservation, published in international indexed journals:

I. Ardenghi N.M.G., Barcheri G., Ballerini C., Cauzzi P., **Guzzon F.** (2016) *Gymnocoronis spilanthoides* (Asteraceae, Eupatorieae), a new naturalized and potentially invasive aquatic alien in S Europe. Willdenowia, 46(2): 265-273.

Abstract: *Gymnocoronis spilanthoides*, a naturalized South American neophyte introduced as an aquarium and aquatic ornamental plant, is recorded for the first time from southern Europe. Two populations were found in irrigation canals and in a rice field in northern Italy. Distribution, invasion status, ecology, and pathways of introduction of the species are presented. Additionally, the modes of dispersal, never investigated before in the European continent, are discussed and the potential invasive behaviour and impacts in the new growing sites are assessed.

II. Orsenigo S., Abeli T., Schiavi M., Cauzzi P., **Guzzon F.**, Ardenghi N.M.G., Rossi G., Vagge I. Morphological characterization of *Cucurbita maxima* Duchesne (Cucurbitaceae) landraces from the Po Valley (northern Italy). Italian Journal of Agronomy (in press).

Abstract: Identifying crop genetic resources represent an important aspect of agricultural biodiversity conservation. However, conservation of landraces is challenging because they often cannot be properly identified, or have already suffered from genetic erosion, or have disappeared. Identification of landraces can be obtained using molecular markers or discriminating qualitative and quantitative morphological traits. The latter methodology is cheap and easily achievable, allowing the registration of landraces in national catalogues. In this study, we carried out a morphological characterisation of different accessions of *Cucurbita maxima* (Cucurbitaceae) cultivated in the Po Valley (N-Italy), locally known as Cappello da prete. The aim was to explore the morphological fruit variability among accessions and to identify potential distinct landraces within the Cappello da prete squash group. Differences between accessions were found indicating that morphological traits can be effectively used to identify these landraces and suggesting a diversification by isolation. Indeed, our morphological analysis shown the existence of two different landraces of Cappello da prete squashes. The adopted procedure can demonstrate that few low-cost traits are useful for the registration of local varieties in the official catalogue of landraces. Our study also demonstrates that morphological characterisation allows a rapid and cost-effective identification of diagnostic morphological traits that, together with historical and cultural information, are fundamental to recognise landraces.

III. Ardenghi N.M.G., Bodino S., Ballerini C., Cauzzi P., **Guzzon F.** (2017) “Lándar”, “Lándra”, “Barlánd” (*Bunias erucago* L.): a neglected crop from the Po Plain (Northern Italy). Economic Botany, Notes on Economic Planta 71(3): 288-295.

Abstract: This paper, through ethnobotanical interviews and the analysis of floristic, linguistic, and culinary literature, both historical and contemporary, identifies for the first time *Bunias erucago* L. as a “neglected crop” in a restricted area of north-western Italy. Here, unlike in other Italian and European regions where this plant is simply harvested from the wild, *B. erucago* is cultivated on a small scale, as it is a key ingredient of different traditional dishes.

IV. Ardenghi N., Rossi G., **Guzzon F.** (2018) Back to beaked: *Zea mays* subsp. *mays* Rostrata Group in Northern Italy, refugia and revival of open pollinated maize landraces in an intensive cropping system. PeerJ, DOI 10.7717/peerj.5123.

Abstract: Crop landraces are fundamental resources to increase the eroded genepool of modern crops in order to adapt agriculture to future challenges; plus, they are of immeasurable heritage and cultural value. Between the 1940s and the 1960s open-pollinated varieties (OPVs) of flint and semi-flint maize in Europe were almost completely replaced by high-yielding hybrid dent cultivars selected in North America. No comprehensive assessment was performed after the 1950s to understand which maize genetic resources survived genetic erosion in northern Italy, an area characterized by a high degree of landraces extinction and introgression, intensive hybrid dent monocultures, as well as being one of the hotspots of maize cultivation at a continental level. Among these landraces, beaked maize represents a peculiar case study for assessing the survival of OPVs in intensive cropping systems. By means of ethnobotanical and literature surveys, the history of *Zea mays* subsp. *mays* Rostrata Group and its current distribution were reconstructed. It emerged that beaked maize originated in the study area and it is one of the oldest genepools available not subjected to formal crop improvement.

We identified 28 landraces of beaked maize currently cultivated, 18 here recorded for the first time. The cultivation of more than half of the 28 landraces has continued throughout the last 80 years in a few fragmented localities that can be regarded as ``refugia". The survival of these landraces from substitution with high-yielding cultivars and unidirectional introgression has been mainly due to active on-farm conservation performed by custodian farmers and secondarily to cultivation in isolated areas (e.g., mountain valleys). After decades of genetic erosion, beaked maize has since the late 1990s experienced a revival, in terms of an increasing number of cultivation localities and the level of product commercialization. This process is mostly spontaneous and only occasionally mediated by governmental institutions; it is linked to the rediscovery of local food products, in this case mainly polenta, a dish made of corn flour, which used to be the staple food across northern Italy. The ex situ conservation of beaked maize and on-farm measures put in place by the farmers to prevent introgression are also assessed. Further research and collecting missions are needed to provide an inventory of open pollinated landraces of other landrace groups that have survived genetic erosion in Europe. To meet this aim, extensive ethnobotanical surveys, such as the one performed here, are very powerful tools in detecting these genetic resources.

Additionally, I presented my research activity through two oral communications in two international conferences:

I. **Guzzon F**, Rossi G, Ardenghi NMG (2018) Rediscover Traditional Food Crops in an Intensive Cropping System; Ethnobotany in Northern Italy:

In northern Italy, the Green Revolution led to a genetic erosion of higher than 90% and an almost complete shift from mixed subsistence farming units to intensive cereals monocultures. Through ethnobotanical surveys we discovered, collected and conserved endemic plant genetic resources (PGR) that survived genetic erosion, being linked to traditional food products once fundamental for food security. We recorded the cultivation by six elderly farmers of *Bunias erucago* L., a leafy vegetable actively cultivated only in the study area. We discovered 24 landraces of beaked maize (*Zea mays* L. subsp *mays* 'Rostrata Group'), a cultivar group locally originated and extremely appreciated until the 1930s for the production of polenta that was the staple food of the area. The cultivation range of beaked maize landraces shrank, due to substitution with hybrid cultivars and related introgression. Nevertheless, they are still cultivated in fragmented 'refugia', isolated from hybrids. Similarly, winter squash (*Cucurbita maxima* Duchesne subsp. *maxima*) landraces with a peculiar fruit morphology, locally known as “priest hat squashes”, cultivated in northern Italy since at least the XIX century, were maintained in gardens being used for typical receipts. We can safely state that: 1) Several neglected crops and landraces can still be found in northern Italy; 2) the role of custodian farmers was pivotal in their survival; 3) ethnobotanical surveys are powerful tools to detect overlooked genetic resources; 4) some of these PGR are still threatened while others are experiencing spontaneous revival processes due to the rediscover of traditional foods.

Presented at the Joint Conference of the Society of Ethnobiology & Society for Economic Botany Food Security, Sovereignty, & Traditional Knowledge 3–7/6/2018, University of Wisconsin-Madison, Madison, Wisconsin, USA.
My participation to the conference was supported by a travel grant of the National Science Foundation of the USA (NSF), grant number: BCS-1822297.

II. **Guzzon F**, Gianella M, Balestrazzi A, Orsenigo S, Pagano A, Rossi G, Mondoni A (2018) Seed heteromorphy influences seed longevity in Aegilops (Poaceae):

The genus *Aegilops* belongs to the secondary genepool of wheat and has a great importance for wheat improvement. *Aegilops* spikelets produce dimorphic seed pairs: seeds are different in both size and germination patterns. We tested seed longevity through controlled ageing tests (CAT), in different seed morphs of five *Aegilops* species. Additionally, the germination phenology in *Ae. neglecta* was investigated using lab and field experiments.
Bigger seeds were shorter-lived and germinated faster than smaller seeds in most of the species. Consistently, field experiments with *Ae. neglecta* showed that bigger seeds of the dimorphic pair germinated soon after dispersal, while smaller seeds germinated a year later, remaining fully viable in the soil.
Our results indicate that different longevities in seed morphs of *Aegilops* may reflect a different soil seed bank persistence, with smaller seeds having higher resilience to ageing than bigger seeds to maintain high viability after dispersal, thereby spreading seedling emergence over two years.
Differences of seed traits detected here between seed morphs in *Aegilops* have important implications for the ex situ seed conservation and reinforce the hypothesis of a bet-hedging germination strategy in the genus.

Finally, we integrated CAT with assessments of antioxidant potential and total phenolic compounds in six *Aegilops* and *Triticum* species in 18 different seed morphs; it emerged that the general antioxidant activity was significantly higher in the smaller seeds of most of the species. Expression of antioxidant genes will be assessed using quantitative Real-Time PCR analysis.

Presented at 2nd Seed Longevity Workshop 30/7-1/8 2018 USDA Agricultural Research Service (ARS), Colorado State University (CSU), Fort Collins, Colorado, USA.

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Finally I am deeply indebted to the dozens farmers, genebank curators, researchers and botanists from all over the world with which I went in contact in these three years and that, many time unconsciously, have provided me with essential inputs to carry out my research. All this disinterested aid makes me thing that a united PGR community can really achieve outstanding goals for a more sustainable and fair world.

Grazie