Genetic erosion in crop wild relatives: wild barley, *Hordeum vulgare* subsp. s*pontaneum,* a case study in Jordan

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1 GENERAL INTRODUCTION

The number of known vascular plant species is currently estimated to be 391,000 (Kew 2016). Of these about 8 %, or at least 31,128 have a documented use for humans, animals or the environment. About 5,500 are known to be used as human food, another 5,300 are reported as gene sources potentially useful in the genetic improvement of crops (Kew 2016). Humans however rely heavily on a very small number of useful plants. Just three – rice, wheat and maize – provide more than 50% of the world's plant-derived calories used by humans. Only twelve crops and five animal species provide 75% of the world's food. Most of the plant species that have been domesticated and gone through selection and breeding to achieve higher yields and desired qualities, have gone through major genetic bottlenecks (Tanksley and McCouch 1997). These have reduced the genetic diversity in their gene pools, which constrains the possibilities of crops to adapt to new or changing environments or to expand the range of their cultivation (Warschefsky et al. 2014). The loss of local varieties can further put the resilience and adaptive capacity of crops and the agricultural ecosystems in which they grow at risk. Farmers and breeders require more genetic diversity to widen the genetic base of crops and adapt them to changing environmental and climatic conditions (FAO 2010). The wild species related to our crops have increasingly been used as resource for this genetic diversity (Hajjar and Hodgkin 2007; Maxted and Kell 2009). These crop wild relatives (CWR) though are affected themselves by genetic erosion and climate change and need to be better conserved (Thuiller et al 2005; Jarvis et al 2008; FAO 2013). The present study is focused on the diversity in these wild relatives and its change over time, using as case study the primary wild relative of barley, *Hordeum vulgare* subsp. *spontaneum* (K. Koch) Thell., one of the first cereal grains that were domesticated and cultivated.

1.1 Plant genetic resources for food and agriculture and crop wild relatives

Any genetic material of plant origin of actual or potential value for food and agriculture are defined and commonly referred to as Plant Genetic Resources for Food and Agriculture (PGRFA) (FAO 2009). PGRFA are the raw material indispensable for crop genetic improvement and are essential in adapting to unpredictable environmental changes and future human needs. The genetic diversity in domesticated plants and their wild relatives has been used since millennia by farmers and breeders to develop and improve landraces and varieties

(Esquinas-Alcazar 1993; McCouch et al. 2013; Bertoldo et al. 2014). They have used the genetic diversity to increase crop yields, improve desired qualities (e.g. nutritional or agronomic) and enhance tolerance to biotic stresses, such as pests and pathogens, and abiotic stresses, such as drought, heat or salinity.

The first global program focused on PGRFA and specifically on their collection and conservation, was coordinated by the International Board for Plant Genetic Resources[1] (IBPGR), founded in 1974. The high importance of PGRFA for food security is today recognized at global level by a number of international agendas, of which two specifically focus on conservation and sustainable use of PGRFA: the International Treaty on PGRFA (ITPGRFA) and the second Global Plan of Action for PGRFA (GPA2). The ITPGRFA provides a legal framework that facilitates the exchange and conservation of crop genetic resources amongst member nations, as well as the fair and equitable sharing of the benefits arising out of their use. The GPA2 lays out a series of agreed priority plans and activities that address conservation and sustainable use of PGRFA and capacity building. Both, the ITPGRFA and the GPA2 highlight the ongoing loss of genetic diversity and the need to develop actions to reduce future genetic erosion. The GPA2 addresses genetic erosion specifically in its activity 16, which proposed the *"developing and strengthening systems for monitoring and safeguarding genetic diversity and minimizing genetic erosion of plant genetic resources for food and agriculture and rules to halt the loss of genetic diversity"*. Although not specifically focused on PGRFA but on all biodiversity, the Convention on Biological Diversity (CBD) recognizes the threat of genetic erosion in PGRFA and addresses it specifically in target 13, *"by 2020, the genetic diversity of cultivated plants and farmed and domesticated animals and of wild relatives, including other socio-economically as well as culturally valuable species, is maintained, and strategies have been developed and implemented for minimizing genetic erosion and safeguarding their genetic diversity"*. This target 13 explicitly mentions CWR, as do also the ITPGRFA in article 5.1d, and the GPA2 in its activity 4, where they call for promoting *in situ* conservation of crop wild relatives and wild food plants.

A CWR is defined as a wild plant taxon that has an indirect use derived from its relatively close genetic relationship to a crop (Maxted et al. 2006). Hajjar and Hodgkin (2007) reviewed the

[1] The IBPGR became the International Plant Genetic Resources Institute (IPGRI) in 1994 and is operating today as Bioversity International (www.bioversityinternational.org).

use of CWR in crop improvement and found a steady increase in the rate of release of cultivars containing genes from CWR. They also found that the range of characteristics used has widened from a strong focus on pest and disease resistance genes to drought and salt tolerance, improved quality and cytoplasmic male sterility. This trend was confirmed by Maxted and Kell (2009), who provide an extensive list of examples of uses of CWR in crop improvement programmes for 14 major food crop genepools. Farmers often tolerate the presence of CWR in their fields and in home gardens because they recognize the value of these species in providing beneficial traits to their crops (Hoyt 1988; Engels 2001; Hughes et al. 2007; Galluzzi et al. 2010).

Based on a very broad working definition of CWR, i.e. any taxon belonging to the same genus as a crop, the total number of CWR taxa existing in the world was estimated to be between 50,000 to 60,000 species. A more precise working definition of a CWR taxon is based on the gene pool (Harlan and de Wet 1971) or taxon group concept (Maxted et al. 2006). Applying these concepts and focusing on the closely related CWR taxon, the number of these CWR taxa is estimated to be about 700 globally (Maxted and Kell 2009). National, regional and global inventories of CWR taxa are being developed (for a review see Dulloo et al. 2015), which are used to set conservation priorities, but data on amount and distribution of genetic diversity harboured in these species and extent of genetic erosion are still mostly unavailable.

1.2 Genetic erosion in crop wild relatives

The term genetic erosion was initially related mainly to cultivated plant species, in particular crop landraces, and referred to loss of landraces caused by their replacement with improved varieties (Baur 1914; Bennett 1968; Brush 2004; Thormann and Engels 2015). This use in the broad sense of loss of landraces or species with time evolved to define the loss rather than in numbers of landraces or species, in terms of intraspecific genetic variation or specific alleles or gene complexes. The FAO technical meeting on the ‘Methodology of the World Information and Early Warning System on Plant Genetic Resources (WIEWS)’ held in Prague in 1999 agreed on a working definition of genetic erosion: "A permanent reduction in the number, evenness and distinctness of alleles, or combinations of alleles, of actual or potential agricultural importance in a defined geographical area" (Serwinski and Faberova 1999). Maxted and Guarino (2006) further generalized the definition into the “permanent reduction in richness (or evenness) of common localized alleles or the loss of combination of alleles over time in a

defined area". They included the aspects of local adaptation and dynamics of diversity in time. Generally, the definitive reduction in diversity needs to be distinguished from the normal addition and disappearance of genetic variability over time in a population (Brush 1999; Brush 2004). With the development of the CBD, the Global Strategy for Plant Conservation (GSPC) and the Aichi biodiversity targets of the CBD's strategic plan 2011 – 2020, the concept of genetic erosion has been extended to biodiversity more widely (Rogers 2004). Whether genetic erosion is considered within the context of agricultural diversity or of natural populations, it usually refers to losses in genetic diversity caused by human-driven or related activities, as these losses are faster in rate or extension than one would expect under natural conditions alone.

The broad range of factors affecting genetic diversity and the inherent temporal component render genetic erosion a complex phenomenon. No coherent set of methods and indicators are yet available to assess its dimensions, and the picture that emerges from existing studies is complex. The range of causes leading to loss and the extent of loss vary, even for the same crop, by geography, national policy environment and agricultural system. Published records about genetic erosion in wild plant species and CWR are very few compared to studies that address genetic erosion in landraces and cultivars (Thormann and Engels 2015). Studies on CWR of major staple crops appear to have addressed mainly rice wild relatives. Several studies report high levels of threat to and extinctions of populations of wild rice (*Oryza rufipogon* Griff., *O. officinalis* Wall. ex G. Watt, *O. granulata* Nees & Arn. ex G. Watt, *O. perennis formosana*) in Asia (Kiang et al. 1979; Morishima and Oka 1995; Akimoto et al. 1999; Gao et al. 2000; Gao 2003) due to invasive species and environmental changes caused by rapid population growth, new agricultural technologies, economic and cultural changes. Populations of African wild rice (*O. barthii* A. Chev., *O. brachyantha* A. Chev. & Roehr., *O. eichingeri* Peter, *Oryza longistaminata* A. Chev. & Roehr., *O. punctata* Kotschy ex Steud) were found threatened by land use change resulting from increasing population pressure that destroyed natural wild rice habitats converting them into agricultural land, and overgrazing (Kiambi et al. 2005).

Several wild *Arachis* species in Latin America were found threatened by extinction based on highly restricted distribution ranges and land use pressures (Jarvis et al. 2003). Wild fruit tree diversity (*Olea europaea* L. var. *oleaster* Hoffmgg. et Link., *Pyrus pyraster* Burgsd., *Pyrus amygdaliformis* Vill.) decreased over the past two decades of the last century on the Italian island Sardinia, due to degradation of the natural environment. Frequent summer fires and

intensive exploitation greatly altered vegetation cover and many genotypes of the crop wild relatives disappeared (Chessa and Nieddu 2005). Ipecac (*Psychotria ipecacuanha* (Brot.) Stokes), an endangered medicinal plant native to the Atlantic rainforest in Southeastern Brazil is mainly threatened by the short distance of plant populations from inhabited areas and poor conservation status of plant populations (De Oliveira and Martins 2002).

1.3 Re-collection and resurrection approaches to assess temporal variation in genetic diversity

Genetic erosion is quantified as the proportion of richness of genetic diversity that no longer exists in current populations when compared with historic populations or that is predicted to be lost in the near future if no remedial measures are taken (Brown 2008). Given this temporal dimension, the genetic diversity needs to be measured in a comparable way in the same geographic space in at least two different time points in order to verify whether genetic erosion has occurred and to estimate its extent. Research on genetic erosion therefore demands time series data which is not always readily and easily available.

Projects such as 'Project Baseline' (Etterson et al. 2016) build a contemporary baseline by establishing a research seed bank for the scientific community for future research. Over 800 populations from 61 wild plant species across a broad geographical range within the USA have been collected from sites that are likely to be preserved into the future, to constitute this baseline. Seeds are stored by maternal lines and will be available over the coming half century to study changes in genetic diversity and evolution under climate change in wild plant populations across time and space using the resurrection approach (Franks et al. 2014): Genotypes from the baseline collection will be grown in a common garden together with seeds that will be re-collected in the same sites, for comparison of ancestors with descendants under common conditions. A growing number of studies has used historical biological specimens from collections conserved in natural history museums, herbaria, botanic gardens and universities and compared with contemporary specimen for studies on population genetics, evolutionary changes or climate change impact (Miller-Rushing et al. 2006; Wandeler et al. 2007; Dosmann and Groover 2012; Vellend et al. 2013). For example, the analysis of herbarium specimens collected from the same geographical region over decades has revealed the impact

of climate change on flowering trends (Primack et al. 2004; Gallagher et al. 2009; Calinger et al. 2013; Li et al. 2013).

Genebanks and plant germplasm collecting missions are a source for baseline data to monitor genetic diversity and erosion in PGRFA, particularly in landraces and CWR (Maxted and Guarino 2006; Franks et al. 2008; van de Wouw et al. 2010; Thormann et al. 2015). They have the added advantage that samples were collected to conserve the then existing diversity, hence samples are larger than museum, herbaria or botanic garden collection samples, which often conserve only one or few individuals of the taxon collected. The collecting missions supported by IBPGR and its successors International Plant Genetic Resources Institute (IPGRI) and Bioversity International represent a particularly useful resource for the study of CWR, as 27% of the collected material were CWR (Thormann et al. 2015). During more than 1,000 collecting trips 226,618 samples of landraces and CWR were collected in 136 countries. Collecting took place from 1975 – 2012, however 90 % of the samples were collected during the first two decades. The IBPGR employed professional collectors as well as some crop specialists to implement the collecting program. A systematic approach to conserve genepool diversity was used that included careful targeting of collecting sites, systematic sampling, the detailed recording of passport data, and general reporting about the collecting trip, which could include additional data about sites and their environments. The material collected was subsequently sent to genebanks for long-term conservation. The original passport data have been extracted from the historical documentation and made available online through the Bioversity Collecting Database (BCD) together with the original reports and collecting sheets (Thormann et al. 2012). The BCD represents therefore a unique historical resource of documented plant collections that can support the establishment of baselines for assessment of extent and drivers of genetic erosion (Thormann et al. 2015).

Two approaches have been used in the study of genetic erosion in PGRFA, i.e. temporal and spatial comparisons. Spatial comparisons investigate populations at the same time in different geographies and environments that are different for one or few key characteristics, e.g. fragmented versus non-fragmented habitats or disturbed versus undisturbed situations. Here space is used as surrogate for time. Brush (1992) for example compared traditional potato diversity in two Peruvian valleys with contrasting levels of modernization and commercialization based on farmer household surveys.

Temporal comparisons are carried out either directly, or indirectly using proxies. Indirect temporal comparison is used where germplasm from earlier times is not available. Here the observations of the current situation are compared with passport data, published observations and/or indigenous and expert knowledge about the situation at the earlier time, e.g. numbers of sub-specific entities, such as landraces, or numbers and size of wild population (Hammer et al. 1996; Hammer and Laghetti 2005; Peroni and Hanazaki 2002; Willemen et al. 2007; Keiša et al. 2008; Davari et al. 2013; Megersa 2014). Historical data can be sourced from formal literature, grey literature (e.g. plant germplasm exploration reports, field books, reports of extension departments), and from experts, farmers and local people.

Direct comparison is possible when germplasm originating from two different time points from the same locations is available (e.g. Akimoto et al. 1999; Gao et al. 2000). Several assessments of genetic erosion in genebank material have been carried out comparing accessions of a specific crop that have been regenerated varying numbers of times within the same genebanks, and a large number of studies have investigated the impact of breeding on genetic diversity of cultivars, using varieties released in different time periods, often grouping them by decades (see Thormann and Engels (2015) for a review of studies on genetic erosion in genebanks and in modern varieties). Khlestkina et al. (2004, 2006) used genebank accessions repeatedly collected from the same regions over time to study variation in genetic diversity in barley and wheat landraces. For comparison with contemporary diversity *in situ* in CWR or on-farm in landraces, germplasm is re-collected from sites that had been collected earlier. The diversity captured in the historical material is resurrected growing side by side with the contemporary samples re-collected from the same sites for direct comparison of genotypic and phenotypic diversity of stored and re-collected seed samples (Davis et al. 2005; Barry et al. 2008; Franks et al. 2008; Bezançon et al. 2009). This approach provides also the necessary material to study evolutionary responses and their genetic basis in specific adaptive traits (see e.g. Franks et al. 2007; Nevo et al. 2012; Vigouroux et al. 2011).

The re-collection and resurrection approach requires as a minimum the availability of quality passport data necessary for collecting site identification and the availability of historical material. Information about sampling strategies adopted at the earlier collecting time will allow to apply comparable sampling methods during re-collecting. Re-sampling studies have been carried out for wild potato species in Arizona and New Mexico (del Rio et al. 1997) and for

various crops in Albania and southern Italy (Hammer et al. 1996). The specific activities to implement the re-collection and resurrection approach for a CWR species can be outlined as follows:

1) *Selection of the historical germplasm collecting mission*: The historical collecting needs to fulfill some requirements in order to represent a meaningful baseline: Samples should cover a meaningful territory in terms of extension and ecogeographical variation. The existence and availability of historical seed material needs to be verified. The descriptions of the collecting sites need to be of sufficient detail and quality to allow—with the support of appropriate software such as Google Earth, gazetteers etc.—to validate quality of location data and to georeference sites if necessary.

2) *Re-collecting*: Permission needs to be obtained from relevant authorities to allow collecting. The original collecting sites should be revisited possibly in the same period of time as in the original collecting. Available information about original sampling strategy should be considered when deciding about sampling sizes and strategy for re-collecting. Any available additional historical information about sites, size of populations and abiotic and biotic conditions that can help interpretation of diversity analyses should be recorded also during re-collection.

3) *Comparison of genetic diversity pattern within and among time points and estimation of change*: The genetic variation within original and re-collected samples is determined with molecular markers. If resurrected and re-collected samples are reared in a common garden, also phenotypic variation can be assessed. The overall diversity pattern within each collecting time can be compared as well as variation within single sites to identify changes in diversity over time.

4) *Identification of potential drivers of change*: While visiting sites, observed factors that affect collected populations can be recorded, and further socio-economic data e.g. about recent land use changes, planned constructions etc., be obtained from local agencies and organizations. Availability of climate data for the study period is required to identify any potential influence of climate change on observed changes in diversity patterns.

1.4 Wild barley diversity, conservation and utilization in barley breeding

The grass genus *Hordeum* (Poaceae) consists of 45 taxa (32 species) and is distributed in temperate and dry regions of the world (von Bothmer et al. 1995; von Bothmer et al. 2003). The genus is morphologically characterized by three single-flowered spikelets (triplets) at each rachis node of the inflorescence. The lateral spikelets are usually either sterile like in 2-row barleys or fertile like in 6-row barleys (von Bothmer et al. 2003). Some *Hordeum* species are annuals with relatively high percentages of inbreeding, while the majority is perennial with varying reproductive systems. The genus is very widespread and four centers of species diversity are recognized (von Bothmer et al. 2003): southern South America with 15 species, western North America (7 species), the Mediterranean (4 species) and Central Asia (3 species). Barley, *Hordeum vulgare* L., is the only cultivated species in the genus. It was one of the first domesticated cereal grains (Zohary and Hopf, 2000) and is of major economic importance today. Barley is the fourth most important cereal crop worldwide in terms of production, yield and area harvested (FAOSTAT). It is cultivated in about 100 countries, from the high plateaus in Tibet to sea level, from Norway down to Chile, often growing in places where other crops do not thrive (Harlan 1995). It is used to feed livestock, as human food, and is malted for beer or whisky production. The Fertile Crescent has been considered the primary center of origin and domestication of barley (Badr et al. 2000; Zohary and Hopf 2000). It is an arc of agricultural diversity that encompasses parts of Northern Palestine, Lebanon, West Syria, South East Turkey, Caucasus and North West Iraq, Iran and Jordan and is well established as the cradle of agriculture origin (Damania 1998). Two Vavilov centres (Asia Minor and Mediterranean center) of crop origin abut (Vavilov 1926) in the Fertile Crescent, and globally it is the region with the highest concentration of CWR species per unit area (Vincent et al. 2013). Several studies report polyphyletic origins of barley (Zohary 1999; Azhaguvel and Komatsuda 2007; Fuller et al. 2012; Allaby 2015; Poets et al. 2015) and suggest additional domestication events of barley in areas east of the Fertile Crescent (Morrell and Clegg 2007), Tibet (Dai et al. 2012), Ethiopia and the Western Mediterranean (Molina-Cano et al. 2005; Orabi et al. 2007).

Wild *Hordeum* species are widely distributed in the northern hemisphere, in South Africa and in southern South America. *Hordeum vulgare* subsp. *spontaneum* (K. Koch) Thell. (hereafter referred to as Spontaneum), is considered the progenitor of cultivated barley and is part of the primary *Hordeum* genepool according to the genepool concept developed by Harlan and de Wet (1971). Members of the primary genepool are usually within the same species and intermate

freely. Cultivated barley and Spontaneum are both classified as subspecies in the species *Hordeum vulgare* and gene flow occurs naturally between them without any breeding barriers (Brown et al. 1978; Jaradat 1989a; Russell et al. 2011; Hübner et al. 2012; Jakob et al. 2014). The secondary genepool—which comprises those wild and weedy forms that have a comparatively good crossability with their cultivated relative, but which is affected by some sterility factors—includes as only species *H. bulbosum* L. All other wild *Hordeum* species are part of the tertiary genepool.

Based on data available in Genesys (Gateway to genetic resources; https://www.genesys-pgr.org), EURISCO (European catalogue of genebank collections; http://eurisco.ipk-gatersleben.de) and WIEWS (FAO's World Information and Early Warning System; http://www.fao.org/wiews-archive/), Spontaneum germplasm is conserved *ex situ* in 24 genebanks with over 16,000 accessions. The five largest collections are held at the Israeli Lieberman Germplasm Bank (6,963), the Canadian Plant Genetic Resources Center (3,787), the International Center for Agricultural Research in Dry Areas ICARDA (1,809), the United States National Small Grains Germplasm Research Facility (1,603) and the German Federal Genebank (949). Most accessions originate from Israeli populations (66%). Other countries of origin are Syria (9%), Turkey (6%) and Jordan (5%) and Iran (3%).

In situ conservation of wild *Hordeum* is reported for the Ammiad reserve in Israel (Vincent et al. 2013). Maxted and Kell (2009) recommended in their report about the 'Establishment of a Global Network for the *In Situ* Conservation of Crop Wild Relatives' that although Spontaneum is a widespread and locally common species (von Bothmer et al. 1995), individual populations are likely to harbour important adaptive traits and selected populations should be actively conserved throughout their geographically and topographically range. Furthermore it should be included in monitoring activities at the national level throughout its range, both within and outside protected areas. The recent wild *Hordeum* gap analysis conducted by Vincent et al. (2013) suggested the establishment of a network of several reserves in the Israel/Jordan region, complemented by additional sites in Europe and Asia, to more effectively conserve the genetic diversity of Spontaneum.

Efforts have been made since the 1970s to characterize Spontaneum germplasm diversity across its distribution range with several biochemical and molecular markers, such as isozymes (e.g. Nevo et al. 1979; Jaradat 1992; Jana and Pietrzak 1988; Volis et al. 2001; Liu et al. 2002), long

primer PCR (Liviero et al. 2002), RAPDs (e.g. Dawson et al. 1993; Baum et al. 1997), AFLPs (e.g. Pakniyat et al. 1997; Turpeinen et al. 2003; Ozkan et al. 2005; Vanhala et al. 2004), RFLPs (Neale et al. 1986; Zhang et al. 1993), SSRs (Saghai-Maroof et al. 1994; Turpeinen et al. 2001; Baek et al. 2003; Ivandic et al. 2002; Hübner et al. 2009; Hübner et al. 2012; Fu and Horbach 2012; Shakhatreh et al. 2016), SNPs (Cronin et al. 2007; Yan et al. 2009; Russell et al. 2011; Hübner et al. 2012; Russell et al. 2014), rDNA (e.g. Gupta et al. 2002; Sharma et al. 2004), (multilocus) sequence data (Lin et al. 2001; Morrell et al. 2005; Jakob et al. 2014). A number of studies focused on phenotypic analysis (Nevo et al. 1984; Jaradat 1989b; Volis et al. 2000; Volis et al. 2002a; Al-Saghir et al. 2009; Shakhatreh et al. 2010). These studies, carried out at varying geographical scales, focused on assessing the amount of genetic diversity in Spontaneum, comparing diversity between wild and cultivated barley, describing the geographical distribution of diversity across the Fertile Crescent and beyond, and investigating the relationship between allelic diversity and ecogeographical variables. Studies concord that genetic diversity found in wild barley is significantly higher than in its cultivated form (Saghai-Maroof et al. 1994; Kilian et al. 2006; Jakob et al. 2014). Studies investigating the diversity across a broad geographical range furthermore concord that the highest genetic variation, characterized also by a substantial number of unique and locally common alleles, lies within the Fertile Crescent, specifically in Israel and Jordan (Fu and Horbach 2012; Jakob et al. 2014). Geographic pattern of diversity at single loci were reported to correlate with temperature or rainfall gradients (e.g. Nevo et al. 1979; Turpeinen et al. 2001; Baek et al. 2003; Batchu et al. 2006). Observed genetic differences in Spontaneum populations located on opposing slopes in the Evolution Canyon in Israel were attributed to adaptation to different microclimates (Yang et al. 2009; Nevo 2014).

Wild *Hordeum* species have been used extensively in research and breeding. The wild relative providing the largest source of genetic diversity for a range of traits is Spontaneum. It represents an important genetic resource for research and breeding on disease resistance traits such as powdery mildew, leaf scald or leaf rust resistance (Fischbeck et al. 1976; Ivandic et al. 1998; Backes et al. 2003; Dreiseitl and Bockelman 2003; Genger et al. 2003; von Korff et al. 2005; Repkova et al. 2006; Steffenson et al. 2007; Schmalenbach et al. 2008), drought and temperature tolerance (Chen et al. 2008; Lakew et al. 2013), yield (von Korff et al. 2006; Schmalenbach et al. 2009), malting quality (Erkkila et al. 1998; von Korff et al. 2008; Schmalenbach and Pillen 2009) or research on the genetics of plant development and yield

formation (Wang et al. 2010; Naz et al. 2014; Maurer et al. 2015; Maurer et al. 2016). Disease resistances have also been identified in the secondary and tertiary genepool. *H. bulbosum* was found to have resistance to Russian wheat aphid *Diuraphis noxia* (Kindler and Springer 1991), powdery mildew *Blumeria graminis* f. sp. *hordei* (Jones and Pickering 1978; Szigat and Pohler 1982; Gustafsson and Claësson 1988; Xu and Snape 1988; Xu and Kasha 1992; Pickering et al. 1995; Pickering and Johnston 2005), mosaic virus (Walther et al. 2000; Ruge et al. 2003; Ruge-Wehling et al. 2006), leaf rust *Puccinia hordei* (Walther et al. 2000), and *Septoria passerinii* (Toubia-Rahme et al. 2003). Two species in the tertiary genepool, *H. chilense* Roemer & Schultes and *H. brevisubulatum* (Trin.) Link subsp. *violaceum* Boiss. & Hohen have shown resistance to barley leaf rust (Patto et al. 2001) and Russian wheat aphid (Kindler and Springer 1991).

1.5 Jordan as a case study

Jordan, officially the Hashemite Kingdom of Jordan, is a Middle East country located at the south western end of the Fertile Crescent. In the north it adjoins to Syria, in the south and southeast to Saudi Arabia, to Iraq in the east, and Israel and Palestine in the west. The country is landlocked except for a 26 kilometer long access to the Gulf of Aqaba. The area of Jordan is about 89,300 square kilometers, of which over 80% are semi-arid and arid areas. Altitude ranges from -400 m at the surface of the Dead Sea up to 1,750 m in the southern highlands.

The climate varies among regions from semi-humid Mediterranean conditions, with more than 500 mm of rainfall, to desert conditions with less than 50 mm, over only 100 km distance. Rainfall occurs in the period from November to March, with a maximum in January, but with large variability between and within regions (Tarawneh and Kadioglu 2003). Jordan is one of the countries with the lowest per capita water supply globally (Al-Qinna et al. 2011). Frequent drought periods occurring in an irregular manner are reported for the years 1970 to 2005 and drought severity, magnitudes and life span increased with time from normal to extreme levels (Al-Qinna et al. 2011). Freiwan and Kadioglu (2008), analyzing climate data from 2000 back to the mid-1920s, identified trends in temperature and precipitation changes. They observed an increase in maximum and minimum temperature. The increase in minimum temperature was more pronounced, resulting in a decreasing daily temperature range. Precipitation showed a statistically not significant decreasing trend. Global climate models are reported to predict the

mean forecasted rainfall quantities to decrease significantly by an average factor of 10.5% until 2040 (Al-Qinna et al. 2011).

The flora of Jordan is rich and diverse comprising over 2,600 vascular plant species (Ghazanfar et al. 2015). It has an important number of wild species of socio-economic importance, including many CWR (Magos Brehm et al. 2016). The Fertile Crescent has in fact been identified globally as the region with the highest concentrations of CWR species per unit area (Vincent et al. 2013). Wild plant diversity is reported to be at risk of genetic erosion in Jordan due to factors such as population pressure, development and urbanization, invasive species, overgrazing, land use legislations and climate change and a national strategy has recently been developed to protect and conserve the wild diversity, focusing specifically on wild socioeconomically important species (Magos Brehm et al. 2016).

Spontaneum is widely distributed across the country and occurs on roadsides and field margins as well as in protected areas (Jana and Pietrzak 1988; Damania 1998; Abdel-Ghani et al. 2004). In addition to Spontaneum, wild species of the secondary and tertiary *Hordeum* genepool occur in Jordan, which are *Hordeum bulbosum* L., *Hordeum marinum* Huds. and its subspecies *gussoneanum* (Parl.) Thell. and *marinum*, and *Hordeum murinum* L. and its subspecies *glaucum* (Steud.) Tzvelev and *leporinum* (Link) Arcang. (von Bothmer et al. 1995).

Spontaneum diversity in Jordan has been studied by Jaradat (1991, 1992), Baek et al. (2003), Sharma et al. (2004), Al-Saghir et al. (2009), Shakhatreh et al. (2010, 2016). All studies revealed high variability in Spontaneum populations. A recent study carried out by Fu and Horbach (2012) on the development of a core subset of the world's largest wild barley collection held at PGRC, found the accessions from Jordan and Israel to be the most diverse genetically. Tyagi et al. (2011) studied seedling vigor in Spontaneum and accessions from the southwestern part of the Fertile Crescent. Two Jordanian and one Turkish accession exhibited the highest positive values for most of the plant vigor traits that were investigated. Baek et al. (2003) studied diversity in Spontaneum in Jordan using the same SSRs that previously had been used by Turpeinen et al. (2001) for study of Spontaneum populations from Israel. While genetic diversity estimates were very similar for both countries, and both studies found associations between ecogeographical variables and gene diversity for a number of loci, the number of unique alleles found in Jordan was notably higher than that found in the Israeli populations. Also rDNA studies found higher genetic diversity in Jordanian populations compared to

populations sampled in Israel (Sharma et al. 2004). Jana and Pietrzak (1988) found both wild and cultivated barleys in Jordan to be a richer source of genetic diversity than those from Turkey.

Barley is an important staple crop in Jordan, and is the predominant crop grown in areas with less than 300 mm of annual rainfall, which are characterized by high inter-seasonal and intra-seasonal variation in amount and distribution of rainfall. In these areas barley is mainly grown as animal feed and both the grain and the straw are utilized (Al-Tabbal 2012). Considering the critical water supply situation in Jordan and the forecasted further reduction of precipitation in the coming decades, barley is likely to remain a critical resource for farmers. Given the high genetic diversity in Spontaneum from Jordan and its general importance for barley improvement, this wild relative represents an important resource for breeders and researchers in Jordan. The recent development of the Jordanian national strategy for economic wild plant conservation has in fact identified the need to further assess distribution of genetic diversity and vulnerability of important CWR, including barley wild relatives (Magos Brehm et al. 2016). And as mentioned above, given the high diversity found in Jordan and Israel, the establishment of a network of several reserves in the Israel/Jordan region was suggested by Vincent et al. (2013). Furthermore, the number of *ex situ* accessions originating from Jordan is rather low when compared with accessions from neighboring countries like Israel and Syria, and as indicated by Vincent et al. (2013), suggests the need for additional collecting.

1.6 Purpose of the study

The purpose of the study was to assess the current pattern of diversity and its changes over time *in situ* in the primary wild barley relative *Hordeum vulgare* subsp. *spontaneum* (K. Koch) Thell. in Jordan, which is located in the center of diversity of this species, and where cultivated barley plays a critical economic role, especially in marginal areas.

The specific objectives of the study were the following:

1. Apply the re-collection and resurrection method to study CWR temporal variation, here in Spontaneum, using the IBPGR collecting documentation as resource to identify and describe the historical collecting used as baseline, to retrieve seed samples from the identified collecting in genebanks, and to locate collecting sites for re-collection;

2. Determine the current amount and distribution of genetic diversity of Spontaneum in Jordan, based on the collecting of maternal lines during the re-collection;
3. Assess the contemporary and past amount and pattern of diversity in the collections, and investigate extent, direction and potential drivers of changes.

2 ORIGINAL PAPERS

The present thesis comprises three papers. The first paper (Thormann et al. 2015), presented in chapter 2.1, relates to the first specific objective of the study and discusses the potential research options that past germplasm collections offer to investigate changes in diversity in CWR over time. It discusses specifically the collections carried out by the IBPGR, which include the *Hordeum* collection carried out in Jordan in 1981 that has served as baseline for the present case study on re-collection of Spontaneum germplasm from the same sites in Jordan for genetic erosion assessment. The re-collection was carried out in 2012. Specific objective two is addressed in the paper in chapter 2.2 (Thormann et al. 2016a). It studies the current extent and distribution of genetic diversity in Spontaneum in Jordan, based on the collection of maternal lines from 32 populations located across the study area in Jordan, and investigates possible contribution of geographical and climatic variation to shaping the observed diversity. The third paper (Thormann et al. 2016b), chapter 2.3, presents results related to objective three. It describes the genotypic and phenotypic differences observed between contemporary and historic Spontaneum populations collected from 18 sites across Jordan in 1981 and 2012, and discusses potential reasons for the observed changes.

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RESEARCH ARTICLE

Plant genetic resources collections and associated information as a baseline resource for genetic diversity studies: an assessment of the IBPGR-supported collections

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Abstract Studying temporal changes in genetic diversity depends upon the availability of comparable time series data. Plant genetic resource collections provide snapshots of the diversity that existed at the time of collecting and provide a baseline against which to compare subsequent observations. The International Board for Plant Genetic Resources conducted collecting missions in 136 countries mainly between 1975 and 1995, with the result that over 200,000 samples of a wide range of taxa were collected and distributed to genebanks around the world for long-term conservation and use. Twenty-seven percent of the collected samples were crop wild relatives and 61 % were landraces. Given their age, geographic and taxonomic scope, these collections have great potential value for establishing historical baselines for monitoring the status of conservation or the erosion of genetic diversity. This article reports on efforts to 'chase down' those samples and to confirm their conservation status and whether they are publicly available. For 35 % of the materials, we were able to recreate a unique link between original passport data and other collecting documentation (collecting sheets and reports) with extant accessions held in genebanks. This information enables a number of important uses, ranging from the identification of potential duplicates in genebanks and the assessment of effectiveness of *ex situ* conservation procedures to the re-collecting and assessing of genetic erosion and temporal variation in landraces and crop wild relatives.

Keywords Collecting missions · Crop wild relatives · *Ex situ* conservation · Genetic erosion · International Board for Plant Genetic Resources · Landraces · Monitoring · Temporal variation

Introduction

The need for monitoring, measuring and reducing the loss of plant genetic diversity is enshrined in global agendas such as the global strategy for plant conservation (Sharrock 2012), the aichi biodiversity targets under the Convention on Biological Diversity (CBD online) or the second Global Plan of Action for Plant Genetic Resources for Food and Agriculture (PGRFA) (FAO 2012). These concerns about changes and loss of genetic diversity are exacerbated by the negative effect on species distribution and survival that climate change is forecasted to have (Jarvis et al. 2008). However, the

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extent of these changes and losses of diversity, evolutionary processes and adaptive responses to climate are not yet well understood (Davis et al. 2005; FAO 2010), and more research is needed to increase our understanding of the effects of climate change, contemporary evolutionary processes and impacts on genetic diversity. Such investigation entails a growing need for baseline data on diversity assessment and monitoring, against which efforts to reduce biodiversity loss can be judged (Magurran et al. 2010).

Project baseline: A Seedbank to study plant evolution[1] (Franks et al. 2008) has been initiated recently to establish a contemporary baseline by systematically collecting, preserving and archiving seeds of wild plant species across sites and time. These seeds will be made available to future biologists for studies of evolutionary responses to anthropogenic and natural changes in the environment over the coming decades. As a source of historical baseline data for contemporary assessments, these documented collections of our bio-cultural heritage, conserved in herbaria, natural history museums and genebanks, are increasingly being used (Miller-Rushing et al. 2006; Wandeler et al. 2007; Vellend et al. 2013; Hart et al. 2014). The analysis of herbarium specimen collected from the same geographical region over decades has revealed the impact of climate change on flowering trends (Primack et al. 2004; Gallagher et al. 2009; Calinger et al. 2013; Li et al. 2013). Research on the evolution of development is increasingly using material from genebanks, long-term experimental plantings and botanical gardens (Dosmann and Groover 2012), for example, to understand the processes that led to the independent domestication of beans in two geographic regions (Kwak and Gepts 2009). Natural history museum specimens are gaining importance for population genetic and evolutionary change studies, comparing the genetic variation from different time periods (Wandeler et al. 2007). Genebanks and plant germplasm collecting missions have been suggested as a source for baseline data (Franks et al. 2008; van de Wouw et al. 2010) to monitor genetic diversity and erosion in agricultural biodiversity, particularly in landraces and crop wild relatives (CWR). Hammer et al. (1996) and Hammer and Laghetti (2005) found a decrease in diversity in their comparisons of number of varieties reported from past collections with the current numbers re-collected in the same sites for a range of different crops in Italy and Albania. Rocha et al. (2008) obtained similar results for common bean, rye and maize in Portugal. Historic and contemporary genotypes of wild cereals (Nevo et al. 2012) and of field mustard (Franks et al. 2007) sampled from the same locations and grown in common gardens showed a reduced flowering time due to droughts and global warming.

Maxted and Guarino (2006) highlighted the collections supported by the International Board for Plant Genetic Resources (IBPGR) as a valuable resource for monitoring and assessing changes in genetic diversity over time. The IBPGR (now called Bioversity International),[2] which was founded in 1974 by the Consultative Group on International Agricultural Research (now the CGIAR Consortium), coordinated a worldwide effort to collect and conserve landraces as a response to the rapidly growing awareness of genetic erosion due to variety and crop replacement, the 'Green Revolution,' land use change and the modernization of agriculture (CGIAR 1972; FAO 1998). During its first two decades (1975–95), over 200,000 samples of landraces and CWR were collected in over 1000 collecting trips[3] in 136 countries. The collecting missions were conducted in areas where landrace and CWR diversity was under risk of erosion or loss and/or where targeted species were being collected that were of major importance to food security. The IBPGR employed collectors and crop specialists as consultants to implement the collecting program and a systematic approach to targeting collecting sites, sampling and passport data recording. The collected material was subsequently conserved in genebanks, whose number grew rapidly from the initial handful of long-term storage facilities in 1975 to nearly 400 with long- or medium-term storage in 1996 (FAO 1998).

[1] Project website available at <http://www.baselineseedbank.org/> (accessed 11 March 2014).

[2] The International Board for Plant Genetic Resources became the International Plant Genetic Resources Institute (IPGRI) in 1991 and in 1994 IPGRI started to operate as an independent CGIAR centre and, since 2006, operates under the name Bioversity International.

[3] A collecting mission often took place in different countries and/or different time periods. Collecting missions were therefore subdivided in single collecting trips, each collecting trip identified by a single target country and a specific collecting period. See Thormann et al. (2012) for more detail.

The collecting missions supported by the IBPGR and the resulting germplasm material were well documented—a necessary condition in order to make use of their historic material today. The collectors usually recorded passport data about each sample in standardized collecting sheets and captured additional data and information in reports about their collecting missions (Thormann et al. 2012). These original passport data were recently extracted from the historic documentation and are now available from the Bioversity Collecting Database (BCD),[4] together with the original reports and collecting sheets, representing a unique historical resource of documented plant collections.

The IBPGR was not only concerned with collecting threatened crop genetic resources; its mandate also included the establishment of a network of base collections for about 40 major crops, and providing advice on the conservation methods and standards to ensure the security of the collections (Hanson et al. 1984; IBPGR 1991). This network, known as the register of base collections (RBC), was established between 1975 and 1990 and included 52 RBC genebanks with which the IBPGR established bilateral (regional or global) base collection agreements. The RBC genebanks were expected to guarantee the availability of the material to the international scientific community and to store germplasm safely and for the long term under storage conditions that corresponded to the genebank standards later published by the Food and Agriculture Organization (FAO) and the International Plant Genetic Resources Institute (IPGRI) in 1994 (FAO/IPGRI 1994). Material collected with IBPGR support was sent to RBC genebanks for long-term conservation after collecting missions were complete.

Given the vast geographical and taxonomic coverage of the IBPGR collecting activities, their focus on landraces and wild relatives, the efforts of ensuring long-term conservation and availability of the original seed samples, and the associated extensive documentation, they are a valuable resource for research about temporal changes in genetic diversity and about vulnerability and adaptability in CWR and landraces. The current content of the BCD and the 52 RBC collections is analyzed in this article, and the possibilities of tracking down original seeds to their current accessions in the genebanks as well as their use for studies of diversity and evolutionary change assessments and monitoring is presented in order to demonstrate the value of such collections and their associated information.

[4] The Bioversity Collecting Database is available at <http://bioversity.github.io/geosite/> (last accessed 15 July 2014).

Materials and methods

The assessment of the current status of the IBPGR collections and the RBC involved a questionnaire survey that was given to 52 RBC genebanks and an analysis of the available passport data in the BCD with a particular focus on CWR. These 52 genebanks were chosen for inclusion in the RBC due to their long-term storage facilities, human capacity, financial sustainability and political stability. As a group, they represented the core characteristics of several hundred genebanks in which germplasm, collected during the IBPGR collecting missions, was conserved according to the IBPGR's procedures. These procedures included the requirement that one sub-sample of the collected material was to be conserved in the country of collecting, while another sub-sample was intended to be conserved in the corresponding IBPGR base collection(s) and/or in the genebank of the organization participating in the collecting mission. The following analysis focuses on the 52 RBC genebanks since (1) these genebanks had specific agreements with the IBPGR for the long-term conservation of collected material; (2) the material sent to these genebanks was considered to be part of the global network; (3) samples of about 80 % of all collected germplasm were sent to the RBC genebanks and (4) the crops and CWR covered under the agreements represent over 75 % of the collected material.

The available list of RBC genebanks with their names and addresses was updated (Thormann and Engels 2001), and representatives, mostly collection curators or plant genetic resource program directors, were contacted for the survey. The main means of retrieving up-to-date contact details included Bioversity International's global network of contacts as well as an Internet search for contacts either in the 'institutes' section of the FAO's World Information and Early Warning System on PGRFA (WIEWS) or directly on the website of the genebanks.

The current version of the BCD contains passport data at the sample level. The original IBPGR collecting database, which was used until 2009, contained records

only at the species batches level—that is, providing the total number of samples collected for a given species—and was subsequently expanded to a sample-level database as described in Thormann et al. (2012). The original IBPGR collecting database included a table with data about the distribution of the collected material to the genebanks. These distribution data provide information on each collecting mission, on all batches of species collected, on how many samples of a collected species had been planned to be shipped to which genebank(s) and on how many samples were reported as received by the respective genebanks. For half of the records about distribution, the numbers of shipped and received samples do match. However, the data also contain records in which only the shipped or only the received samples are recorded. The data about shipment and receipt of samples, therefore, seem incomplete and do not necessarily reflect the real numbers shipped or the number of samples that have effectively been included in the genebank collection as an accession after they were received. This inconsistency can be due to the conditions under which the information was collected, the way in which the material was received or the lag of time between the receipt of samples at the genebank and their subsequent handling. When collectors distributed the samples themselves to the genebanks, communication about the shipments did not always reach the IBPGR Secretariat in Rome and data about the number of samples distributed to the genebanks was seldom included in the collecting mission reports. Shipped material might not have reached a genebank due to phyto-sanitary import restrictions or might not have been received in a healthy condition. Limited genebank staff and insufficient equipment for germination testing could have delayed considerably the processing of the samples after receipt at the genebank and lead to the loss of associated data—for example, the source or donor of the material or parts of the passport data.

The distribution data and our own research on identifying the collected material in genebanks show that not all of the species collected during a specific mission were sent to all of the genebanks that are recorded in the database as recipients of the material from that mission. Unfortunately, this shortcoming occurred without any notes explaining why it happened. In addition, if the samples of a species were distributed to all of the genebanks listed, each genebank did not necessarily receive an equal number of the same samples. The available distribution data therefore most likely do not represent a complete picture of the distribution efforts. Consequently, for the RBC genebank survey, the distribution data were mainly used to check, which genebanks could have received samples from each collecting mission. For each of the 52 genebanks surveyed, we generated a list of samples that could have been distributed to that genebank. We assumed that all of the collected samples for all of the species were equally split and distributed to each genebank, even though, by making this assumption, the lists almost certainly included more samples than were actually received by the genebank.

A questionnaire was emailed in 2012 to each genebank that, according to the records at Bioversity International, had received germplasm samples originating from the IBPGR/IPGRI collecting efforts. This questionnaire served to identify the quantity and availability of the samples obtained from the IBPGR-supported collecting missions. To facilitate the identification of the IBPGR material by the respective genebanks, the lists of samples described earlier were also sent to the respective genebank with detailed passport data. The genebanks were invited to share the passport data of the accessions that they were able to identify as having received from the IBPGR. If the genebanks were unable to identify the accessions deriving from the IBPGR collecting missions, they were requested to send all of the passport data of the respective crop(s)/CWR in their current collection for which they had signed agreements with the IBPGR. These passport data were used by the authors to attempt to match the data from the genebank with the BCD data. When no reply was received from a genebank or replies remained incomplete, we complemented the information about the respective genebank collections using online databases, when available. After verifying that a sample recorded in the BCD corresponded to an accession in a specific genebank, the holding genebank's accession number was added to the BCD. Collected samples for which accession numbers were identified in the genebanks are referred to in this analysis as 'linked samples.'

During the course of this study, we supported the RBC genebank at Plant Gene Resources of Canada (PGRC), which holds one of the three global pearl millet (Pennisetum Rich. spp.) base collections, in the identification of accessions unique to the PGRC pearl millet base collection, as these would then be

prioritized for regeneration. The data for all *Pennisetum* base collections was analyzed to identify all of the possible accessions originating from the same collected sample in other major collections. Detailed passport data were provided for this purpose by the PGRC. Passport data of the other two global base collections held in the United States and at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) were downloaded from the genesys gateway to genetic resources (https://www.genesys-pgr.org/welcome) and the system-wide information network for genetic resources, respectively. Passport data about Pennisetum samples conserved at the French Institut de Recherche pour le Développement (IRD) had been received from the IRD in the past (pers. comm. by I. Thormann).

Results

RBC genebanks and taxon coverage of the agreements

The 52 genebanks that were part of the RBC comprise national and regional genebanks as well as International Agricultural Research Centers (IARC) located in developing and developed countries, as detailed in Table 1. We received answers from 39 (75 %) of the 52 genebanks approached through the survey.

As of 1984, the base collections included all of the major temperate and tropical crops, including the major cereals, food legumes and vegetables that either have a wide distribution, are of considerable economic importance or that have seeds that are suitable to be kept under long-term storage conditions. Seed-producing root and tuber crops such as potato, cassava and sweet potato were included later in the 1980s as well as forage species that are important for livestock or industry. In the case of these latter crops, those that have important germplasm and were considered in danger of erosion were included—for example, beet, sugarcane, cotton and trees species used for fuel wood or environmental stabilization in arid areas (Tao et al. 1989). CWRs for which collections already existed or that were considered of special importance to plant breeders were explicitly included in the RBC, including the genera *Aegilops* L., *Arachis* L., *Allium* L., *Brassica* L., *Citrus* L., Glycine Willd., *Manihot* Mill., *Musa* L., *Oryza* L., *Phaseolus* L., *Sorghum* Moench, *Triticum* L. and *Vigna* Savi. CWR of other major food crops were collected and included by default, such as wild barley or pearl millet. Starting in 1983, field collections were added to the RBC for wild *Allium*, *Musa* and its wild relatives, cocoa and its related species, cassava and its wild relatives, *Citrus* and its wild relatives and sugarcane (IBPGR 1990). Eventually, the agreements with the RBC genebanks covered 80 genera and a total of approximately 250 species (IBPGR 1991) (see Supplementary Table S1). Most agreements included more than one crop or genus. An overview of the repositories forming the register and the respective crop or crops for which the regional or global base collection agreements were signed with the IBPGR is included in Supplementary Table S2.

Table 1 Number of genebanks located in developed and developing countries

Type of genebank\location	Developed country[a]	Developing country[b]
National	26 (20)[e]	15 (10)
Regional[c]	–	2 (1)
IARC[d]	–	9 (8)

[a] Australia, Belgium, Canada, Czech Republic, France, Germany, Greece, Hungary, Italy, Japan, The Netherlands, Poland, Portugal, Russia, Spain, Sweden, United Kingdom and United States

[b] Argentina, Bangladesh, Brazil, China, Ethiopia, India, Jamaica, Kenya, Korea, Malaysia, Mexico, Philippines and Thailand

[c] *CATIE* Centro Agronómico Tropical de Investigación y Enseñanza, Costa Rica, *ICG* International Cocoa Genebank, Trinidad

[d] Eight CGIAR genebanks, *CIAT* Centro International de Agricultura Tropical, *CIMMYT* Centro International de Mejoramento de Maiz y Trigo, *CIP* Centro Internacional de la Papa, *ICARDA* International Centre for Agricultural Research in the Dry Areas, *ICRISAT* International Crop Research Institute for the Semi-Arid Tropics, *ILRI* International Livestock Research Institute, *IITA* International Institute of Tropical Agriculture, *IRRI* International Rice Research Institute and the *AVRDC* World Vegetable Center

[e] The number of genebanks that replied to the survey appears in parentheses

Geographical and taxon composition of the IBPGR collections

A total of 226,618 samples collected between 1975 and 2012 are recorded in the BCD, of which 85 % were collected between 1975 and 1995. They belong to more than 1000 different genera and over 4000 species collected in 136 countries. The collecting trips targeted the landraces and CWRs of major food crops threatened by genetic erosion. Collecting trips focused either on one or a few specific crops and the related wild relatives or entire groups of species such as legumes or grasses in a specific geographic area. However, numerous general missions were also carried out that were not species specific but, rather, targeted a geographical area at the country level or below, collecting a wide range of crops and related species, often including species for which only a few samples were collected and that were not part of the genera covered under the RBC agreements. Nevertheless, most of the collected material—78.3 % (176,486 samples)—belongs to the 80 genera covered under the agreements. The countries from which most of the samples were collected are Thailand and Peru, with more than 10,000 samples, followed by Nepal, India, Pakistan, Spain, Indonesia, Argentina, Syria, Greece, Brazil and Turkey with more than 5000 samples each. Cereals were the most collected type of species with 34 %, followed by forages with 17 %, legumes with 15 % and vegetables with 11 %. Tables 2, 3, 4 and 5 report the number of samples collected, the percentage of CWR and the main countries of collecting for the five most collected genera for the earlier-mentioned crop types as recorded in the BCD.

Some unexpected results appear in Tables 2, 3, 4 and 5. The fact that no CWRs of maize were collected was related to the then prevailing policy of the CIMMYT. The strikingly high percentages of CWR for forage crops is due to the fact that most of the forages were (and possibly still are) non-domesticated species. Furthermore, some bias towards countries and/or crops might have existed due to the personal interests of collectors, the logistical distribution of the regional offices of the IBPGR or specific donor interest.

Distribution to genebanks for conservation

The IBPGR sought to ensure that the germplasm collected under its auspices, either by its full-time collectors or by individuals on a contractual basis, were deposited in base collections as well as in the genebank of the organization participating in the collecting mission or in other selected genebanks worldwide (IBPGR 1987). As a standard, one sub-sample of every collected sample was left in the country of collecting. The splitting of collected samples after collecting and the subsequent shipment to several genebanks was intended to ensure the safe conservation of the collected material. The number of samples recorded as they were distributed to a specific genebank ranges from one to over 13,000. Overall, the collected material was distributed for conservation purposes to more than 400 different institutions, including the national genebanks that held the sub-samples or copies of the original material that remained in the country of collecting. Distribution data show that about 70 % of all samples had been split and sent to more than one genebank after its collection: 39 % was sent to two institutions; 16.5 % to three; 10 % to four and 3 % to five genebanks. In a few cases, samples were recorded as having been distributed to more than five genebanks.

Although standard genebank record keeping should include a record of whom the materials in the genebank were obtained from, this requirement was not completed for a number of the RBC genebanks. Some genebanks that answered the survey were not able to identify any accession that had originated from

Table 2 The five most collected cereal genera recorded in the BCD, the number of samples collected, the percentage of CWR samples and the five main countries of collecting (ISO 3166 country codes)

Genus	Number of samples	% CWR	Country of landraces collecting	Country of CWR collecting
Oryza	25,009	3.5	THA, IDN, NPL, PHL, MDG	MLI, THA, TCD, NGA, CMR
Zea	16,442	0.0	KOR, ARG, BOL PER, BRA	KEN, TCD, MWI, MLI, SDN
Sorghum	10,523	2.6	MLI, ZWE, KEN, UGA, SDN	TUR, IRN, JOR, GRC, SYR
Triticum and Aegilops	7643	41.3	AFG, PAK, EGY, CHN, NPL	GRC, TUR, SYR, PRT, CYP
Pennisetum	6584	11.8	MLI, BFA, NER, ZWE, MWI	MLI, NER, ZMB, MRT, NGA

Table 3 The five most collected forage genera recorded in the BCD, the number of samples collected, the percentage of CWR samples and the five main countries of collecting (ISO 3166 country codes)

Genus	Number of samples	% CWR	Country of landraces collecting[a]	Country of CWR collecting
Trifolium	5,346	89.7	EGY, BGR, ISR	ETH, SYR, ISR, TUR, ITA
Medicago	5,223	95.2	OMN, EGY, YEM, DZA, TUN	LBY, SYR, ITA, ISR, TUR
Desmodium	1,568	89.5	PHL	IDN, THA, ARG, VNM, CHN
Digitaria	865	14.9	GIN, TGO, MLI, BFA, BEN	SWZ, IND, URY, KEN, LSO
Panicum	523	52.0	NPL, MLI, IND, PAK, MNG	MLI, NER, SWZ, BWA, ZMB

[a] Country not listed if less than ten samples were collected

Table 4 The five most collected legume genera recorded in the BCD, the number of samples collected, the percentage of CWR samples and the five main countries of collecting (ISO 3166 country codes)

Genus	Number of samples	% CWR	Country of landraces collecting	Country of CWR collecting[a]
Phaseolus	11,015	5.8	PER, CHL, BRA, PRT, ESP	MEX, ARG, KLA, GTM, PER
Vigna	7560	10.3	PAK, CMR, TGO, ZWE, ZMB	IND, MLI, ZWE, GIN, TZA
Vicia	3663	51.1	EGY, CYP, ESP, GRC, SYR	SYR, TUR, PRT, RUS, ESP
Arachis	2936	13.1	BRA, BOL, ZWE, URY, ZMB	BRA, PRY
Lupinus	1703	44.5	PRT, PER, ESP, EGY, ECU	ESP, PRT, GRC, MAR, ISR

[a] Country not listed if less than ten samples were collected

Table 5 The five most collected vegetable genera recorded in the BCD, the number of samples collected, the percentage of CWR samples and the five main countries of collecting (ISO 3166 country codes)

Genus	Number of samples	% CWR	Country of landraces collecting	Country of CWR collecting[a]
Solanum	5505	15.1	IND, CIV, ESP, BGD, GHA	PER, IND, CHL, ECU, LKA
Abelmoschus	3907	42.5	TGO, BEN, SDN, BFA, LKA	IND, THA, LKA, PNG, NPL
Capsicum	2953	17.7	MEX, ESP, BOL, COL, TUR	GTM, BRA, LKA, BOL, PRY
Cucurbita	2692	5.4	MEX, ESP, PER, ZMB, NPL	GTM, ZMB, MEX
Brassica	1606	16.6	NPL, ESP, GRC, ETH, EGY	ITA, GRC, FRA, ZMB, GBR

[a] Country not listed if less than ten samples were collected

the IBGPR-supported collecting missions 20–25 years before. Based on existing data in the RBC databases, 32 genebanks confirmed that they had conserved about 63,000 samples of IBPGR material.

Linking samples to accession numbers

We obtained passport data from 33 genebanks (63 % of all surveyed genebanks), either through their responses or through our 'downloads' from their online databases. By cross-checking the accession passport data with the genebanks, online databases and our sample level records, we increased the number of so-called linked samples in the BCD by adding more than 15,500 respective accession numbers. Currently, 35 % of all collected samples are linked. For another 35 % of samples that are not yet linked, there is sufficiently detailed passport data available to allow them to be identified in their recipient genebanks.

In total, 73 % of all currently linked samples refer to accessions conserved in the CGIAR genebanks and in the US National Plant Germplasm System (NPGS). Among these, the highest number of samples are linked to the accession numbers in the NGPS, which is possibly due to two factors: (1) that the agreements with the United States include more crops than any other genebank and (2) that it is the only genebank that systematically documented and communicated the

receipt and conservation of samples to the IBPGR. For most of the samples sent to the United States, the original paper records about the collecting missions contain a small sheet of paper produced by the US plant introduction station and returned to the IBPGR documenting the plant introduction number assigned to the sample within the NPGS and the passport data recorded with it.

The effort to link collected samples to the respective accessions in the various genebanks to which they were distributed allows unique material as well as duplicates to be identified. This is illustrated by using the example of the pearl millet (*Pennisetum* spp.) base collections. It was necessary to identify samples that were only conserved in the Canadian pearl millet base collection in order to provide a basis for prioritizing these unique accessions for urgent regeneration. The RBC includes three global base collections for pearl millet,—one is located at the PGRC, one is in the US NPGS and one is at ICRISAT. Sub-samples of *Pennisetum* samples collected with IBPGR support were also conserved in the IRD in France.

The analysis included 4408 *Pennisetum* samples out of a total of 6548 samples recorded in the database. They were collected between 1975 and 1988 during 41 different collecting missions in more than 20 countries, from which samples were recorded as being distributed to the PGRC (Supplementary Table S3). The actual *Pennisetum* collection held in Canada is composed of 3816 accessions (accessed on GRIN-CA on 26 July 2013). We were able to identify and link 3188 accession numbers within the Canadian *Pennisetum* collection that match with the samples in the BCD. For 86.5 % (2759) of these linked samples, we found corresponding duplicates in the United States, ICRISAT and/or the IRD (collectively holding 5716 accessions in total). A total of 294 *Pennisetum* accessions are unique original material conserved only in the PGRC. Of these, 128 accessions are wild *Pennisetum* species, while the others are landraces. ICRISAT has agreed to support the regeneration of the accessions identified as unique in the PGRC (pers. comm. A. Diederichsen).

CWR and geo-referenced samples

The collecting of CWRs was an important objective of the collecting activities, in particular, due to their status of being threatened. In 39 % of all collecting trips, at least half of all of the collected samples were samples of wild species and 25 % of all collecting trips were dedicated solely to the collecting of wild plants. Overall, about 60,000 CWR samples were collected in 115 countries. This number corresponds to 27 % of the total collected samples, which is very high compared to the estimated 2–6 % of CWR recorded globally in *ex situ* collections (Maxted and Kell 2009) or the 11.6 % of wild species in the European Cooperative Programme for Plant Genetic Resources (EURISCO).[5] The countries from which over 2000 CWR samples were collected are (in decreasing order) Syria, China, India, Argentina, Turkey, Libya, Israel and Greece. Most of the CWR samples that were collected are forages, including forage shrubs and trees (53.2 %), followed by wild cereals (10.4 %), wild legumes (9.4 %), wild vegetables and wild root and tubers (both 7.6 %).

Historic collecting information and material needs to include the geographical coordinates of the collecting site or a clear description of the location from which it was collected in order for the material to be used for monitoring of diversity and genetic erosion. Geo-reference data are available in the passport data for 66.2 % of all samples included in the BCD (about 150,000), and the percentage of all CWR samples that include geo-references is even higher (that is, 73 %).

Recommendations exist on the minimum number of individuals it is necessary to sample from a population, depending on the species' breeding system (Brown and Marshall 1995; Crossa and Vencovsky 2011), in order to adequately represent the population's genetic diversity. An analogous recommendation for the number of populations to be sampled per area is less feasible since species differ greatly in a number of life history traits and ecological and genetic attributes and many factors can influence the distribution of genetic diversity across populations of the same species within a region. However, to facilitate collecting, Brown and Marshall (1995) devised a basic sampling strategy of 50 individuals per population and 50 populations per eco-geographic region or collecting mission, if no prior knowledge of genetic diversity is available. This basic sampling strategy should be adapted based on knowledge about factors such as life history, ecology and habitat, migration rates, distribution, abundance and

[5] These data can be verified at <http://eurisco.ecpgr.org/search/advanced_search.html> (last accessed 15 July 2014).

population structure. This threshold of at least 50 geo-referenced samples per species and per collecting trip, which thus coincides with a relatively small geographic region in a country or neighbouring countries, was used to identify CWR collecting trips with a possibly adequate representation of diversity. As such, 21 collecting trips were conducted in which 50 or more samples of one or several wild forage species were collected; seven trips were conducted that targeted one or more wild cereals and five trips were completed that collected one or more wild legume or one or more wild vegetable species. Examples of the CWR collecting trips are provided in Tables 6, 7, 8 and 9. Studies that aim to assess the temporal variation between 'old' and contemporary populations often use a smaller number of samples for comparison (for example, del Rio et al. 1997; Franks et al. 2007; Gao et al. 2000; Nevo et al. 2012). When lowering the threshold to at least 20 geo-referenced collected samples for one or more species, it resulted in 111 collecting events for wild legumes, 40 for wild cereals, 24 for wild legumes and 16 for wild vegetables.

Analyzing the data from a geographical perspective, we can identify countries in which several wild species were collected during different collecting trips with at least 50 geo-referenced samples per collecting mission, such as 11 forage species in Israel and 11 forage and legume species in Syria. For 20 of the geo-referenced samples of wild species, the numbers of species collected per country are provided in Table 10.

A number of CWR have been collected from more than one country across their distribution range (species names as recorded in BCD). *Aegilops triuncialis* L., *Prosopis africana* (Guill. et Perr.) Taub., *Hordeum vulgare* L. subsp. *spontaneum* (K. Koch) Thell., *Lupinus angustifolius* L., *Medicago rigidula* (L.) All. have been collected from two to four countries with 50 or more geo-referenced samples per country. Twenty CWR species have been collected from three to five different countries, with 20 or more samples, in their respective centres of diversity.

Discussion

The IBPGR supported the collecting of over 220,000 landrace and CWR samples across 136 countries, comprising a wide range of taxa, predominantly during the period from 1975 to 1995, and it undertook to secure quality conservation in genebanks for all of the material that it collected. The identification of genebank accessions that originated from those collected samples is one of the requirements to assess the current status of the past collecting efforts and to increase the utilization of this germplasm and its associated data. For nearly 80,000 samples, it was possible to determine the corresponding accession numbers given by the receiving genebanks, thus creating a virtual link between the records from the original samples in the BCD to material recorded in the genebanks. Of these samples, 85 % were linked to the RBC genebanks, mainly the CGIAR centres and the US NPGS.

Efforts to link the samples in the BCD to accession numbers in the genebanks have shown how essential comprehensive and quality passport data are, both at the source in the original collecting documentation (that is, the BCD) as well as in the recipient genebanks. Several key data fields need to be available to allow records from different sources to match, such as the

Table 6 IBPGR collecting trips during which high numbers of wild forage taxa were collected (providing collecting trip number, the country and year of collecting, the species as recorded in the BCD and number of samples collected)

Collecting trip number	Country	Year	Species	Number of samples
CN548C	Niger	1995	*Prosopis africana* (Guill. et Perr.) Taub.[a]	385
CN470	Spain	1989	*Chamaecytisus proliferus* (L. f.) Link	146
CN216	Ethiopia	1982	*Trifolium tembense* Fresen.	118
CN407	Israel	1983	*Trifolium campestre* Schreb.	114
CN407	Israel	1983	*Trifolium tomentosum* L.	107
CN410	Syria	1986	*Lathyrus aphaca* L.	98

[a] Over 100 samples of *P. africana* were also collected from each of Mali, Burkina Faso and Senegal in the same year

Table 7 IBPGR collecting trips during which high numbers of wild cereal taxa were collected (providing collecting trip number, the country and year of collecting, the species as recorded in the BCD and number of samples collected)

Collecting trip number	Country	Year	Species	Number of samples
CN266	Portugal	1983	*Aegilops geniculata* Roth	126
CN367	Greece	1984	*Aegilops lorentii* Hochst.	100
CN306B	Turkey	1984	*Hordeum vulgare* subsp. *spontaneum* (K. Koch) Thell.	98
CN266	Portugal	1983	*Aegilops triuncialis* L.[a]	86
CN306A	Jordan	1984	*Hordeum vulgare* subsp. *spontaneum*	66

[a] More than 50 samples of *A. triuncialis* were also collected in Greece in 1984 and Iran in 1985

Table 8 IBPGR collecting trips during which high numbers of wild legume taxa were collected (providing collecting trip number, the country and year of collecting, the species as recorded in the BCD and number of samples collected)

Collecting trip number	Country	Year	Species	Number of samples
CN125	Spain	1980	*Lupinus angustifolius* L.[a]	83
CN243A1	Australia	1983	*Glycine tomentella* Hayata	76
CN410	Syria	1986	*Vicia peregrina* L.[b]	70
CN326	Australia	1985	*Glycine tabacina* (Labill.) Benth.	64

[a] More than 50 samples of *L. angustifolius* were also collected in Greece in 1984 and in Spain in 1978

[b] During the same trip, more than 50 samples of *V. palaestina* Boiss., *V. sativa* subsp. *sativa*, *V. narbonensis* L. and of *V. hybrida* L. were also collected

Table 9 IBPGR collecting trips during which high numbers of wild vegetable taxa were collected (providing collecting trip number, the country and year of collecting, the species as recorded in the BCD and number of samples collected)

Collecting trip number	Country	Year	Species	Number of samples
CN502B	India	1989	*Abelmoschus esculentus* (L.) Moench	1051
CN502B	India	1989	*Abelmoschus ficulneus* (L.) Wight et Arn.	125
CN502B	India	1989	*Abelmoschus tuberculatus* Pal et Singh	123
CN126B1	Peru	1980	*Solanum lycopersicum* var. *cerasiforme* (Alef.) Voss	86
CN588	Peru	1999	*Solanum pimpinellifolium* L.	66
CN349A3	Chile	1985	*Solanum chilense* (Dunal) Reiche	56

collecting number and other recorded numbers, the collecting date and the country and site of collecting (either as a precise location description or, preferably, as geographical coordinates), possibly supported by the collector's name(s). In addition, information about the donor, collecting mission name, and additional details from the collecting documentation can be relevant for ascertaining a match between an accession and a record with original collecting data.

We observed that for each collecting mission a different set of data fields has to be examined to match the genebank data with the collected sample data. Therefore, it requires careful judgment each time to ascertain matches, and each collecting mission has to be dealt with individually. We relied on data provided directly by the genebanks as well as published data that were downloaded from the Internet. Many genebanks make passport data available today on the Internet, either directly through their own web-enabled databases or by providing their data to larger data portals such as Genesys or EURISCO. However, the data published on the Internet were not always sufficiently detailed for our purposes. Many online databases provide only a subset of the so-called globally agreed multi-crop

Table 10 Number of different wild taxa collected during several collecting trips to the same country (with at least 20 samples per species per collecting trip)

Country of collecting	Number of distinct wild taxa collected with more than 20 samples per collecting trip
Syria	44
Israel	28
Turkey	26
China	23
India	14
Greece	15
Ethiopia	14
Thailand	12
Indonesia	12
Spain	11

passport descriptor (MCPD) fields (FAO/IPGRI 2001) and, thus, do not include additional key data fields necessary for ascertaining matches between collected samples and genebank accessions. The MCPD have recently been updated (Alercia et al. 2012) and now include more descriptors—for example, the collecting mission identifier—which help explain the origin of the accessions. We suggest that online datasets should be extended to include an updated set of MCPD to support the identification of material of common origin.

When a direct contact with a given genebank was established, it was often possible to obtain more complete data than those made available for download from the internet. These data would often include additional data fields. Direct contact with the genebanks was therefore beneficial and often necessary to effectively match samples and accessions, when online data were not sufficiently detailed or the genebank did not publish data online, which is the case with many smaller national genebanks that have been recorded as recipients of IBPGR samples. The unique link that the BCD facilitates between the original passport data of collected samples, the corresponding genebank accession number and the original collecting documentation (collecting sheets and reports) supports and enables a number of important uses:

1. It allows genebanks and genebank users to retrieve additional information from collecting reports about accessions and can thus support decisions about which material to use.
2. It allows genebanks to validate and integrate passport data and to retrieve and integrate original data about the spatial distribution of CWR and landraces.
3. It allows one to identify accessions in different genebanks that have originated from the same collected sample, described earlier in detail for the *Pennisetum* base collections and previously verified for a number of *Hordeum* collecting missions (pers. comm. I. Thormann). These sets of 'duplicate' accessions can be used for research about the effects of splitting seeds after collecting, of conservation conditions and of genebank management practices such as numbers and practices of regeneration cycles on the conservation of genetic diversity *ex situ*. To illustrate, Hirano et al. (2009) compared wheat landraces collected by the IBPGR in Pakistan in 1989 and conserved in Pakistan and Japan. They observed allele changes that pointed to possible unintentional selection pressure during regeneration, indicating the need for increased attention to conservation practices in one of the genebanks and for further research into the maintenance of genetic integrity in genebanks. Similar research is currently being done for barley samples collected in Libya in 1983, in Morocco in 1985 and in Jordan in 1981 and conserved in different genebanks (pers. comm. I. Thormann).
4. Accessions originating from the same sampling event can be prioritized for the rationalization of collections worldwide and can be used to verify the authenticity of these accessions. Genotypic and phenotypic comparison can show whether the accessions are to be considered true biological duplicates that do not have to be conserved in different genebanks from a security perspective (Hazekamp et al. 2014) and have not lost their authenticity through mislabeling or other seed handling procedures (van de Wouw et al. 2011). Our analyses of the distribution data have shown that 70 per cent of all collected samples were conserved in at least two genebanks and multiple distinct accession numbers as unique identifiers have been determined for many of these samples, providing opportunities for further research in this area.

5. The available geo-referenced sample level passport data and the inclusion of a considerable number of linked samples allow re-collections of seeds to be carried out in the old collecting sites for monitoring and assessment of temporal variation in a number of landrace varieties or on a genetic and phenotypic level. Sorghum and pearl millet landraces originally collected in 1976 in Niger and rice collected in 1979 in Guinea Bissau, both with the support of the IBPGR, were re-collected in 2003 from the same villages. The current diversity was assessed and compared with material from the past collections (Barry et al. 2008; Bezançon et al. 2009; Deu et al. 2008, 2010). These studies show that in both countries varietal diversity has been maintained by farmers on a national scale. Further analysis of the old and contemporary pearl millet genotypes shows that significant shifts in adaptive traits has occurred, such as a shorter life cycle and a reduction in plant and spike size in the contemporary samples (Vigouroux et al. 2011). In cases where old seed material might not be available, the original passport data and documentation available from collecting reports can be used to assess changes in varietal diversity (Hammer et al. 1996; Hammer and Laghetti 2005; Teklu and Hammer 2006; Mekbib 2008; Rocha et al. 2008). The results of our detailed analysis of the pearl millet samples provide examples where comparable assessments to those carried out on pearl millet by Bezançon et al. (2009) in Niger could also be carried out in Burkina Faso, Mali, Zambia and Zimbabwe, where collecting trips with over 100 landrace samples have been carried out.
6. Few studies so far have used re-collections of CWR to investigate the effects of climate change (Franks et al. 2007; Nevo et al. 2012) or to examine the extent of genetic erosion (del Rio et al. 1997; Akimoto et al. 1999). The unavailability of data and/or original seed material has been reported as a limiting factor (Keisa et al. 2008). In cases where historical seed samples were unavailable, Keisa et al. (2008), who studied wild *Vicia* species collected in Syria, proposed an indirect genetic erosion assessment based on the documentation of the historic collections.
7. The high percentage of CWR in the database, including the high percentage of geo-referenced records, and the existing collecting mission documentation allow for the extension of monitoring and direct and indirect genetic erosion assessment studies to wild species. Examples of CWR species and collecting trips have been provided earlier in this article. The original sample passport data and documentation from the BCD have recently been used to organize and implement the re-collection of wild barley as well as barley landraces after 31 years in Jordan. The BCD data allowed the material collected in 1981 to be identified in the genebank collections. The respective accessions were requested from the genebanks, and the newly collected seed samples are being compared with original seed samples from the first collecting trip to assess genetic erosion (pers. comm. I. Thormann).
8. Quantifying the changes between the materials conserved in genebanks and the materials re-collected *in situ* not only serves to quantify genetic erosion and inform *in situ* conservation actions, but it also detects changes in adaptive traits. It can also serve genebank managers to validate conservation protocols and develop appropriate re-collection intervals (Greene et al. 2014).

There is room for improving the conservation and use of these materials. The germplasm is conserved in, and across, a large number of genebanks, and the responsibility for its conservation lies with these genebanks. The conservation status of these materials varies considerably across the genera and genebanks involved, depending on the storage conditions and the management practices in the genebanks as well as on the financial and human resources available to a genebank. Identifying which of the accessions are unique, and which are duplicates,—as highlighted in points 3 and 4 above—is a useful step in the process of selecting priority candidates for regeneration. The paper has described how such a selection process was carried out concerning regeneration of the pearl millet collection held at PGRC, to ensure integrity of the accessions. One key to enhance the use and usefulness of *ex situ* PGR collections—both for the kinds of monitoring research described above, but also for crop improvement,—is to improve the quality and availability of documentation of its characteristics. This has been Bioversity's purpose when it extracted all the passport data from the original documentation and

made it available for public use through the online version of the BCD (Thormann et al. 2012). Each sample in the BCD has a unique URL which can be linked to genebank databases, adding a new layer of value-adding information to those genebanks' documentation systems.

Conclusion

To reduce genetic erosion, to more effectively conserve genetic diversity and to predict future ecological impact of global change drivers, we need to better understand genetic diversity distribution patterns and vulnerability and, thus, to learn how these drivers have acted in the past to produce the current plant and crop populations (Vellend et al. 2013). The open access BCD with its large geographical and taxonomic coverage, the large quantity of CWR data and the possibility of identifying not only historical collecting sites but also the original seed material collected from those sites and stored in specialized genebanks represents a unique and largely untapped resource as a baseline for the assessment and monitoring of genetic diversity. It contributes to filling the gaps in baseline data on which studies of neutral and adaptive genetic diversity and evolution, direct and indirect genetic erosion assessment and monitoring need to build. The original collecting data are in the public domain (<http://bioversity.github.io/geosite/>), and a major part of the collected material is conserved in genebanks from which seed samples are usually freely and readily available. Furthermore, this work demonstrates the importance of genebanks maintaining public records (for example, labels) indicating the time and place of collection and the details of the actual collecting mission.

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References

Akimoto M, Shimamoto Y, Morishima H (1999) The extinction of genetic resources of Asian wild rice, *Oryza rufipogon* Griff.: a case study in Thailand. Genet Resour Crop Evol 46:419–425

Alercia A, Diulgheroff S, Mackay M (2012) FAO/Bioversity multi-crop passport descriptors (MCPD V.2). Food and Agriculture Organization. Rome and Bioversity International, Rome

Barry MB, Pham JL, Béavogui S, Ghesquière A, Ahmadi N (2008) Diachronic (1979–2003) analysis of rice genetic diversity in Guinea did not reveal genetic erosion. Genet Resour Crop Evol 55(5):723–733

Bezançon G, Pham JL, Deu M, Vigouroux Y, Sagnard F, Mariac C, Kapran I, Mamadou A, Gérard B, Ndjeunga J, Chantereau J (2009) Changes in the diversity and geographic distribution of cultivated millet (*Pennisetum glaucum* (L.) R. Br.) and sorghum (*Sorghum bicolor* (L.) Moench) varieties in Niger between, 1976 and 2003. Genet Resour Crop Evol 56(2):223–236

Brown AHD, Marshall DR (1995) A basic sampling strategy: theory and practice. In: Guarino L, Ramanatha Rao V, Robert Reid R (eds) Collecting plant diversity: technical guidelines. CAB International, London, pp 75–91

Calinger KM, Queenborough S, Curtis PS (2013) Herbarium specimens reveal the footprint of climate change on flowering trends across north-central North America. Ecol Lett 16:1037–1044

CGIAR (1972) The collection, evaluation and conservation of plant genetic resources. Report of TAC Ad Hoc Working Group held in Beltsville, USA, 20–25 March 1972. Food and Agriculture Organization, Rome

Crossa J, Vencovsky R (2011) Basic sampling strategies: theory and practice. In: Guarino L, Ramanatha Rao V, Goldberg E (eds) Collecting plant genetic diversity: technical guidelines—2011 update. Bioversity International, Rome

Davis MB, Shaw RG, Etterson JR (2005) Evolutionary responses to changing climate. Ecology 86:1704–1714

del Rio AH, Bamberg JB, Huaman Z, Salas A, Vega SE (1997) Assessing changes in the genetic diversity of potato genebanks: *in situ* versus *ex situ*. Theor Appl Genet 95:199–204

Deu M, Sagnard F, Chantereau J, Calatayud C, Hérault D, Mariac C, Pham JL, Vigouroux Y, Kapran I, Traore PS, Mamadou A, Gerard B, Ndjeunga J, Bezançon J (2008) Niger-wide assessment of *in situ* sorghum genetic diversity with microsatellite markers. Theor Appl Genet 116:903–913

Deu M, Sagnard F, Chantereau J, Calatayud C, Vigouroux Y, Pham JL, Mariac C, Kapran I, Mamadou A, Gérard B, Ndjeunga J, Bezançon G (2010) Spatio-temporal dynamics of genetic diversity in *Sorghum bicolor* in Niger. Theor and Appl Genet 120(7):1301–1313

Dosmann M, Groover A (2012) The importance of living botanical collections for plant biology and the 'next generation' of evo-devo research. Front Plant Sci 3:1–5

FAO (1998) The state of the world's plant genetic resources for food and agriculture. Food and Agriculture Organization, Rome

FAO (2010) The second report on the state of the world's plant genetic resources for food and agriculture. Food and Agriculture Organization, Rome

FAO (2012) Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture. Food and Agriculture Organization, Rome

FAO, IPGRI (1994) Genebank standards. Food and Agriculture Organization. Rome and International Plant Genetic Resources Institute, Rome

FAO, IPGRI (2001) Multi-crop passport descriptors. Food and Agriculture Organization. Rome and International Plant Genetic Resources Institute, Rome

Franks SJ, Sim S, Weis AE (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. PNAS 104:1278–1282

Franks SJ, Avise JC, Bradshaw WE, Conner JK, Etterson JR, Mazer SJ, Shaw RG, Weis AE (2008) The resurrection initiative: storing ancestral genotypes to capture evolution in action. Bioscience 58(9):870–873. doi:10.1641/B580913

Gallagher RV, Hughes L, Leishman MR (2009) Phenological trends among Australian alpine species: using herbarium records to identify climate-change indicators. Aust J Bot 57:1–9

Gao L, Chen W, Jiang W, Ge S, Hong D, Wang X (2000) Genetic erosion in northern marginal population of the common wild rice *Oryza rufipogon* Griff. and its conservation, revealed by the change of population genetic structure. Hereditas 133(1):47–53

Greene SL, Kisha TJ, Long Xi Yu, Parra-Quijano M (2014) Conserving plants in genebanks and nature: investigating complementarity with *Trifolium thompsonii* Morton. PLoS One 9(8):e105145. doi:10.1371/journal.pone.0105145

Hammer K, Laghetti G (2005) Genetic erosion: examples from Italy. Genet Resour Crop Evol 52:629–634

Hammer K, Knüpffer K, Xhuveli L, Perrino P (1996) Estimating genetic erosion in landraces: two case studies. Genet Resour Crop Evol 43:329–336

Hanson J, Williams JT, Freund R (1984) Institutes conserving crop germplasm: the IBPGR global network of genebanks. IBPGR Secretariat, Rome

Hart R, Salick J, Ranjitkar S, Xu J (2014) Herbarium specimens show contrasting phenological responses to Himalayan climate. PNAS 111(29):10615–10619

Hazekamp T, Payne TS, Sackville Hamilton NR (2014) Assessing rice and wheat germplasm collections using similarity groups. Genet Resour Crop Evol 61:841–851

Hirano R, Jatoi SA, Kawase M, Kikuchi A, Watanabe N (2009) Consequences of *ex situ* conservation on the genetic integrity of germplasm held at different genebanks: a case study of bread wheat collected in Pakistan. Crop Sci 49:2160–2166

IBPGR (1987) Annual report 1986. International Board for Plant Genetic Resources, Rome

IBPGR (1990) Annual report 1989. International Board for Plant Genetic Resources, Rome

IBPGR (1991) Annual report 1990. International Board for Plant Genetic Resources, Rome

CBD Online. Aichi Biodiversity Targets. Convention on Biological Diversity. http://www.cbd.int/sp/targets/ Accessed 17 July 2014

Jarvis A, Lane A, Hijmans RJ (2008) The effect of climate change on crop wild relatives. Agric Ecosyst Environ 126:13–23

Keisa A, Maxted N, Ford-Lloyd B (2008) The assessment of biodiversity loss over time: wild legumes in Syria. Genet Resour Crop Evol 55:603–612

Kwak M, Gepts P (2009) Structure of genetic diversity in the two major gene pools of common bean (*Phaseolus vulgaris* L., Fabaceae). Theor Appl Genet 118:979–992

Li Z, Wu N, Gao X, Wu Y, Oli KP (2013) Species-level phonological responses to 'global warming' as evidenced by herbarium collections in the Tibetan autonomous region. Biodivers Cons 22:141–152

Magurran AE, Baillie SR, Buckland ST, Dick JM, Elston DA, Scott EM, Smith RI, Somerfield PJ, Watt AD (2010) Long-term datasets in biodiversity research and monitoring: assessing change in ecological communities through time. Trends Ecol Evol 25:574–582

Maxted M, Guarino L (2006) Genetic erosion and genetic pollution of crop wild relatives. In: Ford-Lloyd BV, Dias SR, Bettencourt E (eds) Genetic erosion and pollution assessment methodologies. Proceedings of PGR Forum Workshop 5, Terceira Island, Autonomous Region of the Azores, Portugal, 8–11 September 2004. Published on behalf of the European Crop Wild Relative Diversity Assessment and Conservation Forum, Bioversity International, Rome, pp 35–46

Maxted N, Kell SP (2009) Establishment of a global network for the *in situ* conservation of crop wild relatives: status and needs. FAO Commission on Genetic Resources for Food and Agriculture, Rome

Mekbib F (2008) Genetic erosion of sorghum (*Sorghum bicolor* (L.) Moench) in the centre of diversity. Ethiopia. Genet Resour Crop Evol 55:351–364

Miller-Rushing AJ, Primack RB, Primack D, Mukunda S (2006) Photographs and herbarium specimens as tools to document phonological changes in response to global warming. Am J Bot 93:1667–1674

Nevo E, Fu YB, Pavlicek T, Khalifa S, Tavasi M, Beiles A (2012) Evolution of wild cereals during 28 years of global warming in Israel. PNAS 109(9):3412–3415

Primack D, Imbres C, Primack RB, Miller-Rushing AJ, Tredici PD (2004) Herbarium specimens demonstrate earlier flowering times in response to warming in Boston. Am J Bot 91:1260–1264

Rocha F, Bettencourt E, Gaspar C (2008) Genetic erosion assessment through the re-collecting of crop germplasm: counties of Arcos de Valdevez, Melgaço, Montalegre, Ponte da Barca and Terras de Bouro (Portugal). Plant Genet Resour Newsl 154:6–13

Sharrock S (2012) Global strategy for plant conservation: a guide to the GSPC, all the targets, objectives and facts. Botanic Gardens Conservation International, London

Tao K, Williams JT, van Sloten D (1989) Base collections of crop genetic resources: their future importance in a man-dominated world. Environ Cons 16(4):311–316

Teklu Y, Hammer K (2006) Farmers' perception and genetic erosion of tetraploid wheats landraces in Ethiopia. Genet Resour Crop Evol 53:1099–1113

Thormann I, Engels JMM (2001) IBPGR/IPGRI register of base collections: Annex VII. In: IPGRI (ed) Development of a scientifically sound and financially sustainable global genebank system. Final report on the technical research phase. System-wide Genetic Resources Program, Rome

Thormann I, Gaisberger H, Mattei F, Snook L, Arnaud E (2012) Digitization and online availability of original collecting mission data to improve data quality and enhance the conservation and use of plant genetic resources. Genet Resour Crop Evol 59(5):635–644

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 Genet Resour 8(1):1–15

van de Wouw M, van Treuren R, van Hintum T (2011) Authenticity of old cultivars in genebank collections: a case study on lettuce. Crop Sci 51:736–746

Vellend M, Brown CD, Kharouba HM, Mccune JI, Myers-Smith IH (2013) Historical ecology: using unconventional data sources to test for effects of global environmental change. Am J of Bot 100(7):1294–1305

Vigouroux Y, Mariac C, De Mita S, Pham JL, Gerard B, Kapran I, Sagnard F, Deu M, Chantereau J, Ali A, Ndjeunga J, Luong V, Thuillet A, Saidou A, Bezancon G (2011) Selection for earlier flowering crop associated with climatic variations in the Sahel. PLoS One 6(5):1–9

Wandeler P, Hoeck PEA, Keller KF (2007) Back to the future: museum specimens in population genetics. Trends Ecol Evol 22(12):634–642

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RESEARCH ARTICLE

Geography of Genetic Structure in Barley Wild Relative *Hordeum vulgare* subsp. *spontaneum* in Jordan

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Abstract

Informed collecting, conservation, monitoring and utilization of genetic diversity requires knowledge of the distribution and structure of the variation occurring in a species. *Hordeum vulgare* subsp. *spontaneum* (K. Koch) Thell., a primary wild relative of barley, is an important source of genetic diversity for barley improvement and co-occurs with the domesticate within the center of origin. We studied the current distribution of genetic diversity and population structure in *H. vulgare* subsp. *spontaneum* in Jordan and investigated whether it is correlated with either spatial or climatic variation inferred from publically available climate layers commonly used in conservation and ecogeographical studies. The genetic structure of 32 populations collected in 2012 was analyzed with 37 SSRs. Three distinct genetic clusters were identified. Populations were characterized by admixture and high allelic richness, and genetic diversity was concentrated in the northern part of the study area. Genetic structure, spatial location and climate were not correlated. This may point out a limitation in using large scale climatic data layers to predict genetic diversity, especially as it is applied to regional genetic resources collections in *H. vulgare* subsp. *spontaneum*.

Introduction

Crop wild relatives (CWR) are vital for food security because they provide novel alleles for crop improvement and adaptation [1–3]. Their diversity is threatened by global and climate change [4,5], and more knowledge about the geographical distribution of their genetic variation, and the processes that shape it, is required to more effectively collect, conserve, monitor and use this variation. Genetic data are still lacking for many CWR. Ecogeographical information, which combines environmental and spatial data, is increasingly used as a proxy for genetic diversity to improve collecting, conservation, monitoring and use of CWR [6–11]. This approach assumes that ecogeographical diversity among collecting sites is correlated with

genetic diversity because the distribution of genetic variation in wild plant species is affected by environment (via natural selection) and geographical separation (via isolation by distance). It follows that conserving populations sampled from the widest possible range of ecogeographical conditions is expected to maximize the genetic diversity conserved [12].

Ecogeographical data has been used to identify areas and populations for *in situ* conservation [7,10,13], to assemble core collections [14] and to identify germplasm potentially useful for crop improvement [15,16]. Habitat suitability modelling, also known as species distribution modelling or niche modelling, has been used to identify gaps in existing collections and to prioritize areas for collecting [8,17–19]. Habitat suitability modelling predicts the potential geographical distribution of a species using the known distribution and environmental data, which often come in the form of climatic, edaphic, geophysical and/or land use variables.

Maxted *et al.* [19] have cautioned that the expected correlation between genetic and ecogeographical diversity may not hold for all species and habitats. CWR are often found in ruderal areas and agricultural landscapes where natural, adaptive responses to climate might be altered through anthropogenic influences [20–23]. Of these, the breakdown of isolation by distance due to elevated gene flow may be particularly important.

Barley is the fourth most important cereal crop worldwide in terms of production, yield and area harvested, and is one of the crops in which CWR use in breeding programs is particularly prominent [24]. *Hordeum vulgare* subsp. *spontaneum* (K. Koch) Thell. (hereafter *Spontaneum*) is the progenitor of barley and represents an important genetic resource in barley breeding for traits such as powdery mildew, leaf scald or leaf rust resistance [25–31], yield [32], drought and temperature tolerance [33,34] and agronomic traits such as malting quality [35,36]. Recently, a multi-parental nested association mapping population, using 24 *Spontaneum* donor accessions to induce genetic variation, was set up and tested to study regulation of flowering time in barley [37]. The Fertile Crescent has been considered the primary center of origin and domestication of barley [38,39]. Other studies suggest additional domestication events in areas east of the Fertile Crescent [40], Tibet [41], Ethiopia and the Western Mediterranean [42,43].

Efforts have been made since the 1970s to characterize wild barley germplasm across its distribution range using morphological characters, isozymes and molecular markers [44–55]. The highest genetic variation lies within the Fertile Crescent, and there specifically in Jordan and Israel [55,56]. A few studies have compared diversity in *Spontaneum* between Jordan and neighboring countries. Baek *et al.* [57] found that the number of alleles as well as the percentage of country specific alleles is significantly higher in Jordan than in Israel. Analysis of SNP diversity indicated Jordan and southern Syria as a likely site of domestication [54].

Past studies have investigated the correlation between genetic diversity and environment in *Spontaneum*. They have documented an association between genetic diversity, at single loci, and geography, acrosstemperature or rainfall gradients [44,49,52,58–61]. Genetic differentiation has also been shown to occur, in sympatry, between opposing slopes in the Evolution Canyon in Israel. This has been attributed to adaptation to different microclimates [62,63]. In Jordan, Jaradat [64] characterized kernel protein content and genetic diversity at four esterase loci in 12 wild populations. Ribosomal DNA (rDNA) polymorphism was used to study accessions from 27 collecting sites [65]. The distribution of alleles was found to be correlated with ecogeographical factors such as rainfall, temperature, and geographical location. Baek *et al.* [57] used 18 SSRs to study genetic diversity in accessions from 16 collecting sites and reported associations between ecogeographical variables and allele frequencies at individual loci. Hübner *et al.* [66] studied *Spontaneum* in Israel and attempted to correlate genetic population structure—as opposed to polymorphism or allele frequencies at individual loci—with climate variables. No studies of the correlation between environment and population structure of *Spontaneum* in Jordan have yet been published.

In this study, we sampled *Spontaneum* populations across their range in Jordan and analyzed this collection with a set of 37 SSRs. Our aim was to describe the patterns of genetic diversity and population structure of Jordanian *Spontaneum* and to determine the degree to which the genetic structure estimated with our markers is correlated with spatial and climatic variables derived from global data sources commonly used in conservation and ecogeographical studies [18,67–69].

Material and Methods

Plant material and germination

Single spikes of 12–15 individuals were collected from each of 42 *Spontaneum* populations during a barley collecting mission carried out in 2012, which covered the entire distribution of *Spontaneum* in Jordan. The collecting had been formalized in a letter of agreement between the Jordanian National Center for Agricultural Research and Extension (NCARE) and Bioversity International, which encompassed the permit to collect *Spontaneum* from all visited sites. The collecting was carried out with the continuous participation of NCARE staff and no rare or threatened species was collected. Seeds from each spike were germinated to produce leaf tissue for DNA extraction. Up to eight seeds per spike were rolled into germination paper and placed in an incubator at 25°C for germination. 50–100 mg of 3–5 day old leaf tissue was harvested from one germinated seed per spike. 32 populations (Table 1) (where leaf tissue was available from at least 11 individuals) were used for the study. This resulted in a total of 373 genotypes, with 8–13 individuals per population (S1 Table). The spatial distribution of populations is shown in Fig 1.

Ecogeographical and climate data of collecting sites

Geographical coordinates, altitude, slope, and aspect of the collecting sites were recorded with a GPS Garmin Emap device (datum: WGS84) and habitat type was recorded. Climate data was obtained from the WorldClim database version 1.4 (http://www.worldclim.org), a global and freely available source for climate data layers generated through interpolation of average monthly climate data from weather stations [70]. Layers for current climate conditions (1950–2000) for the 19 bioclimatic variables (Bioclim; see Table 2) were downloaded. Values for the 19 variables were extracted for each collecting site using DIVA-GIS. Collecting sites included ruderal habitats, barley field margins as well as nature reserves, covered an altitudinal range from 87 to 1680 m, a latitudinal range from 30.39875–32.70233 decimal degrees, a longitudinal range from 35.49686111–36.09266667 decimal degrees, an annual precipitation range from 229–491 mm and a mean annual temperature range from 12.5–21.5°C. Collecting site information is provided in S2 Table.

DNA extraction and genotyping

DNA was purified from 3–5 day old leaf tissue with the Qiagen DNeasy® 96 Plant Kit. Thirty-seven EST-derived SSR primers were used for genotyping [71–73] (Table 3). Loci were distributed across all 7 barley chromosomes. PCR was carried out in 5-μl reactions consisting of 2–10 ng genomic DNA, 1x Qiagen Multiplex PCR Master Mix, 225 nM of each primer pair. All fragments were amplified using MJ Research (Waltham, Massachusetts) PTC200 thermocyclers and the following PCR profile: an initial denaturing step of 15 min at 95°C followed by 40 cycles with denaturation at 94°C for 60 s, annealing at 60°C for 30 s and extension at 72°C for 15 s. After 40 cycles, a final extension step was performed at 72°C for 10 min. Amplification products were resolved by capillary electrophoresis on the ABI 3130xl Genetic Analyzer.

Table 1. Collecting site description.

Collecting site number	Latitude	Longitude	Elevation (m)	Number of individuals used in study
1	32.70233	35.72325	94	12
2	32.69611	35.737528	119	13
3	32.67656	35.804833	467	11
4	32.59239	35.666944	87	11
5	32.58686	35.998194	475	12
6	32.51183	35.645444	119	12
7	32.47747	35.969694	608	12
8	32.43733	35.691806	484	12
9	32.37594	35.7365	785	12
10	32.33386	35.91375	957	11
11	32.32931	36.092667	866	12
12	32.32144	35.750139	770	12
13	32.27547	35.891333	564	11
14	32.23847	35.889472	379	13
15	32.14508	35.856639	561	12
16	32.13858	35.646806	141	12
17	32.11461	35.86625	629	14
18	32.06989	35.715139	1044	11
19	32.06694	35.720583	1058	12
20	32.04581	35.775167	911	12
21	32.01156	35.733778	589	11
22	31.77919	35.798833	805	9
23	31.70953	35.960611	709	13
24	31.67036	35.785889	745	12
25	31.56639	35.791417	646	11
26	31.54019	35.773639	677	11
27	31.18831	35.696583	773	12
28	31.04872	35.708861	1222	12
29	30.68108	35.622222	1566	12
30	30.65542	35.610194	1257	12
31	30.42178	35.512	1580	8
32	30.39875	35.496861	1680	11

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Fragment sizes were calculated using GeneScan 400HD (ROX) internal size standards and scored with GeneMapper software (v. 5.0) (Life Technologies, Thermo Fisher Scientific Inc.).

Genetic diversity

Summary statistics of the marker data such as number of alleles, sample adjusted allelic richness, and observed heterozygosity were calculated with GDA [74] and FSTAT version 2.93.2 [75]. The number of multi-locus genotypes was determined with GeneticStudio (http://dyerlab.bio.vcu.edu). Polymorphism information content (PIC) per locus was calculated with PICcalc [76].

Population differentiation among sites

F_{ST} was used to measure differentiation between populations and was calculated with FSTAT. Inter-individual distances were calculated using a simple matching coefficient with DARwin

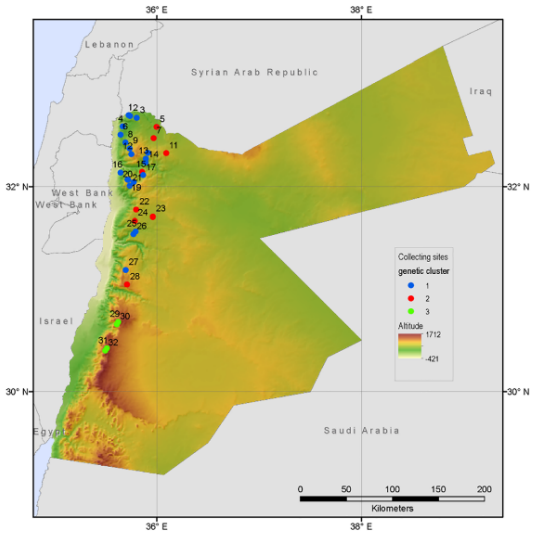

Fig 1. Collecting sites in Jordan.

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software version 5.0.158 [77] and used to build a neighbor-joining tree. Because *Spontaneum* is a highly selfing species, the program InStruct [78] was used to infer population structure. InStruct is an extension of the approach used in STRUCTURE [79] and can specifically account for self-pollination and inbreeding. InStruct was run in mode v = 3 (infer population

Table 2. Coding of bioclimatic variables according to WorldClim at http://www.worldclim.org/bioclim.

Code	Description
Bioclim 1	Annual Mean Temperature
Bioclim 2	Mean Diurnal Range (Mean of monthly (max temp—min temp))
Bioclim 3	Isothermality (Bioclim2/ Bioclim7) (* 100)
Bioclim 4	Temperature Seasonality (standard deviation *100)
Bioclim 5	Max Temperature of Warmest Month
Bioclim 6	Min Temperature of Coldest Month
Bioclim 7	Temperature Annual Range (Bioclim5- Bioclim6)
Bioclim 8	Mean Temperature of Wettest Quarter
Bioclim 9	Mean Temperature of Driest Quarter
Bioclim 10	Mean Temperature of Warmest Quarter
Bioclim 11	Mean Temperature of Coldest Quarter
Bioclim 12	Annual Precipitation
Bioclim 13	Precipitation of Wettest Month
Bioclim 14	Precipitation of Driest Month
Bioclim 15	Precipitation Seasonality (Coefficient of Variation)
Bioclim 16	Precipitation of Wettest Quarter
Bioclim 17	Precipitation of Driest Quarter
Bioclim 18	Precipitation of Warmest Quarter
Bioclim 19	Precipitation of Coldest Quarter

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Table 3. Characteristics of SSR markers.

Marker ID	Location	Ind no.	Allele no.	PIC	Allelic richness
GBM1002	1H	367	12	0.736	5.44
GBM1013	1H	373	7	0.383	3.194
GBM1029	1H	373	6	0.412	3.038
GBM1334	1H	372	5	0.538	3.317
GBM1461	1H	373	20	0.914	9.082
GBM1035	2H	373	6	0.66	4.264
GBM1036	2H	372	5	0.497	3.289
GBM1047	2H	364	7	0.664	4.269
GBM1208	2H	367	7	0.563	3.693
GBM1218	2H	369	4	0.591	3.593
GBM1459	2H	373	6	0.578	3.931
GBM1043	3H	372	5	0.495	3.735
GBM1110	3H	373	11	0.761	5.362
GBM1280	3H	372	5	0.661	4.038
GBM1405	3H	372	8	0.792	5.795
GBM1413	3H	372	5	0.517	3.171
GBM1003	4H	372	9	0.713	5.164
GBM1015	4H	373	19	0.834	7.016
GBM1020	4H	371	7	0.663	3.96
GBM1323	4H	371	7	0.642	4.535
GBM1026	5H	372	4	0.412	2.379
GBM1054	5H	368	7	0.682	4.822
GBM1064	5H	373	5	0.531	3.263
GBM1176	5H	373	7	0.58	3.892
GBM1363	5H	373	5	0.387	2.817
GBM1008	6H	366	9	0.75	5
GBM1021	6H	367	15	0.87	7.503
GBM1063	6H	373	10	0.746	5.26
GBM1075	6H	370	5	0.34	2.792
GBM1212	6H	372	4	0.376	2.208
GBM1404	6H	373	3	0.359	2.453
GBM1033	7H	373	6	0.687	4.268
GBM1060	7H	370	4	0.528	3.008
GBM1326	7H	373	10	0.816	5.926
GBM1419	7H	373	8	0.7	4.675
GBM1464	7H	365	22	0.888	8.074
GBM1516	7H	373	6	0.593	4.033
Mean		371	7.9	0.6	4.4

The following values are presented for each marker: chromosome location (Location), number of individuals scored (Ind no.), number of alleles (Allele no.), polymorphism information content (PIC), allelic richness.

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structure and individual selfing rates) for K = 1–10. For each K, 5 chains were run, with 200,000 MCMC iterations, a burn-in of 100,000 and a thinning interval of 10 steps. Results from independent chains were summarized using CLUMPP [80] and graphical representations of cluster assignments were rendered with DISTRUCT [81]. The *ad hoc* measure of change in likelihood between successive K values, ΔK [82] was calculated to identify the appropriate

number of clusters. As recommended by Gao *et al.* [78], clustering results were compared with results obtained using STRUCTURE v. 2.3.3 [79] and Structure Harvester [83]. STRUCTURE was run with 5 independent runs for each value of K from 1 to 8, with a burn in period of 10^5 followed by 10^5 iterations.

Description of environmental variation in Jordan

We used a procedure developed by Newman and Rissler [84] to delineate distinct environments within the study area. A habitat suitability model was generated for *Spontaneum* with MaxEnt version 3.3.3k [85]. Occurrence data in Jordan was downloaded from Genesys (https://www.genesys-pgr.org). Occurrences showing a geographical coordinate quality rank > 70 [86] were included. The 19 Bioclim layers for current climate conditions (1950–2000), at a resolution of 2.5 arc-minutes, were used. Ten thousand sites were sampled pseudo-randomly from the study area, in proportion to their suitability, as estimated in the habitat suitability model. The environmental data associated with each site was then extracted from all Bioclim layers. Following normalization of each environmental variable, the data set was subjected to k-means clustering such that each pseudo-randomly selected site was assigned to one of k classes. By coloring each site according to habitat, regions within the study area that had similar mean environmental conditions could be visualized.

Association between genetic diversity, geography and environment

Correlations between allelic richness, InStruct clustering results and environmental data were tested using JMP 5.1 (SAS Institute, Cary, NC, USA). Means were compared using the Tukey-Kramer HSD test. Pearson product-moment and Spearman's Rho rank correlation coefficients were calculated. Isolation by distance (IBD) was estimated using R (http://www.r-project.org/). Geographic distances were calculated as straight-line distances with the GeographicDistanceMatrixGenerator version 1.2.3 [87] and log transformed. Genetic distances were calculated as $F_{ST}/(1- F_{ST})$ [88] and as population-wise allelic differences using the FPTEST [89]. Two-tailed Mantel tests were carried out with 10^5 permutations. To test isolation by environment (IBE), environmental distances between sites were estimated. A principal coordinate analysis (PCO) was performed using data from all Bioclim variables and altitude. Environmental distance was then approximated as the simple Euclidean distance between points on the first principal coordinate axis, which accounted for 49% of the environmental variation across sampling sites. The multivariate measure of environmental distance represented a conservative approach aiming to avoid overfitting, as many of the Bioclim parameters covaried significantly. Two-tailed Mantel tests were carried out to estimate IBE. As environmental and geographical distances were significantly correlated, IBD and IBE were also tested using a partial Mantel test. In addition, correlation of the distance matrix calculated with FPTEST to individual Bioclim variables was examined using appropriate Holm-Bonferroni correction [90] to avoid type I error inherent in multiple comparisons.

Results

Genetic diversity

A total of 291 alleles were identified. Alleles per locus ranged from 3 to 22, with an average of 7.9. The mean number of alleles per locus averaged across sites was 2.8. PIC varied from 0.34 to 0.914 with a mean of 0.62. Allelic richness per locus varied from 2.2–9.1. All populations showed low observed heterozygosity (H_o) ranging between 0–0.025. *Spontaneum* is a highly self-pollinating species and previous studies on *Spontaneum* reported similar levels of

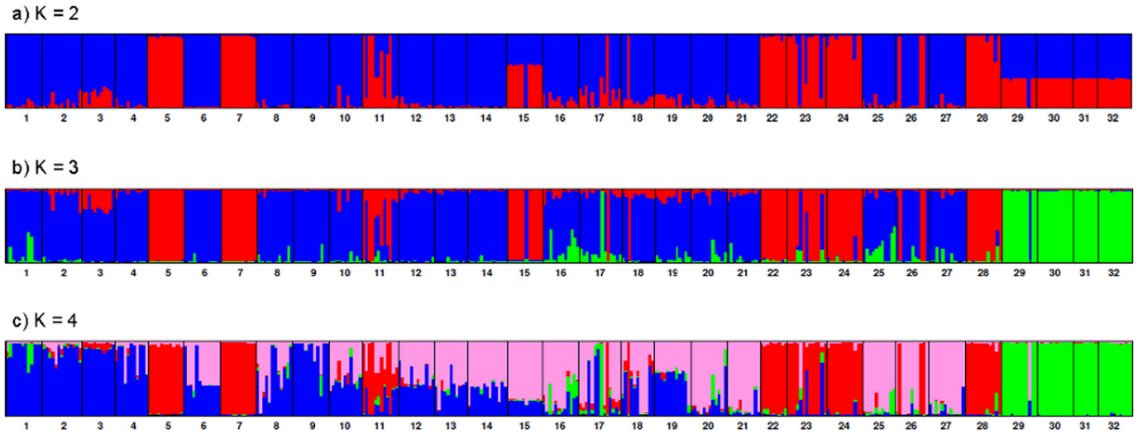

Fig 2. Assignment of individuals to genetic clusters identified by InStruct, for K = 2 to K = 4. Populations are sorted from left to right by decreasing latitude. Clusters are depicted in the following colours: cluster 1 = blue; cluster 2 = red; cluster 3 = green; cluster 4 = pink.

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heterozygosity [66,91]. A total of 370 multi-locus genotypes were identified. Only three populations (5, 15, 18) showed a single multi-locus genotype twice. Allelic richness per population ranged from 1.4 to 3.3 with a mean of 2.63.

Population differentiation among sites

Differentiation among populations measured as F_{ST} was 0.33, i.e. 33% of variation was distributed between populations and 67% within populations, similar to previous studies [57,59,92,93]. The ΔK method [82] applied to InStruct and STRUCTURE results suggested subdivision into three clusters. Fig 2 shows the individual assignment coefficients for K = 2 to K = 4. Partitioning into three genetic clusters produced one group of populations predominantly located in the northwestern part of the collecting area and a second cluster which showed a longitudinal extension from the northeast southwards. A small third cluster was geographically separated in the southern part of the collecting area. The geographical distribution of the three clusters is shown in Fig 1. The InStruct assignment was compatible with the neighbor-joining tree based on inter-individual genetic distances (Fig 3). Assignment coefficients (q) varied across the study area. The average assignment coefficient of individuals to cluster 1 was significantly lower (q = 0.902; p = <0.0001) than those of cluster 2 (q = 0.966) and cluster 3 (q = 0.99). The assignment coefficient was inversely correlated with latitude (Pearson coefficient: r = -0.17; p = 0.001; Spearman's rank coefficient: r = -0.204; p<0.0001) and positively correlated with altitude (Pearson coefficient: r = 0.166; p = 0.0013; Spearman's rank coefficient: r = 0.235; p<0.0001) indicating that the level of admixture was higher in the north. While there were 10 populations whose respective individuals were all strongly assigned to the same genetic cluster ($q \geq 0.8$), the remaining populations contained some individuals either strongly assigned to a different cluster (physical admixture), and/or some genetically admixed individuals ($0.49 < q < 0.8$) (Table 4). Eight populations were physically admixed, with 1–6 individuals assigned to a different cluster. 18 populations contained 1–9 genetically admixed individuals (four of these populations were also physically admixed). In populations assigned to cluster 3, only the population in site 29 showed physical admixture (one individual assigned to cluster

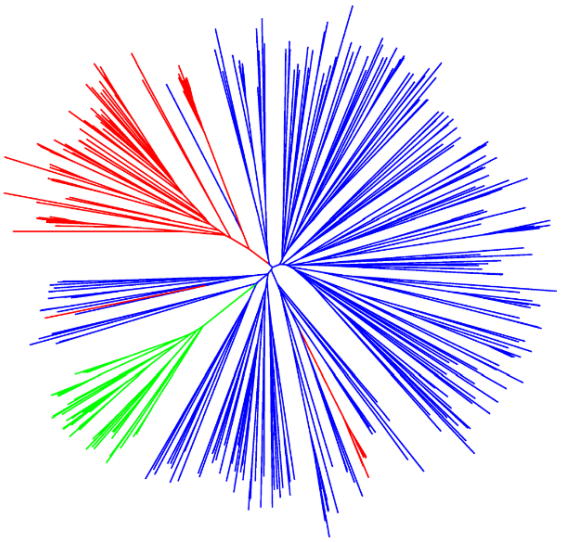

Fig 3. Neighbor-joining tree showing inter-individual genetic distances. Genetic clusters are depicted in the following colours: cluster 1 = blue; cluster 2 = red; cluster 3 = green.

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1), no genetic admixture was identified in any of the four populations of cluster 3. All other physical admixture stems from individuals either assigned to cluster 1 but growing within a site assigned to cluster 2 or vice versa. 88% of the 43 genetically admixed individuals belong to populations assigned to cluster 1, the remaining to cluster 2.

Association between genetic diversity, geography and environment

K-means clustering was used to delineate different environments that might be inhabited by *Spontaneum* in Jordan. Regions of the study area with distinct environmental conditions are depicted in Fig 4. They are predominantly arranged as north-south stripes corresponding to the three main topographical regions described by Al-Eisawi [94] (rift valley along the western border; mountain range extending from the north in Irbid to the south in Ras An-Naqab, and the eastern desert). Although the sampling scheme also followed a north-south transect, populations were sampled from the majority of the distinct environments identified (Fig 4). The geographical distribution of the genetic clusters did not match the geographical distribution of these environmental partitions.

When comparing populations collected from nature reserves and those collected from ruderal habitats, roadsides or field margins, no significant differences in genetic diversity

Table 4. Genetic and physical admixture.

	Physical admixture		Genetic admixture	
Cluster	No. of populations	No. of individuals	No. of populations	No. of individuals
1	4	11	14	38
2	3	5	4	5
3	1	1	0	0

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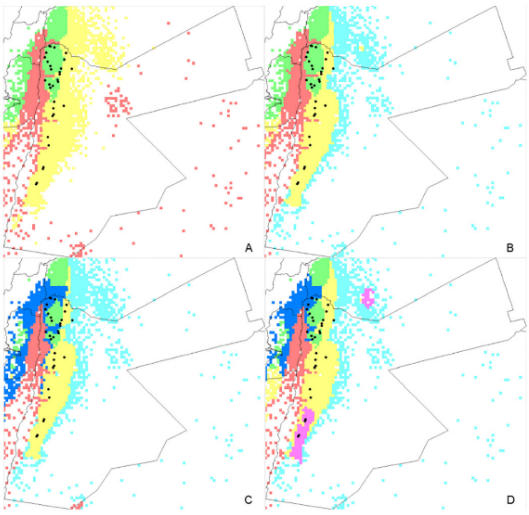

Fig 4. Habitat types in Jordan identified through k-means clustering. Black dots represent the *Spontaneum* collecting sites.

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measures were found. No physical admixture was detected in populations collected in reserves, while they do show genetic admixture. Plants collected in reserves were significantly smaller (37.0 cm) than those collected from ruderal areas or field margins (72.6 cm; p = 0.0047). Also population size observed in reserves was significantly smaller (p = 0.0112, Tukey-Kramer HSD test; based on observed size of all wild populations sampled during the 2012 barley collecting mission). The average habitat suitability, according to the habitat suitability model, was significantly lower in reserves than in the other sites (p = 0.0289).

Average values of geographical, geophysical and Bioclim variables, allelic richness, and selfing rates are compared among clusters in Table 5. Average longitude and Bioclim 6 were the only variables that were significantly different between all three clusters. Cluster 3 collecting sites were significantly different for several variables including: higher elevation, lower latitude, lower values for temperature-related Bioclim variables 1, 5, 8, 9, 10, 11 (these Bioclim variables are highly correlated, r>0.8) and lower selfing rates. Cluster 1 showed significantly higher allelic richness and higher values for Bioclim 13. No significant differences were found for habitat type, aspect, soil type and Bioclim 2, 3, 7, 15, 16 and 19. Bioclim 14, 17 and 18 were zero at all sites. Allelic richness of loci and of populations was weakly correlated with latitude (loci: Spearman's rank coefficient r = 0.079, p = 0.0065; Pearson's coefficient r = 0.147, p<0.0001; populations: Pearson's coefficient r = 0.36, p = 0.0432).

Genetic and geographical distance were significantly correlated (F_{ST} based distance: r = 0.3, p = 0.0003; FPTEST based distance: r = 0.2, p = 0.02), suggesting isolation by distance, when analyzed over all 32 populations, while the Mantel tests for isolation by environment were not significant. Environmental and geographic distance were strongly correlated (r = 0.4, p = 0.0001), indicating possible confounding effects. These were accounted for using a partial Mantel test which confirmed significant IBD among all studied populations (r = 0.25, p = 0.004), but did not find significant IBE (S1 Resource). Several climate variables

Table 5. Comparison of average values for geographical, geophysical and Bioclim variables, allelic richness and selfing rate at collecting sites among genetic clusters.

Variable	Cluster 1			Cluster 2			Cluster 3		
	Level	Mean	p	Level	Mean	p	Level	Mean	p
Allelic richness	A	2.91		B	2.25	<0.0001	B	2.0	<0.0001
Selfing rate	A	0.831	0.0294	A	0.833	0.0220	B	0.0813	
Altitude (m)	B	564	<0.0001	B	749	0.0005	A	1521	
Latitude	A	32.195	<0.0001	A	31.968	<0.0001	B	30.539	
Longitude	A	35.76	0.0015 (1–3)	B	35.9	0.0038 (2–1)	C	35.56	<0.0001 (3–2)
Aspect	A	233.99	ns	A	185.07	ns	A	199.04	ns
Slope	A	3.54	0.0030	B	0.99		A	4.81	0.0024
Bioclim 1	A	18.03	<0.0001	A	16.67	0.0122	B	13.78	
Bioclim 2	A	11.68	ns	A	12.21	ns	A	11.60	ns
Bioclim 3	A	42.94	ns	A	44.10	ns	A	42.82	ns
Bioclim 4	A	625.73	0.0151	AB	620.55		B	598.47	
Bioclim 5	A	32.07	0.0005	A	30.94	0.0249	B	27.96	
Bioclim 6	A	4.9	<0.0001 (1–3)	B	3.25	0.03 (2–1)	C	0.86	0.0332 (3–2)
Bioclim 7	A	27.17	ns	A	27.69	ns	A	27.1	ns
Bioclim 8	A	10.1	0.0002	A	8.68	0.0287	B	6.06	
Bioclim 9	A	25.0	<0.0001	A	23.59	0.0145	B	20.65	
Bioclim 10	A	25.04	<0.0001	A	23.63	0.0130	B	20.65	
Bioclim 11	A	10.1	0.0002	A	8.68	0.0287	B	6.06	
Bioclim 12	A	388.0	0.0448 (1–3)	AB	323.88		B	289.75	
Bioclim 13	A	92.1		B	73.75	0.0314	B	67.0	0.0238
Bioclim 15	A	113.44	ns	A	113.02	ns	A	115.12	ns
Bioclim 16	A	250.1	ns	A	206.88	ns	A	187.0	ns
Bioclim 19	A	250.1	ns	A	206.88	ns	A	187.0	ns

Bioclim 1- Bioclim 19 = Bioclimatic variables as per definition on http://www.worldclim.org/bioclim (see Table 2); ns = non-significant; levels marked with different letters indicate significant difference among cluster averages ($p<0.05$) based on the Tukey HSD test. Numbers in brackets after p values indicate the two clusters being compared.

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were found to be different in cluster 3 compared to cluster 1 and 2 (Table 5). Cluster 3 was furthermore geographically separated from clusters 1 and 2, which are themselves partly overlapping. The correlation analyses were therefore repeated for populations belonging to clusters 1 and 2 only. No significant IBD or IBE was found between cluster 1 and 2, and neither were environmental and geographic distances significantly correlated. No significant correlations existed between single Bioclim variables and distance matrix calculated with FPTEST (S1 Resource).

Discussion

The present study examined the current geography of genetic structure and its correlation with landscape scale climatic and spatial variation in *Spontaneum* populations in Jordan. Correlation analyses showed large scale IBD across the study area but did not reveal a correspondence between climate and genetic structure. Analysis of population structure suggested that the 32 *Spontaneum* populations could be divided into three major, genetically differentiated clusters (Fig 1). Genetic diversity was concentrated in the northern part of the study area, across a range of environments, where populations are characterized by physical and genetic admixture, and high

allelic richness. Allelic richness and admixture decrease towards the south; the southernmost populations are not admixed, exhibit low allelic richness and contain physically smaller plants.

Genetic structure is not correlated with climatic variation inferred from global layers

Three genetic clusters were distributed along a longitudinal gradient in the North (clusters 1 and 2), with a distinct cluster (cluster 3) in the South. The study area was characterized by a longitudinal distribution of distinct habitat types as shown in Fig 4, of which the central mountain range was the most variable. At the large scale across the entire study area, where geographical and environmental distances were strongly correlated, significant IBD implied that physical distance was important for genetic differentiation among populations, but environmental variation was found to have no effect. Results were different at a slightly smaller scale, across the central and northern part of the study area, where clusters 1 and 2 spread across an environmentally heterogeneous landscape. Here, geographical and environmental distances were both uncorrelated with genetic distance either measured by F_{ST} or by population-wise allelic differences.

Spontaneum prefers disturbed, human-made or influenced habitats [20,22], sympatric with its domesticate [95–98]. These habitats favor anthropogenic movement of material—inclusion and transport with cultivated barley seed lots or hitchhiking on livestock fur or human clothing—which interferes with natural diffusion and selection processes. This may alter the expected distribution of genetic diversity across the landscape and lead to weak or nonexistent correlations between ecogeographical and genetic diversity as found in our study. Natural dispersal and selection processes may not have been the principle force shaping genetic structure in some regions of Jordan.

Spontaneum is a highly self-pollinating species. In self-pollinating species much genetic diversity is distributed among populations rather than within populations, population to population variation is greater than in out-crossing species and the genetic structure is more variable [99]. Given their low gene flow and very localized gene transfer, genetic structure has been found at local scale [63,100,101]. This local variation is unlikely to be detected by globally available layers commonly used to represent landscape scale spatial and climatic variation.

Global climate data such as the Bioclim layers provided by WorldClim climatic data are used in a range of studies and applications [11,19,67–69,85,86,102], and the inherent assumption is that they are robust proxies for genetic data, which is often not available. Our results suggest that there may be some limitations on this assumption. Our study did not find a correlation between climate, as represented by commonly used global, interpolated data layers, and genetic structure for *Spontaneum*. Thus global climatic data would not be especially useful for predicting existing genetic diversity in Jordan. A ruderal habitat preference and high self-pollination might explain why the general expectation of tight correlation between genetic and ecogeographical diversity does not hold. If collecting and conservation actions are designed without previous knowledge of genetic structure, it will be important to consider species biology and habitat preferences when using ecogeographical diversity to predict genetic diversity.

Sampling and monitoring genetic diversity within *Spontaneum* populations

All *Spontaneum* populations sampled here, irrespective of cluster assignment, contained many unique multi-locus genotypes. Only three populations showed a single multi-locus genotype twice, and no multi-locus genotype was repeated among populations. Allelic richness, which is a good metric to assess and monitor genetic diversity [103], increased significantly towards the

northern part of the study area. Here, populations were also characterized by admixture. More than half of the populations in clusters 1 and 2 showed considerable genetic admixture as well as physical admixture, a characteristic that was also found by Hübner *et al.* [66] in Israel. Hübner *et al.* [93] observed a fairly high rate of gene flow in *Spontaneum* attributed to sporadic outcrossing events [104] and gene flow through seed dispersal. These mechanisms likely contribute to physical and genetic admixture in Jordan as well.

Due to the reduced level of diversity expected within populations of highly selfing species, germplasm collections are often limited to a few samples per population. The heterogeneity found within populations in this study cautions against such sampling strategies. Modeling studies have shown that collections of highly selfing species need substantially more samples than are commonly recommended to capture existing diversity [105]. The distribution of genetic structure we have described for *Spontaneum* in Jordan prescribes further collecting and monitoring in the northern part of the country, in particular the area occupied by cluster 1.

Ex situ and *in situ* conservation of *Spontaneum*

Natural populations of *Spontaneum* have been reported to harbour large neutral genetic diversity, and also show considerable diversity in disease resistance and quantitative traits of agronomic importance [45,106–108]. Despite evidence of high genetic, adaptive and quantitative diversity in Jordanian *Spontaneum* populations, the number of *ex situ* barley accessions from Jordan in global collections is lower than those from neighboring countries. Although in general the number of *Spontaneum* accessions in *ex situ* collections seems relatively high compared with other CWR samples in genebanks, they are derived from a limited number of populations [109]. Maxted and Kell [24] suggest that, although *Spontaneum* is widespread and locally common [110], individual populations might contain important adaptive traits, thus populations should be actively conserved throughout the geographical range. Vincent *et al.* [111] identified Jordan as one of the countries where wild *Hordeum* should be conserved and suggested the establishment of a network of several reserves in the Israel/Jordan region to more effectively conserve the genetic diversity of wild *Hordeum*. These assessments describe the obvious need to promote *in situ* conservation of *Spontaneum* in Jordan and to enlarge *ex situ* collections. Our description of the distribution of genetic diversity across the Jordanian landscape provides a tool to evaluate the propriety of existing *in situ* conservation activities and supports the application of proper sampling techniques for future *ex situ* acquisitions.

Supporting Information

S1 Resource. Correlation analyses between genetic, geographic and environmental data.
(PDF)

S1 Table. Microsatellite data for 373 *Spontaneum* individuals.
(PDF)

S2 Table. Ecogeographical and genetic data for 32 *Spontaneum* populations collected in Jordan in 2012.
(PDF)

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References

1. Gur A, Zamir D. Unused natural variation can lift yield barriers in plant breeding. PLoS Biology. 2004; 2(10):e245. doi: 10.1371/journal.pbio.0020245 PMID: 15328532
2. Hajjar R, Hodgkin T. The use of wild relatives in crop improvement: a survey of developments over the last 20 years. Euphytica. 2007; 156:1–13.
3. McCouch S, Baute GJ, Bradeen J, Bramel P, Bretting PK, Buckler E, et al. Feeding the future. Nature. 2013; 499:23–24. doi: 10.1038/499023a PMID: 23823779
4. Jarvis A, Lane A, Hijmans RJ. The effect of climate change on crop wild relatives. *Agriculture*, Ecosystems & Environment. 2008; 126:13–23.
5. FAO. Report of the Fourteenth Regular Session of the Commission on Genetic Resources for Food and Agriculture. CGRFA 14/13/report. 2013. Available: http://www.fao.org/nr/cgrfa/cgrfa-meetings/cgrfa-comm/fourteenth-reg/en/.
6. Parra-Quijano M, Draper D, Torres E, Iriondo JM. Ecogeographical representativeness in crop wild relative *ex situ* collections. In: Maxted N, Ford-Lloyd BV, Kell SP, Iriondo JM, Dulloo E, Turok J, editors. Crop wild relative conservation and use. CAB International, Wallingford, UK; 2008. pp. 249–273.
7. Parra-Quijano M, Iriondo JM, Frese L, Torres E. Spatial and ecogeographical approaches for selecting genetic reserves in Europe. In: Maxted N, Dulloo ME, Ford-Lloyd BV, Frese L, Iriondo JM, Pinheiro de Carvalho MAA, editors. Agrobiodiversity Conservation: securing the diversity of crop wild relatives and landraces. CAB International, Wallingford, UK; 2012. pp. 20–28.
8. Parra-Quijano M, Iriondo JM, Torres E. Improving representativeness of genebank collections through species distribution models, gap analysis and ecogeographical maps. Biodivers Conserv. 2012; 21:79–96.
9. Steiner JJ. Exploring the relationship of plant genotype and phenotype to ecogeography. In: Greene SL, Guarino L, editors. Linking genetic resources and geography: Emerging strategies for conserving and using crop biodiversity. Crop Science Society of America Inc., Madison, WI; 1999. pp. 39–50.
10. Dulloo ME, Labokas J, Iriondo JM, Maxted N, Lane A, Laguna E, et al. Genetic reserve location and design. In: Iriondo JM, Maxted N, Dulloo ME, editors. Conserving plant genetic diversity in protected areas. CAB International; 2008. pp. 23–64.
11. Parra-Quijano M, Iriondo JM, Torres E. Review. Applications of ecogeography and geographic information systems in conservation and utilization of plant genetic resources. Spanish J Agric Res. 2012; 10(2):419–429.
12. Maxted N, Van Slageren MW, Rihan J. Ecogeographic surveys. In: Guarino L, Ramanatha Rao V, Reid R, editors. Collecting plant genetic diversity: Technical guidelines. CAB International, Wallingford, UK; 1995. pp. 255–286.
13. Maxted N, Magos Brehm J, Kell S. Resource book for preparation of national conservation plans for crop wild relatives and landraces. Food and Agriculture Organization of the United Nations, Italy; 2013.
14. Parra-Quijano M, Iriondo JM, de la Cruz M, Torres E. Strategies for the development of core collections based on ecogeographical data. Crop Sci. 2011; 51:656–666.
15. Khazaei H, Street K, Bari A, Mackay M, Stoddard FL. The FIGS (focused identification of germplasm strategy) approach identifies traits related to drought adaptation in *Vicia faba* genetic resources. PLoS ONE 2013; 8(5):e63107. doi: 10.1371/journal.pone.0063107 PMID: 23667581
16. Thormann I, Parra-Quijano M, Endresen DTF, Rubio-Teso ML, Iriondo MJ, Maxted N. Predictive characterization of crop wild relatives and landraces. Technical guidelines version 1. Bioversity International, Rome, Italy; 2014.

17. Jarvis A, Williams K, Williams D, Guarino L, Caballero PJ, Mottram G. Use of GIS for optimizing a collecting mission for a rare wild pepper (*Capsicum flexuosum* Sendtn.) in Paraguay. Genet Resour Crop Evol. 2005; 52:671–682.
18. Ramirez-Villegas J, Khoury C, Jarvis A, Debouck DG, Guarino L. A gap analysis methodology for collecting crop genepools: A case study with *Phaseolus* beans. PLoS ONE. 2010; 5(10):e13497. doi: 10.1371/journal.pone.0013497 PMID: 20976009
19. Maxted N, Dulloo ME, Ford-Lloyd BV, Iriondo MJ, Jarvis A. Gap analysis: a tool for complementary genetic conservation assessment. Divers Distrib. 2008; 14:1018–1030.
20. Ellstrand NC. Dangerous liaisons? When cultivated plants mate with their wild relatives. Johns Hopkins University Press, Baltimore, USA; 2003.
21. Andersen MS, de Vicente CM. Gene flow between crops and their wild relatives. Johns Hopkins University Press, Baltimore, USA; 2010.
22. Jain SK. Genetic reserves. In: Frankel OH, Hawkes JG, editors. Crop genetic resources for today and tomorrow. Cambridge University Press, UK; 1975. pp. 379–396.
23. Jarvis S, Fielder H, Hopkins J, Maxted N, Smart S. Distribution of crop wild relatives of conservation priority in the UK landscape. Biol. Cons. 2015; 191:444–451.
24. Maxted N, Kell SP. Establishment of a global network for the in situ conservation of crop wild relatives: status and needs. FAO Commission on Genetic Resources for Food and Agriculture, Rome, Italy; 2009.
25. Fischbeck G, Schwarzbach E, Sobel F, Wahl I. Mehltauresistenz aus Israelischen Populationen der zweizeiligen Wildgerste (*Hordeum spontaneum*). Z. Pflanzenzüchtung. 1976; 76:163–166.
26. Ivandic V, Walther U, Graner A. Molecular mapping of a new gene in wild barley conferring complete resistance to leaf rust (*Puccinia hordei* Otth). Theor Appl Genet. 1998; 97:1235–1239.
27. Backes G, Madsen LH, Jaiser H, Stougaard J, Herz M, Mohler V, et al. Localization of genes for resistance against *Blumeria graminis* f.sp. *hordei* and *Puccinia graminis* in a cross between a barley cultivar and wild barley (*Hordeum vulgare* subsp. *spontaneum*) line. Theor Appl Genet. 2003; 106:353–362. PMID: 12582863
28. Dreiseitl A, Bockelman HE. Sources of powdery mildew resistance in a wild barley collection. Genet Resour Crop Evol. 2003; 50:345–350.
29. Genger RK, Williams KJ, Raman H, Read BJ, Wallwork H, Burdon JJ, et al. Leaf scald resistance genes in *Hordeum vulgare* and *Hordeum vulgare* ssp. *spontaneum*: parallels between cultivated and wild barley. Aust J Agric Res. 2003; 54(12):1335–1342.
30. von Korff M, Wang H, Léon J, Pillen K. AB-QTL analysis in spring barley. I. Detection of resistance genes against powdery mildew, leaf rust and scald introgressed from wild barley. Theor Appl Genet. 2005; 111(3):583–90. PMID: 15902395
31. Repkova J, Dreiseitl A, Lizal P, Kyjovska Z, Teturova K, Psotkova R, et al. Identification of resistance genes against powdery mildew in four accessions of *Hordeum vulgare* ssp. *spontaneum*. Euphytica. 2006; 151:23–30.
32. von Korff M, Wang H, Leon J, Pillen K. AB-QTL analysis in spring barley. II. Detection of favourable exotic alleles for agronomic traits introgressed from wild barley (*Hordeum vulgare* ssp. *spontaneum*). Theor Appl Genet. 2006; 112:1221–1231. PMID: 16477429
33. Chen G, Li C, Shi Y, Nevo E. Wild barley, *Hordeum spontaneum*, a genetic resource for crop improvement in cold and arid regions. Sciences in cold and arid regions. 2008; 1:0115–0124.
34. Lakew B, Henry RJ, Eglinton J, Baum M, Ceccarelli S, Grando S. SSR analysis of introgression of drought tolerance from the genome of Hordeum spontaneum into cultivated barley (*Hordeum vulgare* ssp *vulgare*). Euphytica. 2013; 191(2):231–243.
35. Erkkila MJ, Leah R, Ahokas H, Cameron-Mills V. Allele-dependent barley grain β-Amylase activity. Plant Physiol. 1998; 117:679–685. PMID: 9625721
36. von Korff M, Wang H, Léon J, Pillen K. AB-QTL analysis in spring barley: III. Identification of exotic alleles for the improvement of malting quality in spring barley (*H. vulgare* ssp. *spontaneum*). Mol Breed. 2008; 21(1):81–93.
37. Maurer A, Draba V, Jiang Y, Schnaithmann F, Sharma R, Schumann E, et al. Modeling the genetic architecture of flowering time control in barley through nested association mapping. BMC Genomics. 2015; 16:290. doi: 10.1186/s12864-015-1459-7 PMID: 25887319
38. Zohary D, Hopf M. Domestication of plants in the Old World, 2nd edition. Oxford University Press; 1993.

39. Badr A, Mueller K, Schaefer-Pregl R, El Rabey H, Effgen S, Ibrahim HH, et al. On the origin and domestication history of barley (*Hordeum vulgare*). Mol Biol Evol. 2000; 17(4):499–510. PMID: 10742042

40. Morrell PL, Clegg MT. Genetic evidence for a second domestication of barley (*Hordeum vulgare*) east of the Fertile Crescent. Proc Natl Acad Sci USA. 2007; 104:3289–3294. PMID: 17360640

41. Dai F, Nevo E, Wu D, Comadran J, Zhou M, Qiu L, et al. Tibet is one of the centers of domestication of cultivated barley. Proc Natl Acad Sci USA. 2012; 109(42):16969–16973. doi: 10.1073/pnas.1215265109 PMID: 23033493

42. Molina-Cano JL, Russell JR, Moralejo MA, Excacena JL, Arias G, Powell W. Chloroplast DNA microsatellite analysis supports a polyphyletic origin for barley. Theor Appl Genet. 2005; 110:613–619. PMID: 15723272

43. Orabi J, Backes G, Wolday A, Yahyaoui A, Jahoor A. The Horn of Africa as a center of barley diversification and a potential domestication site. Theor Appl Genet. 2007; 114(6):1117–27. PMID: 17279366

44. Nevo E, Zohary D, Brown AHD, Haber M. Genetic diversity and environmental associations of wild barley, *Hordeum sponateneum*, in Israel. Evolution. 1979; 33(3):815–833.

45. Nevo E, Beiles A, Gutterman Y, Storch N, Kaplan D. Genetic resources of wild cereals in Israel and vicinity. II. Phenotypic variation within and between populations of wild barley, *Hordeum spontaneum*. Euphytica. 1984; 33(3):737–756.

46. Baum BR, Nevo E, Johnson DA, Beiles A. Genetic diversity in wild barley (*Hordeum spontaneum C*. Koch) in the Near East: a molecular analysis using Random Amplified Polymorphic DNA (RAPD) markers. Genet Resour Crop Evol. 1997; 44(2):147–157.

47. Pakniyat H, Powell W, Baird E, Handley LL, Robinson D, Scrimgeour CM, et al. AFLP variation in wild barley (*Hordeum spontaneum C*. Koch) with reference to salt tolerance and associated ecogeography. Genome 1997; 40:332–341. PMID: 18464832

48. Gupta PK, Sharma PK, Balyan HS, Roy JK, Sharma S, Beharav A, et al. Polymorphism at rDNA loci in barley and its relation with climatic variables. Theor Appl Genet 2002; 104:473–481. PMID: 12582721

49. Liviero L, Maestri E, Gulli M, Nevo E, Marmiroli N. Ecogeographic adaptation and genetic variation in wild barley, application of molecular markers targeted to environmentally regulated genes. Genet Resour Crop Evol. 2002; 49:133–144.

50. Ivandic V, Hackett CA, Nevo E, Keith R, Thomas WTB, Forster BP. Analysis of simple sequence repeats (SSRs) in wild barley from the Fertile Crescent: associations with ecology, geography and flowering time. Plant Mol Biol. 2002; 48(5–6):511–527. PMID: 11999832

51. Ozkan H, Kafkas S, Sertac Ozer M, Brandolini A. Genetic relationships among South-East Turkey wild barley populations and sampling strategies of *Hordeum spontaneum*. Theor Appl Genet. 2005; 112(1):12–20. PMID: 16283231

52. Cronin JK, Bundock PC, Henry RJ, Nevo E. Adaptive climatic molecular evolution in wild barley at the Isa defense locus. Proc Nat Acad Sci USA. 2007; 104:2773–2778. PMID: 17301230

53. Wang A, Yu Z, Ding Y. Genetic diversity analysis of wild close relatives of barley from Tibet and the Middle East by ISSR and SSR markers. Comptes Rendus Biologies. 2009; 332:393–403. doi: 10.1016/j.crvi.2008.11.007 PMID: 19304270

54. Russell J, Dawson IK, Flavell AJ, Steffenson B, Weltzien E, Booth A, et al. Analysis of 1000 single nucleotide polymorphisms in geographically matched samples of landrace and wild barley indicates secondary contact and chromosome-level differences in diversity around domestication genes. New Phytologist. 2011; 191(2):564–578. doi: 10.1111/j.1469-8137.2011.03704.x PMID: 21443695

55. Jakob SS, Roedder D, Engler JO, Shaaf S, Oezkan H, Blattner FR, et al. Evolutionary history of wild barley (*Hordeum vulgare* subsp. *spontaneum*) analyzed using multilocus sequence data and paleodistribution modeling. Genome Biol Evol. 2014; 6(3):685–702. doi: 10.1093/gbe/evu047 PMID: 24586028

56. Fu YB, Horbach C. Genetic diversity in a core subset of wild barley germplasm. Diversity. 2012; 4 (2):239–257.

57. Baek HJ, Beharav A, Nevo E. Ecological-genomic diversity of microsatellites in wild barley, *Hordeum spontaneum*, populations in Jordan. Theor Appl Genet. 2003; 106(3):397–410. PMID: 12589539

58. Lin JZ, Brown AHD, Clegg MT. Heterogeneous geographic patterns of nucleotide sequence diversity between two alcohol dehydrogenase genes in wild barley (*Hordeum vulgare* subsp. *spontaneum*). Proc Nat Acad Sci USA. 2001; 98:531–536. PMID: 11149938

59. Turpeinen T, Tenhola T, Manninen O, Nevo E, Nissilä E. Microsatellite diversity associated with ecological factors in *Hordeum spontaneum* populations in Israel. Mol Ecol. 2001; 10(6):1577–1591. PMID: 11412377

60. Chen G, Suprunova T, Krugman T, Fahima T, Nevo E. Ecogeographic and genetic determinants of kernel weight and colour of wild barley (*Hordeum spontaneum*) populations in Israel. Seed Sci. Res. 2004; 14:137–146.

61. Batchu AK, Zimmermann D, Schulze-Lefert P, Koprek T. Correlation between hordatine accumulation, environmental factors and genetic diversity in wild barley (*Hordeum spontaneum C*. Koch) accessions from the Near East Fertile Crescent. Genetica. 2006; 127(1–3):87–99. PMID: 16850216

62. Yang Z, Zhang T, Bolshoy A, Beharav A, Nevo E. Adaptive microclimatic structural and expressional dehydrin 1 evolution in wild barley, *Hordeum spontaneum*, at 'Evolution Canyon', Mount Carmel, Israel. Mol Ecol. 2009; 18:2063–2075. doi: 10.1111/j.1365-294X.2009.04140.x PMID: 19344351

63. Nevo E. Evolution of wild barley at "Evolution Canyon": adaptation, speciation, pre-agricultural collection, and barley improvement. Israel J Plant Sci. 2014; 62(1–2):22–32.

64. Jaradat AA. Genetic diversity of four esterase loci in natural populations of *Hordeum spontaneum* C. Koch from Jordan. Theor Appl Genet. 1992; 84:725–729. doi: 10.1007/BF00224176 PMID: 24201365

65. Sharma S, Beharav A, Balyan HS, Nevo E, Gupta PK. Ribosomal DNA polymorphism and its association with geographical and climatic variables in 27 wild barley populations from Jordan. Plant Sci. 2004; 166:467–477.

66. Hübner S, Hüffken M, Oren E, Haseneyer G, Stein N, Graner A, et al. Strong correlation of wild barley (*Hordeum spontaneum*) population structure with temperature and precipitation variation. Mol Ecol. 2009; 18:1523–1536. doi: 10.1111/j.1365-294X.2009.04106.x PMID: 19368652

67. Castañeda-Álvarez NP, de Haan S, Juárez H, Khoury CK, Achicanoy HA, Sosa CC, et al. Ex Situ Conservation Priorities for the Wild Relatives of Potato (*Solanum* L. Section Petota). PLoS ONE. 2014; 10(4):e0122599. doi: 10.1371/journal.pone.0122599

68. Khoury CK, Castañeda-Alvarez NP, Achicanoy HA, Sosa CC, Bernau V, Kassa MT, et al. Crop wild relatives of pigeonpea [*Cajanus cajan* (L.) Millsp.]: Distributions, ex situ conservation status, and potential genetic resources for abiotic stress tolerance. Biol Conserv 2015; 184:259–270.

69. Kantar MB, Sosa CC, Khoury CK, Castañeda-Álvarez NP, Achicanoy HA, Bernau V, et al. Ecogeography and utility to plant breeding of the crop wild relatives of sunflower (*Helianthus annuus* L.). Frontiers in Plant Sci. 2015; 6:841.

70. Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. Very high resolution interpolated climate surfaces for global land areas. Int J Climat. 2005; 25(15):1965–1978.

71. Thiel T, Michalek W, Varshney RK, Graner A. Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). Theor Appl Genet. 2003; 106:411–422. PMID: 12589540

72. Stein N, Prasad M, Scholz U, Thiel T, Zhang H, Wolf M, et al. A 1000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. Theor Appl Genet. 2007; 114:823–839. PMID: 17219208

73. Varshney RK, Marcel TC, Ramsay L, Russell J, Roder MS, Stein N, et al. A high density barley microsatellite consensus map with 775 SSR loci. Theor Appl Genet. 2007; 114(6):1091–1103. PMID: 17345060

74. Lewis PO, Zaykin D. Genetic Data Analysis: Computer Program for the Analysis of Allelic Data (version 1.1). 2001; Available: http://hydrodictyon.eeb.uconn.edu/people/plewis/downloads/gda-1.1.win32.zip.

75. Goudet J. FSTAT, a program to estimate and test gene diversities and fixation indices (Version 2.9.3). 2001; Available: http://www.unil.ch/popgen/softwares/fstat.htm.

76. Nagy S, Poczai P, Cernák I, Gorji AM, Hegedűs G, Taller J. PICcalc: an online program to calculate polymorphic information content for molecular genetic studies. Biochemical Genet. 2012; 50(9–10):670–672.

77. Perrier X, Jacquemoud-Collet JP. DARwin software. 2006; Available: http://darwin.cirad.fr/.

78. Gao H, Williamson S, Bustamante CD. A Markov chain Monte Carlo approach for joint inference of population structure and inbreeding rates from multilocus genotype data. Genetics. 2007; 176 (3):1635–1651. PMID: 17483417

79. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155:945–959. PMID: 10835412

80. Jakobsson M, Rosenberg NA. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics. 2007; 23 (14):1801–1806. PMID: 17485429

81. Rosenberg NA. Distruct: a program for the graphical display of population structure. Mol Ecol Notes. 2004; 4:137–138.

82. Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol. 2005; 14(8):2611–2620. PMID: 15969739

83. Earl DA, von Holdt BM. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour. 2012; 4(2):359–361.

84. Newman CE, Rissler LJ. Phylogeographic analyses of the southern leopard frog: the impact of geography and climate on the distribution of genetic lineages vs. subspecies. Mol Ecol. 2011; 20:5295–5312. doi: 10.1111/j.1365-294X.2011.05353.x PMID: 22066968

85. Phillips SJ, Anderson RP, Schapire RE. Maximum entropy modeling of species geographic distributions. Ecol Modeling. 2006; 190:231–259.

86. Parra-Quijano M, Torres E, Iriondo JM and López F. CAPFITOGEN Tools User Manual Version 1.2. International Treaty on Plant Genetic Resources for Food and Agriculture, FAO, Rome, Italy; 2014.

87. Ersts PJ. Geographic Distance Matrix Generator (version 1.2.3). American Museum of Natural History, Center for Biodiversity and Conservation. Available: http://biodiversityinformatics.amnh.org/open_source/gdmg.

88. Rousset F. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics. 1997; 145(4):1219–28. PMID: 9093870

89. Fu YB. FPTEST: a SAS routine for testing differences in allelic count. Mol. Ecol. Res. 2010; 10 (2):389–392.

90. Holm S. A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics. 1979; 6:65–70.

91. Morrell PL, Toleno DM, Lundy KE, Clegg MT. Low levels of linkage disequilibrium in wild barley (*Hordeum vulgare* ssp. *spontaneum*) despite high rates of self-fertilization. Proc Nat Acad Sci USA. 2005; 102(7):2442–2447. PMID: 15699350

92. Turpeinen T, Vanhala T, Nevo E, Nissilä E. AFLP genetic polymorphism in wild barley (*Hordeum spontaneum*) populations in Israel. Theor Appl Genet. 2003; 106:1333–1339. PMID: 12748785

93. Hübner S, Günther T, Flavell A, Fridman E, Graner A, Korol A, et al. Islands and streams: clusters and gene flow in wild barley populations from the Levant. Mol Ecol. 2012; 21:1115–1129. doi: 10.1111/j.1365-294X.2011.05434.x PMID: 22256891

94. Al-Eisawi D. Vegetation of Jordan. UNESCO—Cairo office. Regional office for science and technology for the Arab States; 1996.

95. Witcombe JR, Bourgois JJ, Rifaie R. Germplasm collections from Syria & Jordan. Plant Genet Resour Newsletter. 1982; 50:2–8.

96. Jaradat AA. Diversity within and between populations of two sympatrically distributed *Hordeum* species in Jordan. Theor Appl Genet. 1989; 78:653–656. doi: 10.1007/BF00262560 PMID: 24225825

97. Jaradat AA. Ecotypes and genetic divergence among sympatrically distributed *Hordeum vulgare* and *Hordeum spontaneum* from the xeric region of Jordan. Theor Appl Genet. 1989; 78:857–862. doi: 10.1007/BF00266671 PMID: 24226019

98. Nevo E. Origin, evolution, population genetics and resources for breeding of wild barley *Hordeum spontaneum* in the Fertile Crescent. In: Shewry PR, editor. Barley: Genetics, Biochemistry, molecular biology and biotechnology. CAB International, Wallingford, UK. 1992. pp 19–43.

99. Schoen DJ, Brown AHD. Intraspecific variation in population gene diversity and effective population size correlated with the mating system in plants. Proc Nat Acad Sci USA. 1991; 88:4494–4497. PMID: 11607182

100. Nevo E. Genetic diversity and environmental associations of wild barley, *Hordeum spontaneum* in Turkey. Genetica. 1986; 68:203–213.

101. Volis S, Mendlinger S, Ward D. Adaptive traits of wild barley plants of Mediterranean and desert origin. Oecologia. 2002; 133:131–138.

102. Parra-Quijano M., Iriondo J.M., Torres E. Ecogeographical land characterization maps as a tool for assessing plant adaptation and their implications in agrobiodiversity studies. Genet Resour Crop Evol. 2012; 59(2):205–217.

103. Hoban S, Arntzen JA, Bruford MW, Godoy JA, Hoelzel R, Segelbacher G, et al. Comparative evaluation of potential indicators and temporal sampling protocols for monitoring genetic erosion. Evol Appl. 2014; 7(9):984–998. doi: 10.1111/eva.12197 PMID: 25553062

104. Abdel-Ghani AH, Parzies HK, Omary A, Geiger HH. Estimating the outcrossing rate of barley landraces and wild barley populations collected from ecologically different regions of Jordan. Theor Appl Genet. 2004; 109:588–595. PMID: 15083273

105. Hoban S, Strand A. *Ex situ* seed collections will benefit from considering spatial sampling design and species' reproductive biology. Biol Conserv. 2015; 187:182–191.

106. Nevo E. Genetic diversity in wild cereals: regional and local studies and their bearing on conservation *ex situ* and *in situ*. Genet Resour Crop Evol. 1998; 45:355–370.

107. Al-Saghir MG, Malkawi HI, El-Oqlah A. Morphological Diversity in *Hordeum spontaneum* C. Koch of Northern Jordan (Ajloun Area). Middle-East J Sci Res. 2009; 4(1):24–27.

108. Shakhatreh Y, Haddad N, Alrababah M, Grando S, Ceccarelli S. Phenotypic diversity in wild barley (*Hordeum vulgare* L. ssp. *spontaneum* (C. Koch) Thell.) accessions collected in Jordan. Genet Resour Crop Evol. 2010; 57(1):131–146.

109. van Hintum T, Menting F. Diversity in *ex situ* genebank collections of barley. In: von Bothmer R, van Hintum T, Knüpffer H, Sato K, editors. Diversity in barley (*Hordeum vulgare*). Elsevier Science B. V., Amsterdam, the Netherlands; 2003. pp. 247–257.

110. von Bothmer R, Jacobsen N, Baden C, Jørgensen RB, Linde-Laursen I. An ecogeographical study of the genus *Hordeum*. 2nd edition. Systematic and Ecogeographic Studies on Crop Genepools 7. International Plant Genetic Resources Institute, Rome, Italy; 1995.

111. Vincent H, von Bothmer R, Knüpffer H, Amri A, Konopka J, Maxted N. Genetic gap analysis of wild *Hordeum* taxa. Plant Genet Resour-C. 2013; 10(3):242–253.

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RESEARCH ARTICLE

Genotypic and phenotypic changes in wild barley (*Hordeum vulgare* subsp. *spontaneum*) during a period of climate change in Jordan

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Abstract Climate change and other anthropogenic disturbances can lead to the loss of genetic variation and thereby affect evolutionary potential and survival of plant populations in the wild. We examined these predictions in the primary wild relative of barley, *Hordeum vulgare* L. subsp. *spontaneum* (K. Koch) Thell., within its center of diversity, in Jordan. Changes in genotypic and phenotypic diversity were assessed using seed samples collected in 1981 and 2012 from the same 18 sites across Jordan. The overall population structure was conserved, but we observed an increase of within population genetic diversity and a reduction in population differentiation. Phenotypic variation differed among years and sites but the magnitude and direction of change variated among sites. While the sampled region became significantly hotter and drier during this period, simple correlation models did not support association between measures of climate change and the observed genetic and phenotypic changes. Agricultural activities that promote disturbance and demographic fluctuations may affect crop wild relatives that grow in agricultural landscapes, in unexpected ways. The observed increase in genetic diversity within populations might be explained by increased migration or by an advantage of increased genetic variation in the face of variable environmental conditions. This study provides a new perspective on the range of possible responses of crop wild relatives to environmental pressures.

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Introduction

Crop wild relatives (CWR) are an important source of genetic variation and adaptive traits. Their use in crop improvement has been increasing over the past decades (Gur and Zamir 2004; Hajjar and Hodgkin 2007; Maxted and Kell 2009; McCouch et al. 2013). The total number of CWR is estimated to be between 50,000 and 60,000 species, of which 700 are considered of highest global conservation priority (Maxted and Kell 2009). Many of these CWR species are threatened by habitat loss and climate change (Thuiller et al. 2005; Jarvis et al. 2008; FAO 2013) and their genetic diversity is not adequately conserved (Maxted and Kell 2009; Vincent et al. 2013). Although national, regional and global inventories are being developed (Dulloo et al. 2015), data about the extent and distribution of their diversity are frequently unavailable. Likewise, there is little information on temporal changes in genetic diversity in these species (Thormann and Engels 2015). Loss of genetic diversity may impact a species' resilience to changes in environmental conditions, because genetic variation is a prerequisite for adaptation (Frankham et al. 2010). Understanding temporal changes is therefore important in order to assess the vulnerability of CWR populations in situ and to inform conservation actions.

Quantifying temporal changes requires time series data. Genebanks maintain living germplasm and associated provenance information from historical collections of agriculturally important species, which can be used to reveal past diversity and to re-collect contemporary samples for comparison. Genebanks are therefore considered a useful resource for studies of temporal change in genetic diversity (Maxted and Guarino 2006; Franks et al. 2008; van de Wouw et al. 2010; Thormann et al. 2015). Few studies on CWR have used re-collected samples. These have mainly focused on evaluating effectiveness of ex situ conservation and complementarity of ex situ and in situ conservation (del Rio et al. 1997; Che et al. 2011; Greene et al. 2014), or changes in flowering time as an adaptive response to climate change (Franks et al. 2007; Nevo et al. 2012). Two studies monitored the loss of genetic variation over time (genetic erosion) in one wild rice population (Akimoto et al. 1999; Gao et al. 2000).

Barley is the fourth most important cereal crop worldwide in terms of production, yield and area harvested. CWR are important in barley breeding and research (Maxted and Kell 2009). *Hordeum vulgare* L. subsp. *spontaneum* (K. Koch) Thell., the wild progenitor of cultivated barley (hereafter "Spontaneum") represents an important genetic resource for disease resistance traits such as powdery mildew, leaf scald or leaf rust resistance (Fischbeck et al. 1976; Ivandic et al. 1998; Backes et al. 2003; Dreiseitl and Bockelman 2003; Genger et al. 2003; von Korff et al. 2005; Repkova et al. 2006), drought and temperature tolerance (Chen et al. 2008; Lakew et al. 2013), yield (von Korff et al. 2006) and malting quality (Erkkila et al. 1998; von Korff et al. 2008) or research on the genetics of flowering time and root traits (Naz et al. 2014; Maurer et al. 2015). Maxted and Kell (2009) suggested that, although Spontaneum is widespread and locally common (von Bothmer et al. 1995), individual populations might contain important adaptive traits, thus populations should be actively conserved throughout the geographical range. Vincent et al. (2013) identified Jordan as one of the countries where Spontaneum should be conserved and suggested the establishment of a network of reserves in the Israel/Jordan region.

To broaden our understanding of the extent and causes of genetic change in CWR in situ, we used Spontaneum germplasm to analyze temporal changes in genotypic and phenotypic diversity. Population samples collected in Jordan in 1981 were sourced from a genebank and contemporary samples were re-collected from the same sites in 2012. We examined the diversity in both collecting years and asked whether observed changes are associated with climatic changes in the region during this time period.

Materials and methods

Spontaneum germplasm collections

Seed samples of Spontaneum populations were collected in Jordan in 1981 (18 May–2 June) during a collecting trip carried out under a regional FAO project operated by the International Board for Plant

Genetic Resources (IBPGR) (Witcombe et al. 1982) and were re-sampled from the same sites in 2012 (21 May–3 June) in collaboration with the Jordanian National Center for Agricultural Research and Extension (NCARE). Using map coordinates and location descriptions from the 1981 collecting reports, and information provided by the original collector, precise site locations were determined for the re-sampling in 2012. Collecting sites were typically field margins, roadsides or ruderal habitats. Seed samples were collected from the entire populations and contained up to 200 spikes in 1981 and up to 100 spikes in 2012. In 1981, seeds of each sample were divided at random between the three organizations participating in the collecting (Witcombe, 2015, pers. comm.). The 2012 seed samples were conserved at NCARE and the German federal ex situ genebank of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) after collecting.

Seeds from the 1981 collecting mission were obtained from the Nordic Genetic Resource Center NordGen, where the original seed samples had been stored for the long-term in sealed aluminum foil bags, in standard household deep freezers at −18 °C. The collecting years are referred to in the following also as time point t_1 (1981) and time point t_2 (2012).

Field trial

Spontaneum accessions were grown in a standard field trial at IPK during the 2013 growing season and used for DNA extraction and phenotyping. Seeds were sown on 19 April 2013. Each sample was sown in two rows of 1.0 × 1.5 m plots. No irrigation was provided and hand weeding occurred as necessary. Plants were bagged before flowering to avoid cross fertilization with adjacent barley landrace plots and seed loss. A total of 36 accessions, 18 from each collecting year, were used for the study (Fig. 1). Sixteen individual plants per accession were randomly chosen for phenotypic and genotypic data collection. Thinning was carried out between these plants to equalize plant density within plots. Leaf tissue for DNA extractions was collected from all labelled plants, dried at 37 °C and then frozen for later DNA extraction. Sixteen phenotypic traits were measured during the growing season, at harvest, and post-harvest (Table 1) to assess relative phenotypic variation.

DNA extraction and genotyping

DNA was purified using the Qiagen DNeasy® 96 Plant Kit. 38 EST-derived SSR primers (out of 45 examined) were used for genotyping (Thiel et al. 2003; Stein et al. 2007; Varshney et al. 2007) (Online Resource 1). Loci were distributed across all seven barley chromosomes. DNA amplification and fragment size analysis was completed by the Genomics and Bioinformatics Research Group, USDA-ARS, Stoneville, Mississippi. PCR was carried out in 5-μL reactions consisting of 2–10 ng genomic DNA, 1× Qiagen Multiplex PCR Master Mix, 225 nM of each primer pair. Fragments were amplified using the following PCR profile: an initial denaturing step of 15 min at 95 °C followed by 40 cycles with denaturation at 94 °C for 30 s, annealing at 55 °C for 60 s and extension at 72 °C for 60 s. After 40 cycles, a final extension step was performed at 60 °C for 20 min. Amplification products were resolved by capillary electrophoresis on an ABI 3730XL Genetic Analyzer. Fragment sizes were calculated using GeneScan 500 (ROX) internal size standards and scored with GeneMapper software (version 5.0) (Life Technologies, Thermo Fisher Scientific Inc.).

Care was taken to mitigate scoring errors in the microsatellite data. Out of the 45 loci examined, the 38 used were chosen based on polymorphism, low drop-out rates and scoring/amplification consistency. Every sample was inspected manually for allele call fidelity. DNA from four *H. vulgare* L. accessions from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) (BCC844, BCC1500, BCC1411, BCC1390) were used as internal controls for genotype scoring. Unique positioning of controls and blanks on each 96 well plate provided checks for plate identification and orientation as well as for scoring consistency. In the rare case discrepancies were observed, the entire plate was rerun. Samples with ambiguous peaks were also rerun. Because barley, including its sub-species Spontaneum, is an inbreeding species and exhibits extreme heterozygote deficiency, deviations from Hardy–Weinberg equilibrium could not be used to indicate scoring errors. Loci with a high incidence of heterozygotes were suspect and were re-examined. There were 64 out of 21,850 (<0.3 %) missing data points in the SSR data set.

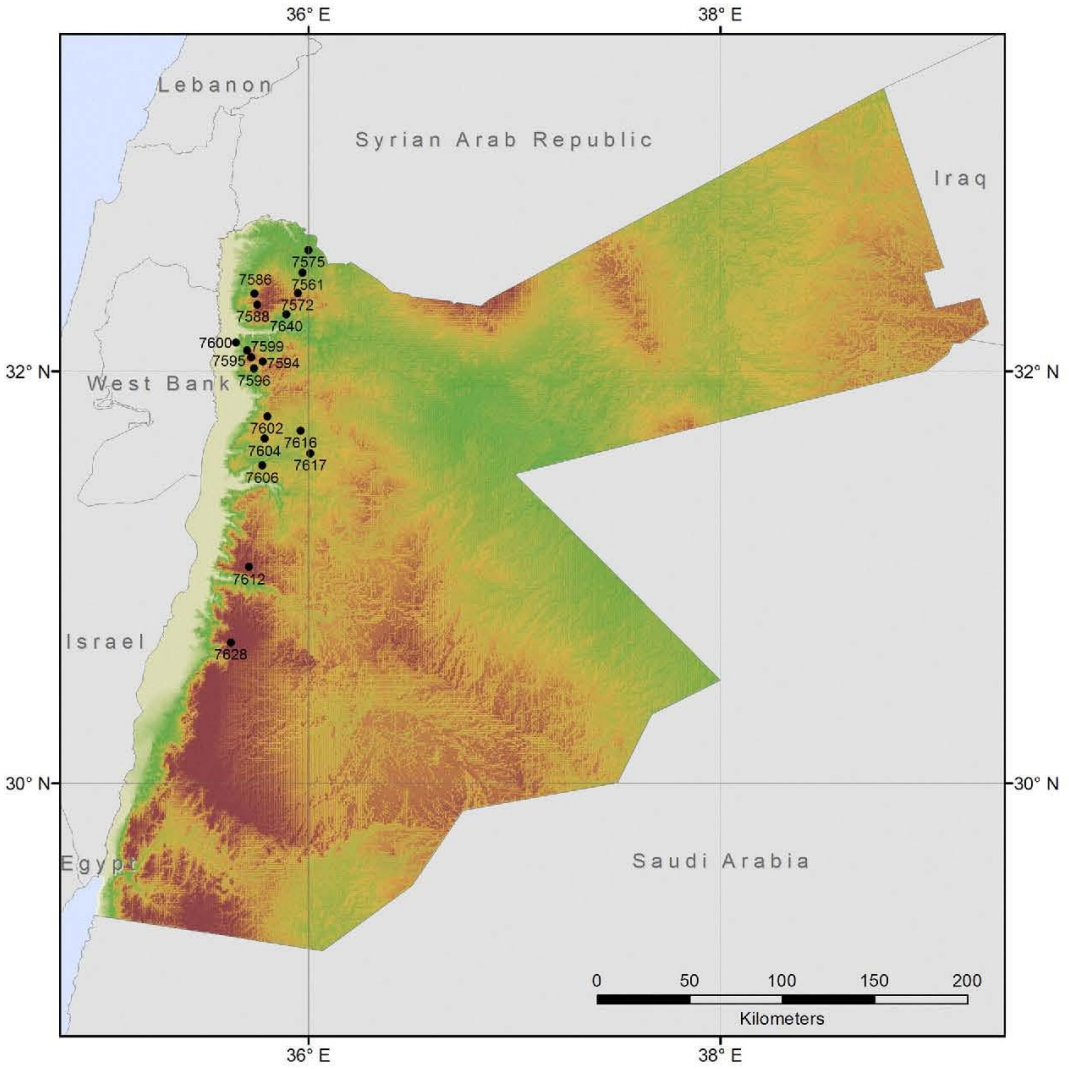

Fig. 1 Collecting sites for wild barley, *Hordeum vulgare* subsp. *spontaneum*

Statistical analyses

Genetic diversity, population differentiation and structure

Summary statistics, such as number of alleles, sample adjusted allelic richness, and observed heterozygosity were calculated with GDA (Lewis and Zaykin 2001) and FSTAT version 2.93.2 (Goudet 2001). The polymorphism information content (PIC) and the number of multi-locus genotypes was determined using GenAlEx 6.502. Population subdivision was quantified using F_{ST} and Jost's D (Jost 2008), which is an alternative to F_{ST} that is unaffected by within-population diversity (Meirmans and Hedrick 2011). D was calculated with the R package *diveRsity*, F_{ST} with FSTAT. Differentiation among populations within a collecting year as well as differentiation within each site between the t_1 and t_2 population was calculated.

Table 1 Phenotypic traits recorded during field trial, harvest and post-harvest

Trait	Level of recording	Unit/categories
Days to emergence	Population	Number of days
Growth habit	Population	Prostrate; intermediate; erect[a]
Leaf hairiness	Individual	Absent; present[a]
Stem pigmentation	Individual	Green; purple (basal only); purple (half or more)[a]
Lemma colour	Individual	Amber (=normal); tan/red; purple; black/grey; other[a]
Awn barbs	Individual	smooth; intermediate; rough[a]
Days from emergence to heading*	Individual	Number of days
Days from emergence to maturity*	Individual	Number of days
Tiller length at harvest*	Individual	cm
Number of tillers*	Individual	Number of tillers
Thousand seed weight (TSW)*	Individual	g
Seed area*	Individual	mm^2
Seed width*	Individual	mm
Seed length*	Individual	mm
Number of seeds harvested per plant*	Individual	Number of seeds
Seed yield per plant*	Individual	g

* Traits used for multivariate phenotypic analysis

[a] According to the IPGRI barley descriptors (IPGRI 2004)

Population structure was inferred using InStruct (Gao et al. 2007). InStruct extends the algorithm used in STRUCTURE (Pritchard et al. 2000) to account for self-pollination and inbreeding, common in Spontaneum. InStruct was run in mode v = 2 (infer population structure and population selfing rates) for K = 1–10. For each K, 5 chains were run, with 200,000 MCMC iterations, a burn-in of 100,000 and a thinning interval of 10 steps. Results from independent chains were summarized using CLUMPP (Jakobsson and Rosenberg 2007) and graphical representations of cluster assignments were rendered with DISTRUCT (Rosenberg 2004). ΔK (Evanno et al. 2005) was calculated to identify the appropriate number of clusters. Clustering of related populations using the neighbor-joining (NJ) method was carried out in GDA, with trees rendered in FigTree.

Isolation by distance (IBD) was estimated using the R package ecodist. Geographic distances were calculated as straight-line distances with the GeographicDistanceMatrixGenerator version 1.2.3 (Ersts Internet 2016) and log transformed. Genetic distances were calculated as $F_{ST}/(1 - F_{ST})$ (Rousset 1997). A two-tailed Mantel test was carried out with 10^5 permutations. Gene flow rates between Spontaneum populations and nearby cultivated barley was estimated using BayesAss Edition 3 (BA3) (Wilson and Rannala 2003).

Climate data analysis

Long-term high resolution daily and monthly climate data sets were generated by the GIS unit of the International Center for Agricultural Research in Dry Areas ICARDA, using European Centre for Medium-Range Weather Forecasts (ECMWF, ERA-40) with bias correction through use of the Weather Research and Forecasting (WRF) model. Spatial downscaling was done in ArcGIS to generate 1 km surfaces. These high resolution 1 km time series gridded datasets were used to extract monthly precipitation and mean temperature values for all collecting sites for 1980 to 2013 for further analysis.

In our analysis, temperature and precipitation trends over the 34-year period from 1980 to 2013 were considered. Annual and monthly precipitation and mean temperatures extracted from the climate layers were tabulated for all years. Precipitation of the

driest and wettest month and quarter, and mean temperature of the hottest and coldest month and quarter were calculated. Temporal trends were analyzed using least squares regression. These data were compared with precipitation and temperature data from Jordanian weather stations for the years 1978 to 2008.

Phenotypic analysis

All phenotypic measurements were tabulated and the 10 traits marked with * in Table 1 were used for phenotypic analysis. Given a single season of field trail in a location outside the species range, our analysis was limited to relative changes in phenotypes and did not focus on single trait values. For this reason we summarized the individual phenotypes as a multivariate statistic through principal component analysis (PCA). We used the first principal component to estimate these multi-trait phenotypes through a single value. We then used a two way ANOVA to ascribe significance to collecting year, site and their interaction. We used a one way ANOVA to estimate phenotypic variation among sites within each collecting year. PCA and ANOVAs were carried out in JMP 5.1.

Correlation analyses

Correlations between climatic, phenotypic and genetic change were tested. Correlation between geographical variables (latitude and elevation) and climatic, phenotypic and genetic change were also tested.

Genetic change was measured as F_{ST} and change in allelic richness. F_{ST} described the differentiation within each site between the t_1 and t_2 population. Change in allelic richness was expressed as difference in allelic richness at population level estimated in each site for both collecting years. A composite measure of phenotypic change was generated through a PCA on average trait differences between collecting years. The average trait value for each of the 10 traits was calculated at each site and for each collecting year. The differences within in each site between average trait values from both collecting years ($t_2 - t_1$) were subjected to PCA and the first principal component was used for regression to climatic and genetic change measures.

Similarly, PCA was used to generate a composite measure of climatic change. Annual, quarterly and monthly precipitation and temperature differences between collecting years were calculated based on values averaged over the collecting year and its preceding year, i.e. values for 1980–1981 were used for t_1 and values for 2011–2012 for t_2. The differences between the 5 precipitation and 5 temperature variables within each site were subjected to PCA and the first principal component was used for regression to phenotypic and genetic change measures. All correlations were tested using least squares regression in JMP 5.1.

Results

Climate changes in Jordan from 1980 to 2013

Precipitation values measured as annual precipitation, precipitation of wettest and driest month and quarter were showing a negative trend over the 34 year period in all 18 sites. The decreases over time in precipitation of driest month and quarter were statistically significant in most sites. Temperature increased significantly over the 34 year period in all 18 sites for all five temperature variables. Trends in precipitation and temperature are visualized and statistics tabled in Online Resource 2. These trends confirm that the precipitation and temperature values of the two collecting years are part of a broader trend and do not represent anomalous values.

Precipitation and temperature values for both collecting years are summarized in Figs. 2 and 3. Changes in precipitation and temperature values within single sites are visualized in Online Resource 3. These show that increases in annual and warmest quarter temperature as well as decrease in annual, wettest quarter and month precipitation were more pronounced in the southern sites.

Temperature and precipitation data at 16 weather stations in Jordan showed the same trends as the interpolated data at the collecting sites. Annual mean temperature measured at the weather stations trended significantly upwards, while annual precipitation fell during the period 1978 to 2008 (data not shown).

Genetic diversity

Population and collecting year specific values for genetic diversity measures are summarized in Tables 2 and 3. Overall, genetic diversity was higher

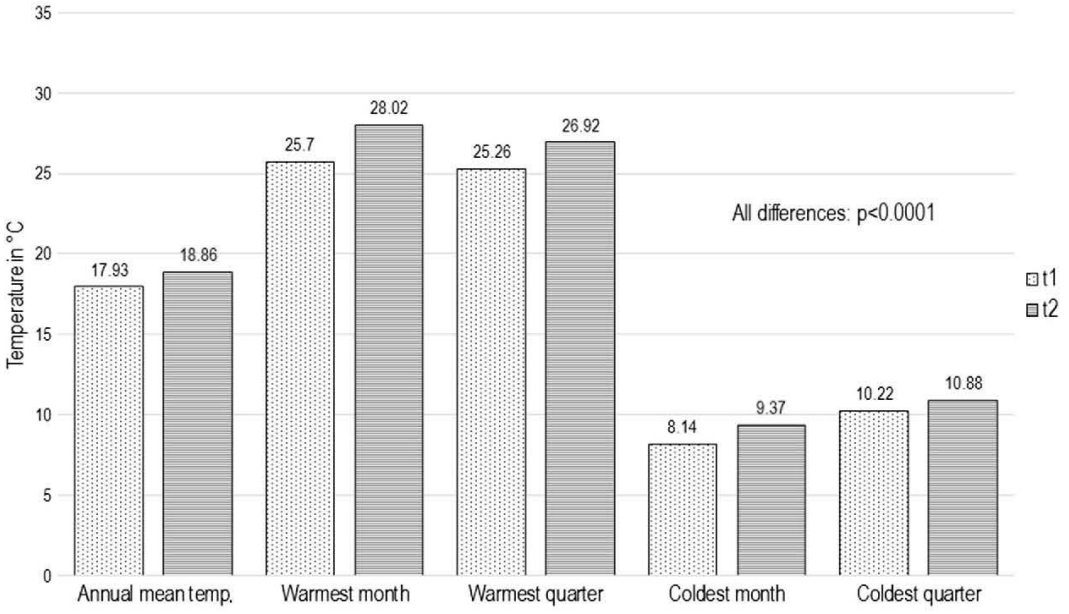

Fig. 2 Differences in temperature between collecting years, where t_1 is averaged over 1980 and 1981, t_2 over 2011 and 2012. *p* values of differences are according to Tukey–Kramer HSD tests

in 2012 than in 1981. The total number of alleles was 248 (t_1) and 273 (t_2). Twenty alleles were unique to 1981 (with frequencies between 0.002 and 0.052) and 45 alleles were unique to 2012 (frequencies between 0.002 and 0.075). The total number of multi-locus genotypes increased from 127 (t_1) to 206 (t_2). Mean allelic richness per population significantly increased from 1.98 (t_1) to 2.32 (t_2). The mean number of alleles per population significantly increased from 75.2 (t_1) to 92.6 (t_2) (Table 2). Also the average number of collecting sites in which a specific allele was found increased from 4.62 to 5.69 ($p = 0.0075$). The mean number of distinct multi-locus genotypes per population significantly increased from 7.17 (t_1) to 11.56 (t_2) (Table 2). Most multi-locus genotypes were unique to a site and collecting year. In 1981 only one genotype was found in two sites, likewise in 2012. Only two genotypes were shared among collecting years, all others were unique to their respective collecting time.

Population differentiation and structure

Population subdivision decreased as evidenced by a reduction in D and F_{ST} and by increased genetic and physical admixture. D decreased from 0.475 (t_1) to 0.423 (t_2), F_{ST} from 0.575 (t_1) to 0.441 (t_2) (Table 2). D and F_{ST} values calculated at each site between t_1 and t_2 populations varied from 0.04 to 0.38 (D) and 0.115 to 0.886 (F_{ST}) (Table 3), indicating that populations have changed to varying degrees across the study area. Physical admixture, where an individual strongly assigned to one cluster resides in a site dominated by individuals belonging to another cluster, did not occur in 1981. Four individuals, from four different populations (7575, 7588, 7604, 7616), were genetically admixed, i.e. had a cluster assignment coefficient $q < 0.8$. In 2012, two populations (7616, 7617) were physically admixed with one and two individuals respectively. Six individuals were genetically admixed, and occurred in two populations, one in population 7604 and five in population 7616.

InStruct suggested subdivision into two clusters in both collecting years. The assignments were nearly identical with the exception of two populations (7616, 7617) that 'switched' cluster between years (Fig. 4). The clusters identified by InStruct were consistent with the NJ tree (Online Resource 4). No correlation between climate or geographical

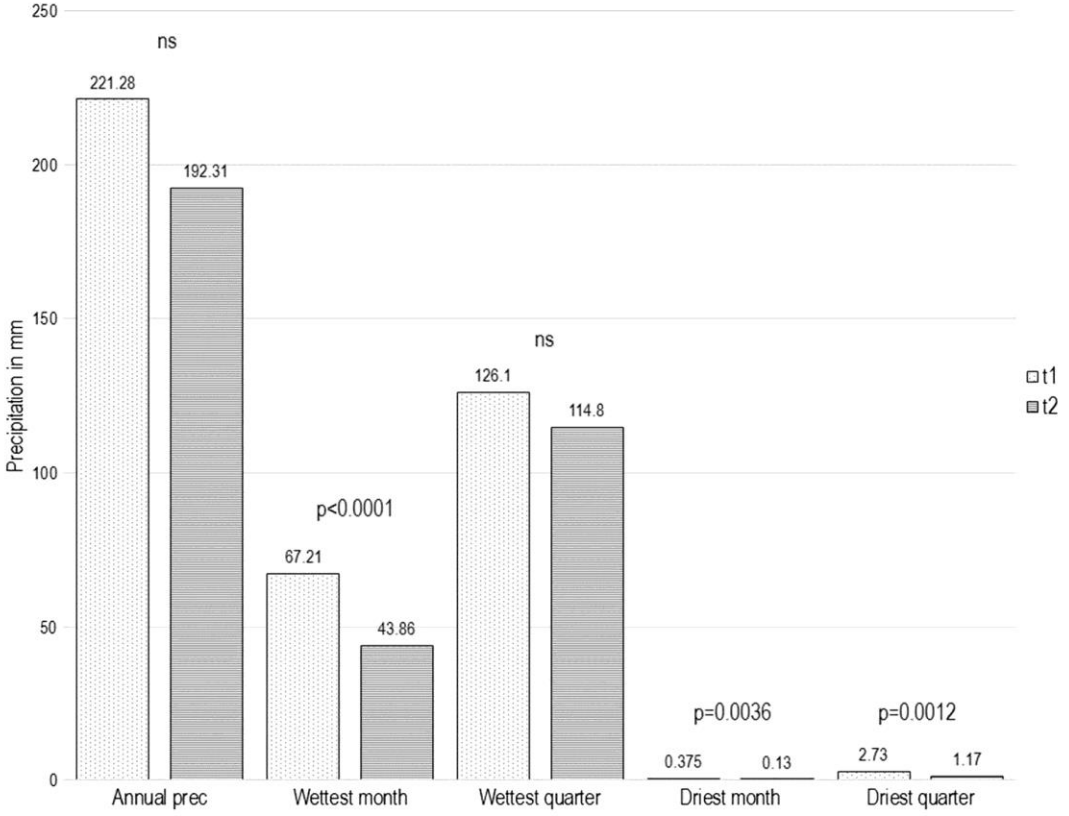

Fig. 3 Differences in precipitation between collecting years, where t_1 is averaged over 1980 and 1981, t_2 over 2011 and 2012. *p* values of differences are according to Tukey–Kramer HSD tests

Table 2 Genetic diversity measures by collecting year

Variable	1981	2012	Significance[a]
Total number of alleles	248	279	N/A
Number of alleles unique to collecting year	20	45	N/A
Total number of multi-locus genotypes	127	206	N/A
Mean allelic richness per population	1.98	2.32	0.0314
Mean number of alleles per population	75.2	92.6	0.0267
Mean number of multi-locus genotypes per population	7.17	11.56	0.0002
D	0.475 CI (95 %): 0.453–0.507	0.423 CI (95 %): 0.406–0.440	
F_{ST}	0.575 CI (95 %): 0.553–0.598	0.441 CI (95 %): 0.414–0.47	

[a] Tukey–Kramer HSD

Table 3 Genetic diversity measures by population

ID	Site	IPK Accession number 1981 samples[a]	IPK Accession number 2012 samples	D between collecting years	F_{ST} between collecting years	Allelic richness 1981	Allelic richness 2012	Multi-locus geno-types 1981	Multi-locus geno-types 2012	Alleles 1981	Alleles 2012	Alleles present in both collecting years
1	7575	HOR 22328	HOR 22645	0.0876	0.289	2.12	1.76	8	7	84	68	54
2	7561	HOR 22320	HOR 22630	0.0885	0.364	1.28	2.36	6	14	49	94	41
3	7572	HOR 22327	HOR 22641	0.1441	0.25	2.44	2.71	11	12	96	108	68
4	7586	HOR 22335	HOR 22656	0.2479	0.66	1.26	1.65	8	7	50	63	29
5	7588	HOR 22336	HOR 22657	0.2797	0.361	1.97	2.75	4	14	77	110	49
6	7640	HOR 22353	HOR 22689	0.185	0.267	2.39	2.64	7	13	97	105	71
7	7599	HOR 22340	HOR 22664	0.1815	0.308	1.89	2.66	4	14	72	106	55
8	7600	HOR 22341	HOR 22665	0.3235	0.49	2.68	1.77	13	10	105	70	44
9	7595	HOR 22338	HOR 22660	0.1566	0.19	3.06	2.54	11	13	122	100	75
10	7594	HOR 22337	HOR 22658	0.2696	0.394	1.73	2.59	7	12	66	102	43
11	7596	HOR 22339	HOR 22661	0.06	0.115	2.79	2.78	14	15	111	117	91
12	7602	HOR 22342	HOR 22666	0.1228	0.504	1.04	2.35	3	13	40	93	35
13	7616	HOR 22347	HOR 22674	0.2771	0.316	2.46	2.89	6	11	98	114	65
14	7604	HOR 22343	HOR 22667	0.1074	0.374	2.12	2.08	8	7	86	86	64
15	7617	HOR 22348	HOR 22676	0.3759	0.866	1.01	2.04	2	8	39	86	29
16	7606	HOR 22344	HOR 22668	0.1586	0.307	1.78	2.69	9	16	71	105	56
17	7612	HOR 22346	HOR 22672	0.1678	0.66	1.12	1.83	4	13	44	73	32
18	7628	HOR 22352	HOR 22684	0.0405	0.456	1.20	1.69	4	9	47	66	42

[a] Seed samples obtained from NordGen were accessioned in IPK

variables, population differentiation and cluster assignment was found.

Migration rates between Spontaneum and nearby cultivated barley were not significant in any collecting year. The 95 % credible ranges of migration rates overlapped and rates were close to zero, suggesting negligible migration (see Online Resource 5 for migration rates). No significant IBD was evident in either collecting year.

Phenotypic analysis

Qualitative traits such as leaf hairiness, stem pigmentation, lemma colour and type of awn barbs did not show any difference between populations and collecting years. Quantitative traits varied within sites and across the study area in both collecting years (Table 4 and Online Resource 6). Tiller length at harvest, number of tillers, number of seeds harvested per plant,

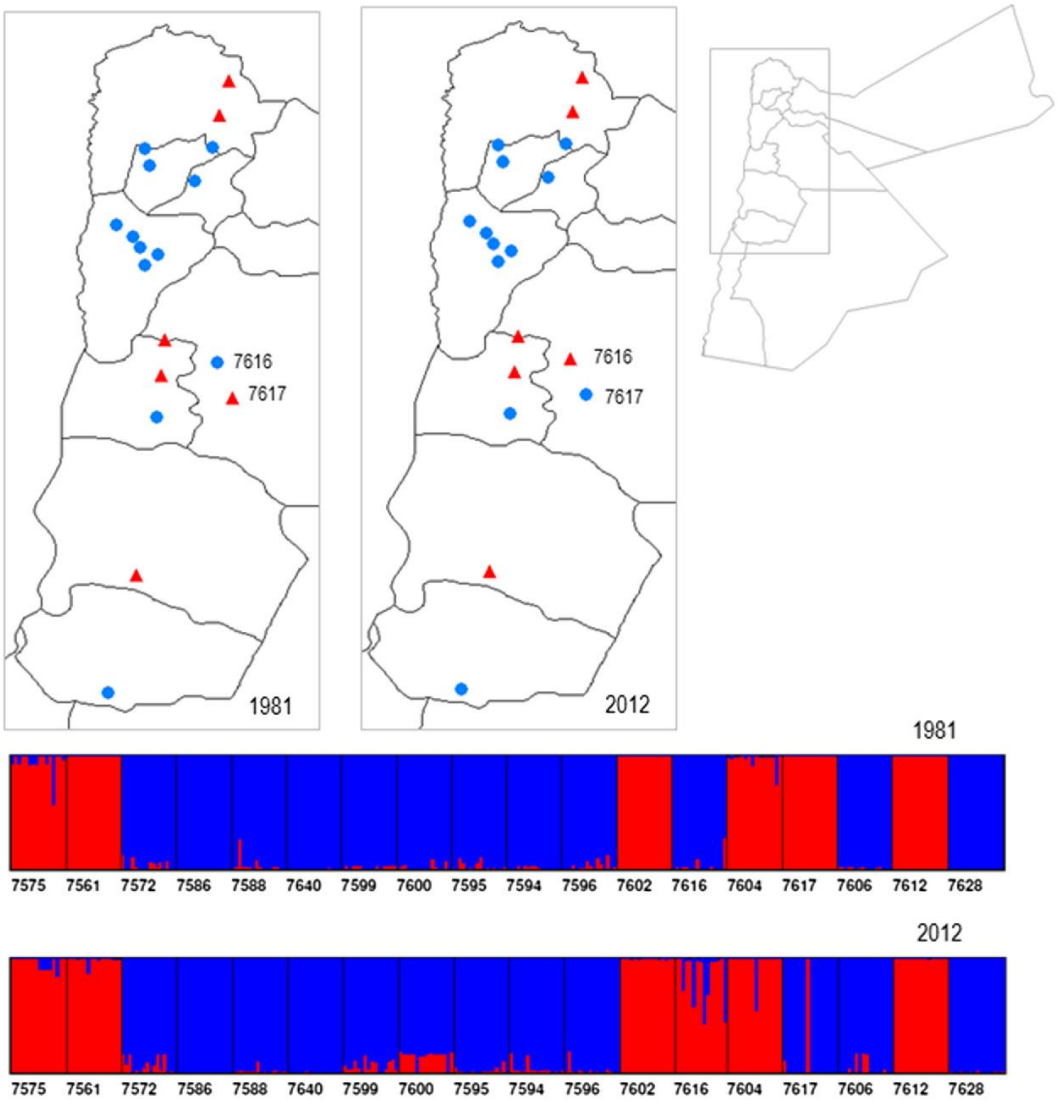

Fig. 4 Genetic clustering of Spontaneum populations in Jordan

and seed yield per plant showed comparatively larger differences between collecting years than other traits. Growth habit was largely prostrate in plants from 1981 collections, while an intermediate growth habit prevailed among collections from 2012.

The first principal coordinate, which estimated the multi-trait phenotypes, represented 37.3 % of phenotypic variation. ANOVA showed that phenotype differed among sites. This was true for both collecting years (Table 5). Considered individually, the majority of sites differed between years, but the magnitude and direction of difference was not the same across the study area (Fig. 5). Furthermore, relative change in phenotype among collecting years was significant (Table 6).

Correlation analyses

Overall climate change, expressed as the first principal component (explaining 69 % of variation) was strongly correlated with latitude ($R^2 = 0.8$, $p < 0.0001$). No other significant correlation between climate, phenotypic and genetic change, and between phenotypic or genetic change and latitude or elevation were found.

Discussion

Genetic erosion studies commonly use correlative models to assess the impact of environmental pressures on populations. These studies assume that environmental pressures tend to fragment habitats, leading to increased population structure, reduced genetic variation, and increased vulnerability to extinction (Williams et al. 2008; Urban 2015). In the present study we used resurrection and re-collection approaches (Davis et al. 2005; Franks et al. 2008) to compare genotypic and phenotypic changes over time. We compared wild barley populations from Jordan,

Table 4 Phenotypic trait values by collecting year, providing percentages for categorical variables and means followed by standard deviation for quantitative variables

Trait	1981	2012
Days to emergence[a]	9 days: 33 %	9 days: 72 %
	10 days: 67 %	10 days: 28 %
Growth habit[a]	Prostrate: 67 %	Prostrate: 22 %
	Intermediate: 33 %	Intermediate: 78 %
Leaf hairiness	Present: 100 %	Present: 100 %
Stem pigmentation	Green: 61.46 %	Green: 63.54 %
	Basal purple: 38.19 %	Basal purple: 35.76 %
	Purple (half or more): 0.35 %	Purple (half or more): 0.69 %
Lemma colour	Amber: 100 %	Amber: 100 %
Awn barbs	Rough: 100 %	Rough: 100 %
Days from emergence to heading	88.71 (30.13)	90.53 (29.48)
Days from emergence to maturity	116.64 (31.99)	118.65 (32.97)
Tiller length (cm)	75.72 (22.94)	69.95 (21.85)
Number of tillers	11.87 (8.61)	9.1 (6.69)
TSW	30.61 (6.97)	31.29 (7.65)
Seed area	35.82 (5.36)	35.7 (5.69)
Seed width	2.89 (0.25)	2.93 (0.27)
Seed length	14.15 (1.32)	13.97 (1.45)
Number of seed harvested per plant	81.69 (70.74)	62.14 (57.51)
Seed yield per plant (g)	2.5 (2.12)	1.95 (1.85)

[a] Traits 'days to emergence' and 'growth habit' were recorded at population level, all other traits at individual level

Table 5 One way ANOVA on multi-trait phenotypes for each collecting year to test effect of site within year

Source of variation	Number of parameters	Degrees of freedom	Sum of Squares	F Ratio	Prob > F
Sites in 1981	17	17	331.31	6.7	<0.0001
Sites in 2012	17	17	195.02	4.9	<0.0001

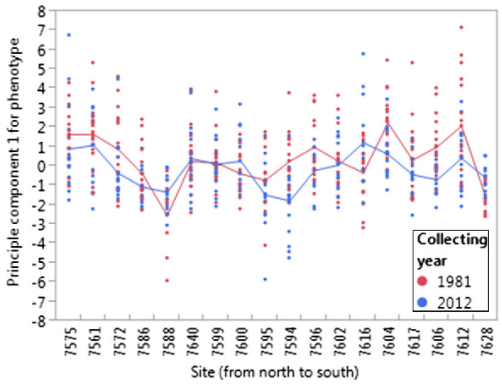

Fig. 5 Differences in magnitude and direction of phenotypic variation within sites between collecting years

collected in identical locations 31 years apart. Climate changed significantly over the study period, becoming hotter and dryer. While population structure was largely maintained, we observed an increase in genetic diversity and a concurrent reduction in population differentiation across the study area. Sampling sites showed a surprising degree of demographic dynamics and genetic diversity among years. While phenotypic variation within and among sites was comparable in both collecting years, multi-trait phenotypes differed between years. These responses are not common for populations evolving under environmental pressures. Unexpected responses to variable environments were reported also in other studies. Shaw and Shaw (2014) found that increased environmental variation can lead to an increase in variance in additive genetic fitness. Bet-hedging adaptation observed that maintenance of phenotypic and adaptive diversity is one strategy to maintain fitness in variable environments (Kind and Masel 2007; Olofsson et al. 2009).

Study system and sampling

Differences in sampling protocols between historical and contemporary collections have been suggested as possible explanations for genetic changes over time (del Rio et al. 1997; Barry et al. 2008). The sample sizes collected in 1981 and 2012 are considered sufficient to adequately represent the diversity of a population (Brown and Marshall 1995; Hoban and Schlarbaum 2014). The 1981 seed material used here represented original seed samples, which had not been regenerated and had been conserved under standard long-term storage conditions. This eliminates the possibility that genetic diversity has been affected by inappropriate regeneration and/or other management practices (Rao et al. 2006; Dulloo et al. 2008). Thus seed handling and differences in sampling protocols are not likely to explain our result of reduced genetic differentiation and increased genetic diversity. Another concern related to seed storage is the potential selective loss of genotypes during long-term conservation. To our knowledge, no study has yet demonstrated conclusively that selective mortality occurs during seed storage. *Hordeum* seeds are desiccation tolerant and comparatively long lived, and

Table 6 Two way ANOVA on multi-trait phenotypes of both collecting years to test effect of year, site and their interaction

Source of variation	Number of parameters	Degrees of freedom	Sum of Squares	F Ratio	Prob > F
Collecting year	1	1	29.15	11.13	0.0009
Site	17	17	403.42	9.06	<0.0001
Collecting year × site	17	17	129.05	2.9	<0.0001

germination percentages (under field conditions) were within a normal range (76.3 % for samples from 1981, >80 % for samples from 2012) thus major genotypic changes due to long term storage seem unlikely. Our study has furthermore included a relatively large number of sites covering a range of ecogeographical conditions to provide a good estimate of the overall distribution of diversity and population structure across Jordan. We used 38 SSRs, while 20 SSRs are considered sufficient to provide resolution and representation of diversity for genetic erosion studies (Hoban et al. 2014). The overall population structure across the study area remained the same across collecting years. We therefore assume that the collections used in this study provide comparable snapshots of diversity existing at the respective collecting time and that observed differences are not significantly affected by sampling bias or seed conservation practices.

Reduced differentiation and increased genetic diversity

Spontaneum is one of many CWR species that thrive in agricultural landscapes and in ruderal habitats (Jain 1975; Ellstrand 2003; Jarvis et al. 2015). Changes in land use, grazing intensity and movement of barley seed by farmers potentially impact its population structure. The populations analyzed in this study were exposed to a changing environment, which was not only characterized by climate change, but also by increasing human activity.

The decades between the two collecting times were characterized by increased urbanization and infrastructure development due to rapid population growth in Jordan, land use changes such as conversion of rangelands into cultivated land, and a generalized intensification of agricultural activities (Khresat et al. 1998; Al-Bakri et al. 2001, 2008; FAO 2006; NCARTT 2007). The reported number of sheep and goats, which are the predominant livestock species in Jordan, nearly doubled between 1981 and 2012, from 1,6 million to 3,0 million (FAOSTAT 2015). Because Spontaneum seeds easily adhere to animal fur, bird feathers and human clothing (von Bothmer 1992), opportunities for dispersal likely increased with the intensification and expansion of grazing and agriculture. We need to consider in this context that Spontaneum is wide spread across the study area and our study populations were and are embedded in landscapes where many other 'non-sampled' populations occur and adjacent populations could disperse into the study sites. The decrease in differentiation among our study populations, therefore, may be attributable to, and indicative of, a generalized increase in seed dispersal among local populations since 1981 and not between sampled sites.

Spontaneum belongs to a minority of plant species (~10–15 %) that are highly selfing (Goodwillie et al. 2005). Its average outcrossing rate is 1–2 % (Brown et al. 1978; Abdel-Ghani et al. 2004), but outcrossing up to 10 % has been reported (Nevo 1992). Although selfers are expected to have lower intra-population genetic diversity compared to outcrossers (Hamrick and Godt 1996), even under very high selfing rates, genetic and genotypic diversity can remain elevated (Chauvet et al. 2004; Jorgensen and Mauricio 2004; Nordborg et al. 2005; Siol et al. 2008; Hughes and Simons 2015). Spontaneum populations from the Fertile Crescent usually show high intra-population genetic diversity (Baek et al. 2003; Hübner et al. 2009), as we have observed in Jordan, and actually found an increase in genetic diversity, measured as increased allelic richness as well as increase in overall as well as population level multi-locus genotypes. Increases in the number of multi-locus genotypes over time and low numbers of shared multi-locus genotypes among populations have been observed also in other highly selfing species like *Medicago truncatula* Gaertn. (Bonnin et al. 1996; Siol et al. 2007) and *Arabidopsis thaliana* (L.) Heynh. (Abbott and Gomes 1989). The higher number of multi-locus genotypes found in 2012 might partly be explained by increased dispersal that is—as discussed above—likely to have taken place given the observed reduction in differentiation.

As Spontaneum primarily occurs alongside its domesticate in Jordan (Witcombe et al. 1982; Jaradat 1989; Nevo 1992), it is possible that the increase in allelic richness might be attributable to increased gene flow with cultivated barley. We could not, however, provide any support for this hypothesis as gene flow rates were negligible. Although gene flow from cultivated barley to Spontaneum has been reported in Israel by Hübner et al. (2012), the high level of genetic diversity present in Spontaneum consistently reported across the literature is not considered to be of recent development and influenced by introgression, but

rather it is presumed to predate the domestication of barley (Brown et al. 1978). Generally, little knowledge exists about how genetic diversity in highly selfing species is maintained (Hughes and Simons 2015). Bedada et al. (2014) found that the genetic differentiation observed in Spontaneum at Evolution Canyon in Israel would not fit a simple selectionist model and suggested that the pattern may be influenced by neutral demographic effects. Higher than expected within population genetic diversity observed in *Mycelis muralis* (L.) Wallr. was suggested to arise from efficient seed dispersal (seeds are wind-dispersed) that counteracts population turnover and thus maintains genetic diversity within populations (Chauvet et al. 2004). Temporal genotype-by-environment interaction was suggested to maintain genetic diversity over time in the obligate selfer *Lobelia inflata* L. (Hughes and Simons 2015).

Phenotypic variation

Phenotypic variation between populations was observed in both collecting years, a typical characteristic reported by other studies that investigated phenotypic diversity (Nevo et al. 1984; Van Rijn et al. 2000; Volis et al. 2000; Shakhatreh et al. 2010). We also observed a relative change in phenotype among the collecting years. Because our data do not include multi-year and multi-location data, the observed differences in phenotype could be attributable to maternal effects, as the maternal environments differed significantly (Kirkpatrick and Lande 1989; Mousseau and Fox 1998). Grime and Hunt (1975) and Poorter and Remkes (1990) found that species originating from less favorable environments have inherently lower relative growth rates than species from more favorable conditions. If current conditions in Jordan are more stressful than in the past, maternal seed provisioning may be compromised, and smaller, less fertile plants might be expected. On the other hand, Elberse et al. (2003) and van Rijn et al. (2000) found that differences in growth and morphology among Spontaneum populations from Israel were not significantly affected by maternal environments. To determine whether observed differences between collecting years are heritable adaptations or are mainly maternal effects would require a complex set of field trials in controlled environments meant to mimic conditions in 1981 and 2012. Adaptive change in quantitative traits has been reported in Spontaneum populations from Israel. Collected 28 years apart and tested under different irrigation regimes, these populations had shown a reduction in flowering time (Nevo et al. 2012).

Responses to environmental variation

Predominantly selfing species have been considered to be at higher risk of extinction than out-crossing species due to their reduced potential for adaptation (Stebbins 1957)—this is one proposed explanation for the small number of selfing species. Selfers are expected to have lower intra-population genetic diversity compared to outcrossers (Hamrick and Godt 1996), and their effective population size is half that of outcrossers. A single individual can establish a new population, decreasing genetic diversity via founder effect. Extinction and re-colonization dynamics can also lead to reduced genetic diversity in selfing species. These same demographic dynamics, however, might also be considered to reduce extinction risk because selfing species can quickly colonize and increase their range size. Hereford (2010) did not find significant differences in adaptation between selfing and out-crossing species and the adaptive potential of selfing species was identified as issue for further research (Wright et al. 2013).

The increase in genetic diversity change in phenotype observed in our study could not be explained by simple correlative analysis that links genetic and phenotypic change with climate or geographic variation. The range of observed responses to climate change has been shown to be varied and complex (Davis and Shaw 2001; Etterson and Shaw 2001; Thuiller et al. 2008; Hoffmann and Sgrò 2011; Alsos et al. 2012). The observed pattern of increased diversity, reduced differentiation and maintenance of phenotypic variation in the present study have contributed empirical evidence of an additional possible response in a CWR that is exposed to a variable and changing environment. More complex models are being developed to better predict future vulnerability and extinction risk (Williams et al. 2008; Chevin et al. 2010). These models must incorporate factors such as species interactions, dispersal differences, and evolutionary processes, to address biological and environmental complexities more realistically than simple correlative models can, and will require more empirical results to be validated.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abbott RJ, Gomes MF (1989) Population genetic structure and outcrossing rate of *Arabidopsis thaliana* (L.) Heynh. Heredity 62:411–418

Abdel-Ghani AH, Parzies HK, Omary A, Geiger HH (2004) Estimating the outcrossing rate of barley landraces and wild barley populations collected from ecologically different regions of Jordan. Theor Appl Genet 109:588–595

Akimoto M, Shimamoto Y, Morishima H (1999) The extinction of genetic resources of Asian wild rice, *Oryza rufipogon* Griff.: a case study in Thailand. Genet Resour Crop Evol 46:419–425

Al-Bakri JT, Taylor JC, Brewer TR (2001) Monitoring land use change in the Badia transition zone in Jordan using aerial photography and satellite imagery. Geogr J 167(3):248–262

Al-Bakri JT, Ajlouni M, Abu-Zanat M (2008) Incorporating land use mapping and participation in Jordan. Mt Res Dev 28(1):49–57

Alsos IG, Ehrich D, Thuiller W, Eidesen PB, Tribsch A, Schönswetter P, Lagaye C, Taberlet P, Brochmann C (2012) Genetic consequences of climate change for northern plants. Proc R Soc B 279(1735):2042–2051

Backes G, Madsen L, Jaiser H, Stougaard J, Herz M, Mohler V, Jahoor A (2003) Localization of genes for resistance against *Blumeria graminis* f.sp. *hordei* and *Puccinia graminis* in a cross between a barley cultivar and wild barley (*Hordeum vulgare* subsp. *spontaneum*) line. Theor Appl Genet 106:353–362

Baek HJ, Beharav A, Nevo E (2003) Ecological-genomic diversity of microsatellites in wild barley, *Hordeum spontaneum*, populations in Jordan. Theor Appl Genet 106(3):397–410

Barry MB, Pham JL, Béavogui S, Ghesquière A, Ahmadi N (2008) Diachronic (1979–2003) analysis of rice genetic diversity in Guinea did not reveal genetic erosion. Genet Resour Crop Evol 55(5):723–733

Bedada G, Westerbergh A, Nevo E, Korol A, Schmid KJ (2014) DNA sequence variation of wild barley *Hordeum spontaneum* (L.) across environmental gradients in Israel. Heredity 112:646–655

Bonnin I, Huguet T, Gherardi M, Prosperi JM, Olivieri I (1996) High level of polymorphism and spatial structure in a selfing plant species, *Medicago truncatula* (Leguminosae), shown using RAPD markers. Am J Bot 83:843–855

Brown AHD, Marshall DR (1995) A basic sampling strategy: theory and practice. In Guarino L, Ramanatha Rao V, Reid R (eds) Collecting plant genetic diversity: technical guidelines. International Plant Genetic Resources Institute (IPGRI), Rome; Plant Production and Protection Division, FAO, Rome; World Conservation Union (IUCN), Gland; CABI Publishing, Wallingford, pp 75–91

Brown AHD, Zohary D, Nevo E (1978) Outcrossing rates and heterozygosity in natural populations of *Hordeum spontaneum* Koch in Israel. Heredity 41(1):49–62

Chauvet S, van der Velde M, Imbert E, Guillemin ML, Mayol M, Riba M, Smulders MJ, Vosman B, Ericson L, Bijlsma R, Giles BE (2004) Past and current gene flow in the selfing, wind-dispersed species *Mycelis muralis* in Western Europe. Mol Ecol 13:1391–1407

Che YH, Yang YP, Yang XM, Li XQ, Li LH (2011) Genetic diversity between ex situ and in situ samples of *Agropyron cristatum* (L.) Gaertn. based on simple sequence repeat molecular markers. Crop Pasture Sci 62:639–644

Chen G, Li C, Shi Y, Nevo E (2008) Wild barley, *Hordeum spontaneum*, a genetic resource for crop improvement in cold and arid regions. Sci Cold Arid Reg 1:0115–0124

Chevin L-M, Lande R, Mace GM (2010) Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. PLoS Biol 8(4):e1000357

Davis MB, Shaw RG (2001) Range shifts and adaptive responses to quaternary climate change. Science 292:673–679

Davis MB, Shaw RG, Etterson JR (2005) Evolutionary responses to changing climate. Ecology 86(7):1704–1714

Del Rio AH, Bamberg JB, Huaman Z, Salas A, Vega SE (1997) Assessing changes in the genetic diversity of potato gene banks: 2—in situ vs ex situ. Theor Appl Genet 95:199–204

Dreiseitl A, Bockelman HE (2003) Sources of powdery mildew resistance in a wild barley collection. Genet Resour Crop Evol 50:345–350

Dulloo ME, Hanson J, Jorge MA, Thormann I (2008) Regeneration guidelines: general guiding principles. In: Dulloo ME, Thormann I, Jorge MA, Hanson J (eds) Crop specific regeneration guidelines [CD-ROM]. CGIAR System-wide Genetic Resource Programme (SGRP), Rome

Dulloo ME, Fiorino E, Thormann I (2015) Research on conservation and use of crop wild relatives. In: Redden R, Yadav SS, Maxted N, Dulloo ME, Guarino L, Smith P (eds) Crop wild relatives and climate change. Wiley-Blackwell. ISBN: 978-1-118-85433-4. Chapter 7:108–112

Elberse IAM, van Damme JMM, van Tienderen PH (2003) Plasticity of growth characteristics in wild barley (*Hordeum spontaneum*) in response to nutrient limitation. J Ecol 91:371–382

Ellstrand NC (2003) Dangerous liaisons? when cultivated plants mate with their wild relatives. Johns Hopkins University Press, Baltimore

Erkkila MJ, Leah R, Ahokas H, Cameron-Mills V (1998) Allele-dependent barley grain β-amylase activity. Plant Physiol 117:679–685

Ersts PJ [Internet] (2016) Geographic Distance Matrix Generator (version 1.2.3). American Museum of Natural History, Center for Biodiversity and Conservation. http://biodiversityinformatics.amnh.org/open_source/gdmg. Accessed on 19 Jan 2016

Etterson JR, Shaw RG (2001) Constraint to adaptive evolution in response to global warming. Science 294:151

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14(8):2611–2620

FAO (2006) Country pasture/forage resource profiles: Jordan. Food and Agriculture Organization of the United Nations, Rome

FAO (2013) Report of the fourteenth regular session of the commission on genetic resources for food and agriculture. CGRFA 14/13/report. http://www.fao.org/nr/cgrfa/cgrfa-meetings/cgrfa-comm/fourteenth-reg/en/

FAOSTAT (2015) http://faostat3.fao.org/home/E Statistical Division of the Food and Agriculture Organization of the United Nations, Rome

Fischbeck G, Schwarzbach E, Sobel F, Wahl I (1976) Mehltauresistenz aus Israelischen Populationen der zweizeiligen Wildgerste (*Hordeum spontaneum*). Z Pflanzenzücht 76:163–166

Frankham R, Ballou JD, Briscoe DA (2010) Introduction to conservation genetics, 2nd edn. Cambridge University Press, Cambridge

Franks SJ, Sim S, Weis AE (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. PNAS 104:1278–1282

Franks SJ, Avise JC, Bradshaw WE, Conner JK, Etterson JR, Mazer SJ, Shaw RG, Weis AE (2008) The resurrection initiative: storing ancestral genotypes to capture evolution in action. Bioscience 58(9):870–873

Gao L, Chen W, Jiang W, Ge S, Hong D, Wang X (2000) Genetic erosion in northern marginal population of the common wild rice *Oryza rufipogon* Griff. and its conservation, revealed by the change of population genetic structure. Hereditas 133(1):47–53

Gao H, Williamson S, Bustamante CD (2007) A Markov chain Monte Carlo approach for joint inference of population structure and inbreeding rates from multilocus genotype data. Genetics 176(3):1635–1651

Genger RK, Williams KJ, Raman H, Read BJ, Wallwork H, Burdon JJ, Brown AHD (2003) Leaf scald resistance genes in *Hordeum vulgare* and *Hordeum vulgare* ssp. *spontaneum*: parallels between cultivated and wild barley. Aust J Agric Res 54(12):1335–1342

Goodwillie C, Kalisz S, Eckert C (2005) The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. Ann Rev Ecol Evol Syst 36:47–79

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (Version 2.9.3). http://www.unil.ch/popgen/softwares/fstat.html

Greene SL, Kisha TJ, Yu L-X, Parra-Quijano M (2014) Conserving plants in gene banks and nature: investigating complementarity with *Trifolium thompsonii* Morton. PLoS One 9(8):e105145

Grime JP, Hunt R (1975) Relative growth rate: its range and adaptive significance in a local flora. J Ecol 63:393–422

Gur A, Zamir D (2004) Unused natural variation can lift yield barriers in plant breeding. PLoS Biol 2(10):e245

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

Hamrick JL, Godt MJW (1996) Effects of life history traits on genetic diversity in plant species. Philos Trans R Soc Lond B 351:1291–1298

Hereford J (2010) Does selfing or outcrossing promote local adaptation? Am J Bot 97(2):298–302

Hoban S, Schlarbaum S (2014) Optimal sampling of seeds from plant populations for ex situ conservation of genetic biodiversity, considering realistic population structure. Biol Conserv 177:90–99

Hoban S, Arntzen JA, Bruford MW, Godoy JA, Hoelzel R, Segelbacher G, Vila C, Bertorelle G (2014) Comparative evaluation of potential indicators and temporal sampling protocols for monitoring genetic erosion. Evol Appl 7(9):984–998

Hoffmann AA, Sgrò CM (2011) Climate change and evolutionary adaptation. Nature 470:479–485

Hübner S, Hüffken M, Oren E, Haseneyer G, Stein N, Graner A, Schmid K, Fridman E (2009) Strong correlation of wild barley (*Hordeum spontaneum*) population structure with temperature and precipitation variation. Mol Ecol 18:1523–1536

Hübner S, Günther T, Flavell A, Fridman E, Graner A, Korol A, Schmid KJ (2012) Islands and streams: clusters and gene flow in wild barley populations from the Levant. Mol Ecol 21:1115–1129

Hughes PW, Simons AM (2015) Microsatellite evidence for obligate autogamy, but abundant genetic variation in the herbaceous monocarp *Lobelia inflata* (Campanulaceae). J Evol Biol 28:2066–2077

IPGRI (2004) Descriptors for barley (*Hordeum vulgare* L.). International Plant Genetic Resources Institute, Rome

Ivandic V, Walther U, Graner A (1998) Molecular mapping of a new gene in wild barley conferring complete resistance to leaf rust (*Puccinia hordei* Otth). Theor Appl Genet 97:1235–1239

Jain SK (1975) Genetic reserves. In: Frankel OH, Hawkes JG (eds) Crop genetic resources for today and tomorrow. Cambridge University Press, Cambridge, pp 379–396

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23(14):1801–1806

Jaradat AA (1989) Diversity within and between populations of two sympatrically distributed *Hordeum* species in Jordan. Theor Appl Genet 78:653–656

Jarvis A, Lane A, Hijmans RJ (2008) The effect of climate change on crop wild relatives. Agric Ecosyst Environ 126:13–23

Jarvis S, Fielder H, Hopkins J, Maxted N, Smart S (2015) Distribution of crop wild relatives of conservation priority in the UK landscape. Biol Conserv 191:444–451

Jorgensen S, Mauricio R (2004) Neutral genetic variation among wild North American populations of the weedy plant *Arabidopsis thaliana* is not geographically structured. Mol Ecol 13:3403–3413

Jost L (2008) G_{ST} and its relatives do not measure differentiation. Mol Ecol 17:4015–4026

Khresat SA, Rawajfih Z, Mohammad M (1998) Land degradation in north-western Jordan: causes and processes. J Arid Environ 39:623–629

Kind OD, Masel J (2007) The evolution of bet-hedging adaptations to rare scenarios. Theor Popul Biol 72(4):560–575

Kirkpatrick M, Lande R (1989) The evolution of maternal characters. Evolution 43(3):485–503

Lakew B, Henry RJ, Eglinton J, Baum M, Ceccarelli S, Grando S (2013) SSR analysis of introgression of drought tolerance from the genome of *Hordeum spontaneum* into cultivated

barley (*Hordeum vulgare* ssp. *vulgare*). Euphytica 191(2):231–243
Lewis PO, Zaykin D (2001) Genetic Data Analysis: Computer program for the analysis of allelic data (version 1.1). http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php
Maurer A, Draba V, Jiang Y, Schnaithmann F, Sharma R, Schumann E, Kilian B, Reif JC, Pillen K (2015) Modeling the genetic architecture of flowering time control in barley through nested association mapping. BMC Genom 16:290
Maxted M, Guarino L (2006) Genetic erosion and genetic pollution of crop wild relatives. In: Ford-Lloyd BV, Dias SR, Bettencourt E (eds) Genetic erosion and pollution assessment methodologies: proceedings of PGR forum workshop 5, Terceira Island, Autonomous Region of the Azores, Portugal, 8–11 Sept 2004. Published on behalf of the European Crop Wild Relative Diversity Assessment and Conservation Forum, Bioversity International, Rome, pp 35–46
Maxted N, Kell SP (2009) Establishment of a global network for the in situ conservation of crop wild relatives: status and needs. FAO Commission on Genetic Resources for Food and Agriculture, Rome
McCouch S, Baute GJ, Bradeen J, Bramel P, Bretting PK, Buckler E et al (2013) Feeding the future. Nature 499:23–24
Meirmans PG, Hedrick PW (2011) Assessing population structure: F_{ST} and related measures. Mol Ecol 11:5–18
Mousseau TA, Fox CW (1998) The adaptive significance of maternal effects. Trends Ecol Evol 13:403–407
Naz AA, Arifuzzaman M, Muzammil S, Pillen K, Jeon J (2014) Wild barley introgression lines revealed novel QTL alleles for root and related shoot traits in the cultivated barley (*Hordeum vulgare* L.). BMC Genet 15:107
NCARTT (2007) The second country report on the state of the plant genetic resources for food and agriculture. The Hashemite Kingdom of Jordan. National Center for Agricultural Research and Technology Transfer NCARTT, Amman
Nevo E (1992) Origin, evolution, population genetics and resources for breeding of wild barley *Hordeum spontaneum* in the Fertile Crescent. In: Shewry PR (ed) Barley: genetics, biochemistry, molecular biology and biotechnology. CAB International, Wallingford, pp 19–43
Nevo E, Beiles A, Gutterman Y, Storch N, Kaplan D (1984) Genetic resources of wild cereals in Israel and vicinity: II—phenotypic variation within and between populations of wild barley, *Hordeum spontaneum*. Euphytica 33(3): 737–756
Nevo E, Fu YB, Pavlicek T, Khalifa S, Tavasi M, Beiles A (2012) Evolution of wild cereals during 28 years of global warming in Israel. PNAS 109(9):3412–3415
Nordborg M, Hu TT, Ishino Y, Jhaveri J, Toomajian C et al (2005) The pattern of polymorphism in *Arabidopsis thaliana*. PLoS Biol 3(7):e196
Olofsson H, Ripa J, Jonzén N (2009) Bet-hedging as an evolutionary game: the trade-off between egg size and number. Proc R Soc B 276:2963–2969
Poorter H, Remkes C (1990) Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. Oecologia 83:553–559
Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959
Rao NK, Hanson J, Dulloo ME, Ghosh K, Nowell D, Larinde M (2006) Manual of seed handling in genebanks. In Handbooks for Genebanks No. 8. Bioversity International, Rome
Repkova J, Dreiseitl A, Lizal P, Kyjovska Z, Teturova K, Psotkova R, Jahoor A (2006) Identification of resistance genes against powdery mildew in four accessions of *Hordeum vulgare* ssp. *spontaneum*. Euphytica 151:23–30
Rosenberg NA (2004) Distruct: a program for the graphical display of population structure. Mol Ecol Notes 4:137–138
Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145(4):1219–1228
Shakhatreh Y, Haddad N, Alrababah M, Grando S, Ceccarelli S (2010) Phenotypic diversity in wild barley (*Hordeum vulgare* L. ssp. *spontaneum* (C. Koch) Thell.) accessions collected in Jordan. Genet Resour Crop Evol 57:131–146
Shaw RG, Shaw FH (2014) Quantitative genetic study of the adaptive process. Heredity 112:13–20
Siol M, Bonnin I, Olivieri I, Prosperi JM, Ronfort J (2007) Effective population size associated with self-fertilization: lessons from temporal changes in allele frequencies in the selfing annual *Medicago truncatula*. J Evol Biol 20:2349–2360
Siol M, Prosperi JM, Bonnin I, Ronfort J (2008) How multilocus genotypic pattern helps to understand the history of selfing populations: a case study in *Medicago truncatula*. Heredity 100:517–525
Stebbins GL (1957) Self-fertilization and population variability in higher plants. Am Nat 91:337–354
Stein N, Prasad M, Scholz U, Thiel T, Zhang H, Wolf M et al (2007) A 1000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. Theor Appl Genet 114:823–839
Thiel T, Michalek W, Varshney RK, Graner A (2003) Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). Theor Appl Genet 106:411–422
Thormann I, Engels JMM (2015) Genetic diversity and erosion: a global perspective. In: Ahuja MR, Jain SM (eds) Genetic diversity and erosion inplants—indicators and prevention, vol 1. Springer, Berlin, pp 263–294
Thormann I, Fiorino E, Halewood M, Engels J (2015) Plant genetic resources collections and associated information as baseline resource for genetic diversity studies: an assessment of the IBPGR supported collections. Genet Resour Crop Evol 62(8):1279–1293
Thuiller W, Lavorel S, Araújo MB, Sykes MT, Prentice IC (2005) Climate change threats to plant diversity in Europe. PNAS 102(23):8245–8250
Thuiller W, Albert C, Araújo MB, Berry PM, Cabeza M, Guisan A, Hickler T, Midgley GF, Paterson J, Schurr FM, Sykes MT (2008) Predicting global change impacts on plant species' distributions: future challenges. Perspect Plant Ecol Evol Syst 9:137–152
Urban MC (2015) Accelerating extinction risk from climate change. Science 348(6234):571–573

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 Genet Resour: Charact Util 8(1):1–15

Van Rijn CPE, Heersche I, van Berkel YEM, Nevo E, Lamers H, Poorter H (2000) Growth characteristics in *Hordeum spontaneum* populations from different habitats. New Phytol 146:471–481

Varshney RK, Marcel TC, Ramsay L, Russell J, Roder MS, Stein N et al (2007) A high density barley microsatellite consensus map with 775 SSR loci. Theor Appl Genet 114(6):1091–1103

Vincent H, von Bothmer R, Knüpffer H, Amri A, Konopka J, Maxted N (2013) Genetic gap analysis of wild *Hordeum* taxa. Plant Genet Resour: Charact Util 10(3):242–253

Volis S, Mendlinger S, Orlovsky N (2000) Variability in phenotypic traits in core and peripheral populations of wild barley *Hordeum spontaneum* Koch. Hereditas 133: 235–247

von Bothmer R (1992) The wild species of *Hordeum*: relationships and potential use for improvement of cultivated barley. In: Shewry PR (ed) Barley: genetics, biochemistry, molecular biology and biotechnology. CAB International, Wallingford, pp 3–18

von Bothmer R, Jacobsen N, Baden C, Jørgensen RB, Linde-Laursen I (1995) An ecogeographical study of the genus *Hordeum*. Systematic and ecogeographic studies on crop genepools, vol 7, 2nd edn. International Plant Genetic Resources Institute, Rome

von Korff M, Wang H, Léon J, Pillen K (2005) AB-QTL analysis in spring barley. I. Detection of resistance genes against powdery mildew, leaf rust and scald introgressed from wild barley. Theor Appl Genet 111(3):583–590

von Korff M, Wang H, Leon J, Pillen K (2006) AB-QTL analysis in spring barley II. Detection of favourable exotic alleles for agronomic traits introgressed from wild barley (*Hordeum vulgare* ssp. *spontaneum*). Theor Appl Genet 112:1221–1231

von Korff M, Wang H, Léon J, Pillen K (2008) AB-QTL analysis in spring barley: III—identification of exotic alleles for the improvement of malting quality in spring barley (*H. vulgare* ssp. *spontaneum*). Mol Breed 21(1):81–93

Williams SE, Shoo LP, Isaac JL, Hoffmann AA, Langham G (2008) Towards an integrated framework for assessing the vulnerability of species to climate change. PLoS Biol 6(12):e325

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191

Witcombe JR, Bourgois JJ, Rifaie R (1982) Germplasm collections from Syria and Jordan. Plant Genet Resour Newslett 50:2–8

Wright SI, Kalisz S, Slotte T (2013) Evolutionary consequences of self-fertilization in plants. Proc R Soc B 280:20130133

3 GENERAL DISCUSSION

3.1 Genetic erosion assessment through re-collection

Monitoring of biodiversity for documenting loss of diversity, estimating genetic erosion and assessing conservation and use effectiveness are core activities of biodiversity conservation and conservation biology (Marsh and Trenham 2008). For agricultural biodiversity, the re-collection and resurrection method is particularly appropriate, as seed samples collected during germplasm collecting missions and stored in genebanks can serve as baseline material. Reference to the use of these collections was made by Maxted and Guarino (2006), van de Wouw et al. (2010) and Hoban et al. (2014). Maxted and Guarino (2006) had specifically mentioned the extensive collecting missions supported by the IBPGR and its successors, IPGRI and Bioversity International, on which the studies in this thesis are based. The documentation associated with these missions has recently been digitized, the original sample passport data has been extracted and been made available online through the Bioversity Collecting Database (BCD) (Thormann et al. 2012). This paved the way for carrying out a thorough analysis of the over 220,000 sample passport data records with a focus on their use for genetic diversity assessment, which is discussed in chapter 2.1. Special focus was placed on CWR, for which this dataset was found to be particularly relevant, as 27% of all collected and documented samples are CWR.

Some assessments of temporal variation in landrace diversity used IBPGR supported collections as baseline, such as the studies based on re-collection of sorghum and pearl millet diversity in Niger (Deu et al. 2008; Bezançon et al. 2009; Deu et al. 2010; Vigouroux et al. 2011) and the study of rice landraces in Guinea (Barry et al. 2008). The present genetic erosion study on Spontaneum in Jordan is the first study using the IBPGR collections as baseline for the assessment of genetic erosion in CWR.

Few previous studies on CWR have used re-collected samples and mainly focused on evaluating effectiveness of *ex situ* conservation and complementarity of *ex situ* and *in situ* conservation in wild potato, *Agropyron* and clover (del Rio et al. 1997; Che et al. 2011; Greene et al. 2014), or on investigating changes in flowering time as adaptive response to climate change in wild mustard, barley and wheat (Franks et al. 2007; Nevo et al. 2012). Two studies monitored genetic erosion in a wild rice population (Akimoto et al. 1999; Gao et al. 2000). A recent review of genetic monitoring studies where the same wild populations of animals or

plants were sampled over time showed that this approach is not widely used in plants (Hansen et al. 2012). Among the identified 44 studies only three regarded plant species, which were common beech in Spain (Jump et al. 2006), wild wheat in Israel (Li et al. 2001) and field mustard in the US (Franks et al. 2007).

Chapter 1.3 outlines four major activities that are required for re-collection and resurrection studies making use of past germplasm collecting missions, namely 1) selection of historical germplasm collecting mission, 2) re-collecting, 3) comparison of genetic diversity pattern within and among time points and estimation of change, and 4) identification of potential drivers of change. These activities are discussed in the following in more detail, based on the experience and results gained in the present genetic erosion study.

1) Selection of historical germplasm collecting mission

As outlined in the introduction (section 1.3), the availability of sufficiently detailed data about past collecting missions is a fundamental condition for establishing baselines. The unavailability of data and/or original seed material was reported as a limiting factor in particular for CWR (Keiša et al. 2008; Etterson et al. 2016). Where data but no historical seed samples are available, an indirect genetic erosion assessment method based on the documentation of the historic collections was proposed (Keiša et al. 2008) to estimate the threat of genetic erosion. The two fundamental characteristics of the BCD, which support its use for genetic diversity assessment, are therefore the availability of sufficiently detailed and geo-referenced sample level passport data that allows the identification of both, original collecting sites as well as genebank accessions that stem back from the collecting missions. Nearly three quarters (73%) of the CWR sample records contain geo-references. For 35 % of all collected samples, accession numbers have currently been identified in genebanks to which the samples had been sent after collecting. For many other samples passport data are sufficiently detailed to allow identification of genebank accessions.

The first fundamental step in the genetic erosion study consisted in thorough and extensive data mining, focusing not on the quantity of data available but in particular on the level of details provided about the location of the collecting sites. The BCD proved to be a very valid instrument to identify suitable collecting missions and retrieve collected germplasm in genebanks. It provides indications to which genebanks collected samples were distributed after

collecting, which is a particularly useful feature for the retrieval of germplasm. Within the context of a wider assessment of barley collecting missions and the retrieval of barley samples in genebanks, the *Hordeum* collecting mission to Jordan carried out in 1981 (Witcombe et al. 1982) was selected for the present genetic erosion study for several reasons: (i) Spontaneum and barley landrace samples were both collected during this collecting mission, in some occasions even from the same collecting site. This provided the opportunity to make better use of the investment in a re-collecting mission by collecting Spontaneum and landraces at the same time. The landraces would then be used for an analogous genetic erosion study. The same type of genetic and phenotypic data as for Spontaneum (described in chapter 2.3) was generated and the same type of analyses applied. (ii) The collecting mission had covered most parts of the Spontaneum distribution range and barley cultivation in Jordan. The samples would therefore represent a range of agro-ecological environments. (iii) The collecting mission report contained latitude and longitude data as well as location descriptions, which provided a very good basis for identification of collecting sites. (iv) Seed samples of the collection had been conserved at the Nordic Genetic Resource Center (NordGen) under long-term storage conditions and without regeneration, hence represented the most original seed samples available from this collection. Further data mining in the System-wide Information Network for Genetic Resources of the CGIAR (SINGER) database (now incorporated in the Genesys gateway to genetic resources available at www.genesys-pgr.org/) and through passport data exchange with NCARE, allowed to identify accessions deriving from this collecting mission also in ICARDA and NCARE. The material had been repatriated from ICARDA to Jordan after opening of the national genebank at NCARE in Amman in the late 1990s. The samples in these two genebanks would have represented fallback collections, in case of unavailability of samples from NordGen.

2) Re-collecting

The preparation of a re-collecting mission requires as a minimum the geographical identification of the original collecting sites. Availability of any additional information about the original collecting, in particular the sampling strategy that determined sample size and method of sampling, will help to minimize differences between the original and re-collected samples that potentially could influence the diversity collected. Based on the available georeferences and location data, and advice provided by the original collector John Witcombe, it was assessed with the use of gazetteers and Google Earth how well the coordinates and the

location description matched each other and all historical collecting sites could be identified. In most cases there was a fairly good match. In cases of discrepancies between coordinates and location description, location description was preferred over coordinates as recommended by Witcombe. NCARE researchers carried out scouting trips to various regions in April and early May 2012 to verify existence of sites, to anticipate the best collecting time and to set up an appropriate itinerary for the collection expedition. Anticipated ripening stages in the various regions suggested to start collecting in the south of Jordan as seeds are expected to ripen earlier there. Then the sites located in the west, north and east of Amman would be collected. During re-collecting, in addition to the bulk samples, single spikes of at least 12 randomly chosen individuals from each Spontaneum population were collected to carry out a detailed analysis of the contemporary genetic diversity present in Jordan (focus of chapter 2.2). The original collecting in 1981 had targeted only sites in disturbed habitats and field margins. In addition to re-collecting from original collecting sites, samples were taken also in reserves managed either by the Ministry of Agriculture or the Royal Society for Conservation of Nature.

Differences in sampling protocols between historical and contemporary collections have been suggested as possible explanations for genetic changes over time (del Rio et al. 1997; Barry et al. 2008). It is therefore relevant to seek information about sampling strategies employed in the historical collection to avoid introduction of potential sampling bias during re-collecting. Information about the sampling strategy was not contained in the original collecting documentation from 1981, but indications were provided by Witcombe (Witcombe 2015, personal communication). Populations in 1981 were generally abundant, sometimes very large. Seed samples were collected from the entire population and contained up to 200 spikes, as collected material needed to be split after collecting among three participating institutions. Samples of up to 100 spikes were collected from each site in 2012 sampling the entire population. These sample sizes were considered sufficient to adequately represent the diversity of a population (Brown and Marshall 1995; Hoban and Schlarbaum 2014) and sampling bias was ruled out as possible reason for observed changes.

Prior informed consent (PIC) is required today for each plant collecting exploration (Moore and Williams 2011). Access to PGR is under the control of the national government in countries that are parties to the CBD, but PIC is also required in countries that do not have specific national legislation on access to genetic resources. PIC needs to be requested from respective

national authorities in the country where the collecting should take place. For re-collecting in Jordan, a formal letter of agreement (LOA) had been stipulated between Bioversity International and NCARE, which detailed the purpose of the collecting and NCARE's active involvement. The LOA was considered by national authorities as sufficient to grant permission to collect. It might however be useful at the stage of selecting a collecting mission, to obtain preliminary information about the possibility and the required timeframe to obtain PIC from the target country, in particular for countries that are not party to the ITPGRFA and where access is known to be difficult.

The resurrection approach relies on the ability to revive a random sample of genetic diversity present in the historical population and on the comparability between the historical and contemporary sample. While sampling bias was discussed earlier, seed handling during storage and potential selective loss of genotypes during long-term conservation are further caveats about re-collection and resurrection studies. Inappropriate seed handling or regeneration can affect genetic integrity of accessions (Rao et al. 2006; Dulloo et al. 2008a). As the 1981 seed material used in the genetic erosion study represented original seed samples, which had not been regenerated and had been conserved under standard long-term storage conditions, any impact on genetic diversity through inappropriate seed handling was ruled out. Regarding potential selective loss of genotypes, no study has yet demonstrated conclusively that selective mortality occurs during seed storage. *Hordeum* seeds are desiccation tolerant and comparatively long lived, and germination percentages (under field conditions) were within a normal range (76.3% for samples from 1981, > 80% for samples from 2012) thus major genotypic changes due to long term storage seemed unlikely in the present study.

3) Comparison of genetic diversity pattern within and among time points and estimation of change

The most common molecular markers currently used to assess genetic diversity in plants are microsatellites and they have proved to be very informative also in *Hordeum* (Matus and Hayes 2002). It is estimated that 20 SSRs and 50 individuals from two collecting times can provide sufficient basis to detect genetic erosion (Hoban et al. 2014), while power of detecting change increases with increase in individuals as well as in markers. The genetic erosion study used a set of 38 SSRs, which have previously been used in other studies on wild and cultivated barley

(Hübner et al. 2009; Haseneyer et al. 2010). Populations stemming from 18 collecting sites were compared and 16 individuals per population were genotyped, providing a total of 288 individuals per collecting time. The availability of 18 populations for each collecting time allowed to go beyond a comparison of the overall genetic diversity present in each collecting time. The relatively large number of sites distributed across the range of Spontaneum in Jordan was used to also assess population structure, distribution of diversity across the study area and genetic change in each collecting site.

The studied populations were grown in a common garden in IPK, which allowed to collect also phenotypic data. Given that the common garden was limited to a single season, and was carried out in one location only outside the study area, the analysis was limited to relative changes in multi-trait phenotypes and did not focus on single trait values. In general, re-collection studies provide very useful material for investigating single adaptive traits (see Franks et al. (2007) and Nevo et al. (2012) as examples for flowering time). Experiments under controlled conditions or in multiple site-by-season combinations are then required.

4) Identification of potential drivers of change

Data on factors impacting populations and potentially presenting threats to their genetic diversity can be systematically recorded during collecting. The measures to estimate the threat of genetic erosion first developed by Goodrich (1987), modified by Guarino (1995) and adapted to specific studies by De Oliveira and Martins (2002) and by Keisa et al. (2008) can serve as useful basis. When cultivated species are collected for comparison, household surveys and farmer interviews (Peroni and Hanazaki 2002; Davari et al. 2013) are useful tools to collect information that can support the analysis of the comparison and interpretation of results. Especially the latter requires extensive advanced preparation and additional time.

In several of the historical collecting sites that were visited in 2012, Spontaneum populations did not exist anymore or barley cultivation had been abandoned most likely due to urbanization, change from field crop to fruit tree cultivation or land degradation. As climate is expected to influence wild plant populations, particular emphasis was placed on climate data. Rainfall and temperature time series data was obtained both from ICARDA's GIS unit as well as Jordanian national weather stations to verify if potential changes in climate have had an impact on any observed changes in the studied populations. Based on the results obtained in the present study

it is suggested to include the collection of information about agricultural and seed exchange practices used in the area of study in future assessments, when CWR are targeted which thrive in disturbed habitats and agricultural areas. These practices are an important variable that can affect population structure and diversity and should be considered in conservation actions (Mariac et al. 2006; Thormann et al. 2016b).

3.2 Structure of genetic diversity in wild barley

Geographical distribution of diversity in Spontaneum has mostly been studied across two or more countries. Jakob et al. (2014) studied population structure at a macro-geographical level across the natural distribution range from the Mediterranean to the Middle East. They identified three population clusters, one in the Levant (including Israel, Lebanon, Jordan, Syria, and Greece), one in Turkey and one east of Turkey (including Iran, Uzbekistan, Tajikistan). The cluster in the Levant was identified as the most genetically diverse, as had also been found in previous studies on geographic pattern across the distribution range from Israel to Tajikistan (Morrell et al. 2003), on a comparison between populations from Turkmenistan and the Middle East (Volis et al. 2001), as well as in a study on ecological niche modeling of Spontaneum, covering a range from North Africa, through the Fertile Crescent, into Central Asia (Russell et al. 2014).

A number of studies investigated the correlation of geographic pattern of diversity at single loci with single ecogeographical variables, mostly related to rainfall or temperature (e.g. Nevo et al. 1979; Nevo et al. 1986a; Nevo et al. 1986b; Dawson et al. 1993; Nevo et al. 1998; Turpeinen et al. 2001; Ivandic et al. 2002; Baek et al. 2003; Ozkan et al. 2005; Batchu et al. 2006). Very few analyses of population structure exist like the study on Spontaneum populations in Israel by Hübner et al. (2009). They studied 50 populations, using 42 SSRs, attempting to correlate genetic population structure—as opposed to polymorphism or allele frequencies at individual loci—with climate variables. Their study identified seven genetic clusters across their investigation area in Israel and suggested that a combination of elevation, midday temperature and rainfall has contributed to shaping population structure of Spontaneum in Israel. No similar study had been carried out on populations from Jordan.

The study presented in chapter 2.2 (in the following referred to as current structure study) was therefore specifically conceived to investigate the current population structure in Jordanian Spontaneum and hence to provide a reference to the subsequent study on genetic erosion in Spontaneum diversity in Jordan, subject of chapter 2.3. For this purpose the re-collecting in 2012 was extended in two ways. Samples were not only collected from historical collecting sites, as those were mainly located in disturbed habitats. Samples were also collected from sites located in reserves managed by the Ministry of Agriculture (MoA) or the Royal Society for Nature Conservation (RSNC), thereby including populations from undisturbed habitats. Furthermore, in addition to bulk samples collected for comparison with historical samples, single spikes were collected to constitute a set of single maternal lines for the analysis of current population structure in Jordan as a baseline.

Spontaneum diversity in Jordan had been studied by Jaradat (1991, 1992), Baek et al. (2003), Sharma et al. (2004), Al-Saghir et al. (2009), Shakhatreh et al. (2010, 2016). All studies revealed high variability in Spontaneum populations. The most recent studies were carried out on 16 populations collected in 1996, using 18 SSRs (Baek *et al.* 2003) and on a set of 103 accessions, sourced from the ICARDA genebank (without information about collecting dates), using 11 SSRs (Shakhatreh et al. 2010; Shakhatreh et al. 2016). None of these studies inferred population structure within their respective samples from the genetic data. The former addressed the correlation of allele frequencies at specific loci with single climate variables such as annual rainfall or mean January and August temperatures. Gene diversity at some loci was found to be correlated with altitude, rainfall and temperature. The recent study by Shakhatreh et al. (2016) had grouped the 103 wild barley accessions a priori in six populations according to latitude, longitude, altitude and rainfall, hence no structure was inferred from the genetic data.

Differently to these preceding studies, the current structure study aimed to describe the patterns of genetic diversity and population structure of 32 populations. All individuals were genotyped with 37 SSRs. Genetic diversity in the analyzed populations was found to be higher within than among populations, confirming previous findings in Jordan (Baek et al. 2003; Shakhatreh et al. 2010; Shakhatreh et al. 2016). Populations were characterized by a high number of multi-locus genotypes of which very few were shared among sites, by admixture and high allelic richness. Genetic diversity was concentrated in the northern part of the study area. The analysis of

population structure suggested that the 32 populations could be divided into three major, genetically differentiated clusters. These clusters were distributed along a longitudinal gradient in the North (clusters 1 and 2), with a distinct cluster (cluster 3) in the South. Like in Israel (Hübner et al. 2009) large scale IBD was evident across the study area, but environmental variation was found to have no effect. At a slightly smaller scale, excluding the small southern cluster and focusing on the central and northern part of the study area, an environmentally heterogeneous landscape, neither geographical nor environmental distance was correlated to genetic distances.

The temporal comparison of 18 populations discussed in the genetic erosion study in chapter 2.3 also showed higher within than among population diversity and high number of multi-locus genotypes, of which very few were shared among sites, both in 1981 and 2012. Similar observations were made by Shakhatreh et al. (2016) who reported a high number of alleles unique to single genotypes. Nevo et al. (1986a, b) described a mosaic pattern of allele distribution in Spontaneum in Israel, Turkey and Iran with alleles being unique or locally common to a population or region, or widespread and common, but hardly any ubiquitous alleles among different regions. Low numbers of shared multi-locus genotypes among populations have been observed also in other highly selfing species like *Medicago truncatula* Gaertn. (Bonnin et al. 1996; Siol et al. 2007) and *Arabidopsis thaliana* (L.) Heynh. (Abbott and Gomes 1989).

The distribution and structure of genetic diversity in wild plant populations is commonly expected to be correlated with ecogeographical variation. Based on this hypothesis, ecogeographical data has been used for example to identify areas and populations for *in situ* conservation (Dulloo et al. 2008b; Parra-Quijano et al. 2012; Maxted et al. 2013), to assemble core collections (Parra-Quijano et al. 2011) and to identify germplasm potentially useful for crop improvement (Khazaei et al. 2013; Thormann et al. 2014). The current structure study investigated therefore also whether ecogeographical variation would explain observed distribution and structure of genetic diversity, based on this hypothesis.

The climate data used for the study was downloaded from the WordClim database, a global and freely available resource for climate data. It is a common source for climate data used in gap analysis studies, species distribution modelling and other ecogeographical studies, based on the inherent assumption that they are robust proxies for genetic data, which is often not available.

Genetic structure in Spontaneum was not correlated to climate variation as represented by these global climate layers. Given the low gene flow and very localized gene transfer, a characteristic for highly self-pollinating species such as Spontaneum, genetic structure has however been found at very local scale (Nevo 1986; Volis et al. 2002b;Yang et al. 2009; Nevo 2014). This local scale structure though is very unlikely to be detected by the commonly used landscape scale climate and spatial layers. Landscape scale climatic data would thus not be very useful for predicting distribution of genetic diversity in Jordan.

Spontaneum is one of many CWR species that thrive in agricultural landscapes and in ruderal habitats (Jain 1975; Ellstrand 2003; Jarvis et al. 2015). This ruderal habitat preference might also explain why the general expectation of tight correlation between genetic and ecogeographical diversity was not observed. Spontaneum occurs in disturbed habitats and in sympatry with cultivated barley. These habitats favor anthropogenic movement of material—inclusion and transport with cultivated barley seed lots or hitchhiking on livestock fur or human clothing—which interferes with natural diffusion and selection processes. This may alter the expected distribution of genetic diversity across the landscape and lead to weak or nonexistent correlations between ecogeographical and genetic diversity as found in the study. Natural dispersal and selection processes appeared not to be the principle force shaping the genetic structure of Spontaneum in some regions of Jordan. Similar observations and conclusions were drawn from the genetic erosion study discussed below.

3.3 Temporal variation in wild barley

A relatively limited number of studies on CWR exist that compare samples collected from the same or analogues locations at different points in time. These studies are very diverse regarding temporal sampling protocols, number of sites and purpose of the studies. Purposes span from evaluating effectiveness of *ex situ* conservation in genebanks and botanic gardens and the complementarity of *in situ* and *ex situ* conservation (del Rio et al. 1997; Che et al. 2011; Rucinska and Puchalski, 2011; Lauterbach et al. 2012; Greene et al. 2014), to investigating changes in adaptive traits as response to climate change, in both cases flowering time (Franks et al. 2007; Nevo et al. 2012) and monitoring of genetic diversity to understand extent of genetic change and erosion as well as ecological and genetic processes affecting population biology (Akimoto et al. 1999; Gao et al. 2000; Gomma et al. 2011). Time frames span from a focus on

a short time period of a few years (Franks et al. 2007) to several decades (Del Rio et al. 1997; Che et al. 2011; Nevo et al. 2012; Lauterbach et al. 2012). Geographic extension ranges from very few sites – even a single site (Gao et al. 2000; Franks et al. 2007; Greene et al. 2014) to over 20 sites in Northern China (Che et al. 2011). Studies based comparisons primarily on molecular marker data. No phenotypic data were included, except for the studies that focused specifically on an adaptive trait change.

Given the diverse approaches, comparison among studies is difficult. This applies also to a comparison of the genetic erosion study with a similar investigation that addressed changes over time in Spontaneum in Israel. Historical and contemporary samples of 10 populations collected 28 years apart were compared (Nevo et al. 2012). Although the composition of the study material is similar, the protocols for validating the locations and sampling methods, the *ex situ* storage conditions, regeneration cycles and germination rates are not provided by Nevo et al. (2012). Data for allele counts is analyzed in a similar way, but results are different. A reduction of alleles was observed in Israel, an increase was recorded in Jordan. While the Israeli study focused on the allele states at a modest number of SSR loci, the genetic erosion study sought to estimate diversity and differentiation as a multi-locus measure using F_{ST} (Wright's measure of differentiation among populations) and D_{ST} (Jost's relative measure of differentiation). Treatment of phenotypic data differed considerably. The Israeli study was set up to explore the temporal effect of one specific trait, i.e. flowering time, thought to influence fitness during climate change. The genetic erosion study looked at change over time as relative, multivariate data to identify any relative change in morphology. Given a single season of field trial in a location outside the study range, focus on particular change in a single trait value was not reasonable.

The comparison of the Jordanian Spontaneum populations collected in 1981 and 2012 showed that population structure was largely maintained across the study area. On the other hand, the populations collected in 2012 showed higher genetic diversity and a concurrent reduction in population differentiation across the study area, compared to the 1981 populations. Phenotypic variation within and among sites was comparable in both collecting years, but multi-trait phenotypes differed between years. The climate changed significantly over the study period becoming hotter and dryer. However, the observed changes could not be explained by

commonly used correlative analyses that link genetic and phenotypic change with climatic or geographic variation, similarly to the results discussed in the previous section.

As in the discussion of current structure in Spontaneum, it needs to be considered here that Spontaneum thrives in agricultural landscapes and ruderal habitats (Jain 1975; Ellstrand 2003; Jarvis et al. 2015). Changes in land use, grazing intensity and movement of barley seed by farmers can potentially impact its population structure. The presence and spread of weedy pearl millet relatives for example was found to be influenced by farmers' seed exchange practices in Niger (Mariac et al. 2006). The decades between the two collecting missions in 1981 and 2012 in Jordan were characterized by increased urbanization and infrastructure development due to rapid population growth, land use changes and a generalized intensification of agricultural activities including a doubling of livestock (Khresat et al. 1998; Al-Bakri et al. 2001; FAO 2006; Al-Bakri et al. 2008; NCARTT 2007; FAOSTAT 2015). Because Spontaneum seeds easily adhere to animal fur, bird feathers and human clothing (von Bothmer 1992), opportunities for dispersal likely increased with the intensification and expansion of grazing and agriculture. Spontaneum is widespread across the study area and the study populations were and are embedded in landscapes where many other 'non-sampled' populations occur, and adjacent populations could disperse into the study sites. The decrease in differentiation among the populations, therefore, may be attributable to, and indicative of, a generalized increase in seed dispersal among local populations since 1981.

The common garden planted at IPK in 2013 allowed to describe multi-trait phenotypes and assess whether any relative change in multi-trait phenotype can be observed. As data was generated only for a single season in one location, no single trait values were assessed. The data indicated a significant relative change in phenotype, where plants from 2012 had shorter and less tillers, and produced less seeds than in 1981. Additional common gardens will be required to investigate whether the observed shift was mainly due to maternal effects or presents heritable adaptations.

The increase in genetic diversity was a rather unexpected response for populations evolving under environmental pressures. It could not be explained through geneflow between Spontaneum and cultivated barley, as geneflow rates were found insignificant. Other studies observed that increased environmental variation can lead to an increase in variance in additive

genetic fitness (Shaw and Shaw 2014) or to maintenance of phenotypic and adaptive diversity as a strategy to maintain fitness (Kind and Masel 2007; Olofsson et al. 2009).

The increase in genetic diversity and change in phenotype observed in the genetic erosion study have shown that responses to environmental variation can be compound and multi-faceted and require more complex models, as they could not be explained by the common correlative analysis that links genetic and phenotypic change with climate or geographic variation. More complex models are being developed to better predict future vulnerability and extinction risk (Williams et al. 2008; Chevin et al. 2010). These models must incorporate factors such as species interactions, dispersal differences, evolutionary processes, and—in cases of CWR growing in agricultural areas—agricultural practices and seed exchange mechanisms, to address biological and environmental complexities more realistically, and will require more empirical results to be validated.

3.4 Future prospects

The inherent complexity of genetic erosion, the dynamism and broad range of possible threats, and the lack of comparable quantitative assessments due to widely varying assessment methods and scales, are reasons for the difficulty in producing comparable measurements and drawing a clear picture of the overall status and trends in genetic erosion in PGRFA. Genetic erosion assessments should therefore be addressed in a more systematic way, which includes regular monitoring in agricultural and natural systems, and harmonized and standardized estimation of trends and impact.

The re-collection and resurrection approach is an effective method that is used to study evolutionary and plastic responses of plant populations to environmental change (Franks et al 2014). Here it was applied successfully to study genetic erosion in Spontaneum originating from Jordan, revealing an unexpected pattern of response (increased diversity, reduced differentiation and maintenance of phenotypic variation) in a CWR that is exposed to a variable and changing environment. The same approach has been used to study temporal changes in the barley landraces that were collected during the same collecting mission in 1981 and 2012 in Jordan and has proved equally effective in assessing changes. The data on the landrace comparison has been submitted for publication.

Spontaneum belongs to the group of CWR that thrive in disturbed habitats and agricultural areas, where the range of factors shaping their population structure and impacting on their evolution are different from those affecting CWR in pristine habitats. The results of the studies suggest that in future investigations on CWR growing in disturbed habitats and agricultural areas, agricultural and farmer practices should also be investigated to further inform interpretation of results and conservation actions that might be required to maintain the diversity in the species under study.

The analysis of the data in the BCD (Thormann et al. 2015) has shown that there are many collections that could serve as baseline for studies similar to the genetic erosion study here presented on Spontaneum from Jordan. Further similar studies on other CWR species as well as on Spontaneum collected in other countries will contribute to improving our knowledge about the extent, causes and mechanisms leading to genetic erosion and micro evolutionary responses to environmental changes over time. The BCD could be harnessed to increase the still relatively small amount of empirical data about the likely rates and types of plant responses to changing climatic and other environmental conditions which is required for more effective conservation and for making species and trait-specific predictions.

4 SUMMARY

Crop wild relatives (CWR) have been recognized as important source of genetic diversity and adaptive traits for plant breeding and climate change adaptation over the past decades. Their *in situ* diversity though is threatened by climate change, invasive species, land degradation and other anthropogenic factors. Knowledge about occurrence and distribution of CWR genetic diversity is insufficient and genetic erosion in CWR is ongoing.

In this context, the purpose of this study was to assess the current pattern of diversity and its changes over time *in situ* in the primary wild barley relative *Hordeum vulgare* subsp. *spontaneum* (K. Koch) Thell. (referred to as Spontaneum hereafter) in Jordan, which is located in the center of diversity of this species, and where barley plays a critical economic role, especially in marginal areas. The re-collection and resurrection approach was applied to study genetic erosion in CWR, where ancestral and descendant genotypes sampled from the same location are grown together in a common environment. Maternal lines sampled from 32 populations during the re-collection were analyzed to assess the current genetic structure in Spontaneum. The contemporary and past amount and pattern of diversity of 18 populations sampled from the same locations in Jordan in 1981 and 2012 were then compared and the extent, direction and potential drivers of changes investigated.

The Collecting Mission database (BCD) hosted by Bioversity International was analyzed and used as resource to identify and retrieve data about past Spontaneum collecting missions and to select the 1981 *Hordeum* collecting mission to Jordan. This database provides original passport data and related documentation for samples of CWR and landraces collected during more than 1,000 collecting trips in 136 countries mainly from 1975 – 1995. The 1981 collecting sites were resampled in 2012 and material from both collections grown in a common garden at the German Federal Genebank of the Leibniz Institute for Plant Genetics and Crop Plant Research (IPK) in 2013.

The analysis of current population structure identified three distinct genetic clusters. Populations were characterized by admixture and high allelic richness, and genetic diversity was concentrated in the northern part of the study area. Correlation analyses showed large scale isolation by distance (IBD) across the study area but did not reveal a correspondence between climate and genetic structure. Spontaneum prefers disturbed, human-made or influenced

habitats, sympatric with its domesticate. These habitats favor anthropogenic movement of material—inclusion and transport with cultivated barley seed lots or hitchhiking on livestock fur or human clothing—which interferes with natural diffusion and selection processes. This may alter the expected distribution of genetic diversity across the landscape and may have led to the observed weak or nonexistent correlations between ecogeographical and genetic diversity. Natural dispersal and selection processes have therefore probably not been the principle force shaping genetic structure in Jordan.

The comparison of populations collected in 1981 and 2012 showed that population structure was largely maintained across the study area. However, the populations from 2012 showed higher genetic diversity and a concurrent reduction in population differentiation. Phenotypic variation within and among sites was comparable in both collecting years, but multi-trait phenotypes differed between years. The climate changed significantly over the study period becoming hotter and dryer. The observed increase in genetic diversity and change in phenotype showed that responses to environmental variation can be compound and multi-faceted. They could not be explained by common correlative analyses that link genetic and phenotypic change with climate or geographic variation and require more complex models to interpret results and better predict vulnerability and extinction risk. The changing environment to which the populations were exposed, was not only characterized by climate change, but also by increasing human activity, and opportunities for dispersal likely increased with the intensification and expansion of grazing and agriculture. The decrease in differentiation may, therefore, be attributable to, and indicative of, a generalized increase in seed dispersal among local populations since 1981.

The results suggest that in future studies on CWR growing in disturbed habitats and agricultural areas, agricultural and farmer practices should also be investigated to further inform interpretation of results and development of conservation actions that might be required to maintain the diversity in the species under study.

The re-collection and resurrection method has shown to be effective in assessing change over time in Spontaneum and the BCD provides data for many more CWR collections to which this approach could be applied to generate more data and knowledge about extent of genetic erosion and the impact of factors which is required to improve monitoring and conservation actions.

5 ZUSAMMENFASSUNG

Die genetische Vielfalt der verwandten Wildarten der Kulturpflanzen (Crop Wild Relatives; CWR) stellt eine immer wichtiger werdende Ressource für die Pflanzenzüchtung und Anpassung an den Klimawandel dar. Die CWR Vielfalt in situ ist jedoch von Klimaveränderung, invasiven Arten, Bodenverschlechterung und anderen anthropogenen Faktoren bedroht. Die Kenntnisse über das Vorkommen und die Verteilung der genetischen Vielfalt in CWR ist unzureichend und die genetische Erosion in CWR schreitet fort.

In diesem Zusammenhang war das Ziel dieser Studie, die heutige Struktur der genetischen Vielfalt in der Gerstenwildart *Hordeum vulgare* subsp. *spontaneum* (K. Koch) Thell. (im Folgenden Spontaneum genannt) und ihre zeitliche Veränderung in situ in Jordanien zu untersuchen. Jordanien liegt im Ursprungszentrum Spontaneums und der Kulturgerste. Gerste hat dort besonders in Randgebieten eine große wirtschaftliche Bedeutung. Die „re-collection and resurrection“ Methode wurde angewandt, um genetische Erosion in dieser Wildart zu untersuchen. Dabei werden frühere und gegenwärtige Genotypen, die an denselben Orten gesammelt wurden, gemeinsam im Versuchsfeld angebaut und verglichen. In dieser Studie wurden dazu 2012 neue Spontaneumproben an denselben Sammelorten genommen, an denen bereits 1981 gesammelt worden war. Die gegenwärtige genetische Struktur wurde zunächst anhand von Einzelähren untersucht, die 2012 von 32 Populationen gesammelt wurden. Die heutige und frühere Verteilung und Struktur der genetischen Vielfalt wurde anhand von jeweils 18 Populationen aus 1981 und 2012 verglichen und das Ausmaß der Veränderung und mögliche Faktoren untersucht.

Die von Bioversity International erstellte Sammelreisen Datenbank (Bioversity Collecting Mission Database; BCD) wurde analysiert und genutzt, um *Hordeum* Sammelreisen zu identifizieren und die Sammelreise von 1981 nach Jordanien auszuwählen. Die Datenbank enthält originale Passport Daten und dazugehörige Dokumentation über CWR und Landsortenproben, die hauptsächlich zwischen 1975 – 1995 in über 1,000 Sammelreisen gesammelt worden waren. Originalsammelgut von 1981 und die 2012 neu gesammelten Proben wurden 2013 im Versuchsfeld an der Genebank des Leibniz Instituts für Pflanzengenetik und Kulturpflanzenforschung (IPK) angebaut.

Die Analyse der gegenwärtigen Populationsstruktur identifizierte drei genetische Cluster. Populationen waren durch Vermischung und hohe allelische Variation gekennzeichnet, und genetische Vielfalt war vorwiegend im nördlichen Teil des Untersuchungsareals zu finden. Korrelationsanalysen zeigten Isolation durch Entfernung (Isolation by Distance; IBD) über das gesamte Areal, aber ergaben keinen Hinweis auf einen Zusammenhang zwischen Klima und genetischer Struktur. Spontaneum wächst bevorzugt in landwirtschaftlichen und Ruderalgebieten. Hier ist Samentransport durch menschliche Aktivitäten begünstigt, z.B. mittels Beimischung zu Gerstensaatgut, oder durch Anhaften an das Fell von Weide- und Nutztieren oder auch Kleidung, was mit natürlichen Diffusions- und Selektionsprozessen interferiert. Dies könnte die erwartete geographische Verteilung der genetischen Vielfalt verändern und zu einer schwachen oder gänzlich abwesenden Korrelation zwischen klimatischer, geographischer und genetischer Variation führen, wie in dieser Studie. Natürliche Verbreitungs- und Selektionsprozesse waren daher wohl nicht die hauptsächlichen Faktoren, die die genetische Struktur Spontaneums in Jordanien geprägt haben.

Der Vergleich der 1981 und 2012 gesammelten Populationen hat gezeigt, dass die Populationsstruktur weitgehend erhalten wurde. Die heutigen Populationen besitzen jedoch eine größere allelische Variation, bei gleichzeitiger Verringerung der Populationsdifferenzierung. Phänotypische Variation in und zwischen den Sammelorten war in beiden Jahren vergleichbar, aber der Phänotyp insgesamt zeigte Veränderungen. Das Klima hat sich während des untersuchten Zeitraums signifikant verändert, und ist trockener und heißer geworden. Die größere genetische Variation und die Veränderung des Phänotyps zeigen, dass Reaktionen auf Umweltvariabilität komplex und vielschichtig sein können. Die beobachteten Veränderungen konnten nicht anhand geläufiger Korrelationsanalysen erklärt werden und erfordern komplexere Modelle, um diese Ergebnisse zu interpretieren und Vulnerabilität und Extinktionsgefahr einzuschätzen. Die Umwelt der untersuchten Populationen war nicht nur von Klimaveränderung geprägt, sondern auch von intensiveren menschlichen Aktivitäten, und die Möglichkeiten der Verbreitung erhöhten sich sehr wahrscheinlich mit der Intensivierung der Land- und Weidewirtschaft. Die verringerte Populationsdifferenzierung kann daher die Folge und ein Hinweis auf vermehrten Austausch zwischen Populationen seit 1981 sein.

Basierend auf den Ergebnissen dieser Studie erscheint es angebracht, in zukünftigen Untersuchungen an CWR, die bevorzugt in landwirtschaftlichen und Ruderalgebieten

vorkommen, auch ackerbauliche und bäuerliche Praktiken mit einzubeziehen. Diese können die Interpretation der Ergebnisse und die Entwicklung von notwendigen Maßnahmen zur Erhaltung der CWR Vielfalt unterstützen.

Die „re-collection and resurrection“ Methode erwies sich als sehr angebracht, um zeitliche Veränderungen in Spontaneum zu untersuchen. Die BCD enthält viele Daten für weitere Wildarten, die mit derselben Methode untersucht werden können, um zusätzliche Daten zum Ausmaß der genetischen Erosion und ihrer Auswirkungen zu generieren, die zur Verbesserung von Monitoring und Konservierungsaktivitäten notwendig sind.

6 REFERENCES

Abbott RJ, Gomes MF (1989) Population genetic structure and outcrossing rate of *Arabidopsis thaliana* (L.) Heynh. Heredity 62:411–418.

Abdel-Ghani AH, Parzies HK, Omary A, Geiger HH (2004) Estimating the outcrossing rate of barley landraces and wild barley populations collected from ecologically different regions of Jordan. Theoretical and Applied Genetics 109:588–595.

Akimoto M, Shimamoto Y, Morishima H (1999) The extinction of genetic resources of Asian wild rice, *Oryza rufipogon* Griff.: A case study in Thailand. Genetic Resources and Crop Evolution 46:419–425.

Al-Bakri JT, Taylor JC, Brewer TR (2001) Monitoring land use change in the Badia transition zone in Jordan using aerial photography and satellite imagery. The Geographical Journal 167(3):248–262.

Al-Bakri JT, Ajlouni M, Abu-Zanat M (2008) Incorporating land use mapping and participation in Jordan. Mountain Research and Development 28(1):49–57.

Allaby RG (2015) Barley domestication: the end of a central dogma? Genome Biology 16:176.

Al-Qinna MI, Hammouri NA, Obeidat MM, Ahmad FY (2011) Drought analysis in Jordan under current and future climates. Climatic Change 106:421–440.

Al-Saghir MG, Malkawi HI and El-Oqlah A (2009) Morphological diversity in *Hordeum spontaneum* C. Koch of northern Jordan (Ajloun Area). Middle-East Journal of Scientific Research 4(1):24–27.

Al-Tabbal JA (2012) Genetic variation, heritability, phenotypic and genotypic correlation studies for yield and yield components in promising barley genotypes. Jounal of Agricultural Science 4(3):193–210.

Azhaguvel P, Komatsuda T (2007) A phylogenetic analysis based on nucleotide sequence of a marker linked to the brittle rachis locus indicates a diphyletic origin of barley. Annals of Botany 100:1009–1015.

Backes G, Madsen LH, Jaiser H, Stougaard J, Herz M, Mohler V, Jahoor A (2003) Localization of genes for resistance against *Blumeria graminis* f.sp. *hordei* and *Puccinia graminis* in a cross between a barley cultivar and wild barley (*Hordeum vulgare* subsp. *spontaneum*) line. Theoretical and Applied Genetics 106:353–362.

Badr A, Mueller K, Schaefer-Pregl R, El Rabey H, Effgen S, Ibrahim HH, Pozzi C, Rohde W, Salamini F (2000) On the origin and domestication history of barley (*Hordeum vulgare*). Molecular Biology and Evolution 17(4):499–510.

Baek HJ, Beharav A, Nevo E (2003) Ecological-genomic diversity of microsatellites in wild barley, *Hordeum spontaneum*, populations in Jordan. Theoretical and Applied Genetics 106(3):397–410.

Barry MB, Pham JL, Béavogui S, Ghesquière A, Ahmadi N (2008) Diachronic (1979–2003) analysis of rice genetic diversity in Guinea did not reveal genetic erosion. Genetic Resources and Crop Evolution 55(5):723–733.

Batchu AK, Zimmermann D, Schulze-Lefert P, Koprek T (2006) Correlation between hordatine accumulation, environmental factors and genetic diversity in wild barley (*Hordeum spontaneum* C. Koch) accessions from the Near East Fertile Crescent. Genetica 127(1–3):87–99.

Baum BR, Nevo E, Douglias AJ, Beiles A (1997) Genetic diversity in wild barley (*Hordeum spontaneum* C. Koch) in the Near East: a molecular analysis using Random Amplified Polymorphic DNA (RAPD) markers. Genetic Resources and Crop Evolution 44:147–157.

Baur E (1914) Die Bedeutung der primitiven Kulturrassen und der wilden Verwandten unserer Kulturpflanzen für die Pflanzenzüchtung; Jahrbuch Deutsche Landwirt. Gesell. (Saatzuchtabteilung).

Bennett E (1968) Record of the FAO/IBP technical conference on the exploration, utilization and conservation of plant genetic resources, Rome, Italy 18–26 September 1967. Food and Agriculture Organization of the United Nations, Rome, Italy.

Bertoldo JG, Coimbra JLM, Guidolin AF, Braatz de Andrade LR, Nodari RO (2014) Agronomic potential of genebank landrace elite accessions for common bean genetic breeding. Scientia Agricola 71(2):120–125.

Bezançon G, Pham JL, Deu M, Vigouroux Y, Sagnard F, Mariac C, Kapran I, Mamadou A, Gérard B, Ndjeunga J, Chantereau J (2009) Changes in the diversity and geographic distribution of cultivated millet (*Pennisetum glaucum* (L.) R. Br.) and sorghum (*Sorghum bicolor* (L.) Moench) varieties in Niger between 1976 and 2003. Genetic Resources and Crop Evolution 56(2):223–236.

Bonnin I, Huguet T, Gherardi M, Prosperi JM, Olivieri I (1996). High level of polymorphism and spatial structure in a selfing plant species, *Medicago truncatula* (Leguminosae), shown using RAPD markers. American Journal of Botany 83:843–855.

Brown AHD (2008) Indicators of genetic diversity, genetic erosion and genetic vulnerability for plant genetic resources for food and agriculture. Thematic background study. Food and Agriculture Organization of the United Nations, Rome, Italy.

Brown AHD, Zohary D, Nevo E (1978) Outcrossing rates and heterozygosity in natural populations of *Hordeum spontaneum* Koch in Israel. Heredity 41:49–62.

Brown AHD, Marshall DR (1995) A basic sampling strategy: theory and practice. In: Guarino L, Ramanatha Rao V, Reid R (eds) Collecting plant genetic diversity: Technical guidelines. International Plant Genetic Resources Institute (IPGRI), Rome, Italy; Plant Production and Protection Division, FAO, Rome, Italy; World Conservation Union (IUCN), Gland, Switzerland; CABI Publishing, Wallingford, UK. Pp 75–91.

Brush SB (1992) Ethnoecology, biodiversity and modernization in Andean potato agriculture. Journal of Ethnobiology 12:161–185.

Brush SB (1999) Genetic erosion of crop populations in centers of diversity: a revision. In: Serwinski J, Faberova I (eds) Proceedings of the Technical Meeting on the Methodology of the FAO World Information and Early Warning System on Plant Genetic Resources, held at the Research Institute of Crop Production, Prague, Czech Republic 21 – 23 June 1999. Food and Agriculture Organization of the United Nations, Rome, Italy.

Brush SB (2004) Farmers' bounty: Locating crop diversity in the contemporary world. Yale University Press, New Haven (CT) and London.

Calinger KM, Queenborough S, Curtis PS (2013) Herbarium specimens reveal the footprint of climate change on flowering trends across north-central North America. Ecology Letters 16:1037–1044.

Che YH, Yang YP, Yang XM, Li XQ, Li LH (2011) Genetic diversity between *ex situ* and *in situ* samples of *Agropyron cristatum* (L.) Gaertn. based on simple sequence repeat molecular markers. Crop & Pasture Science 62:639–644.

Chen G, Li C, Shi Y, Nevo E (2008) Wild barley, *Hordeum spontaneum*, a genetic resource for crop improvement in cold and arid regions. Sciences in cold and arid regions 1:0115–0124.

Chessa C, Nieddu G (2005) Analysis of diversity in the fruit tree genetic resources from a Mediterranean island. Genetic Resources and Crop Evolution 52:267–276.

Chevin LM, Lande R, Mace GM (2010) Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. PLoS Biology 8(4):e1000357.

Cronin, JK, Bundock PC, Henry RJ, Nevo E (2007) Adaptive climatic molecular evolution in wild barley at the Isa defense locus. PNAS 104:2773–2778.

Dai F, Nevo E, Wu D, Comadran J, Zhou M, Qiu L, Chen Z, Beiles A, Chen G, Zhang G (2012) Tibet is one of the centers of domestication of cultivated barley. PNAS 109(42):16969–16973.

Damania AB (1998) Diversity of major cultivated plants domesticated in the Near East. In: Damania AB, Valkoun J, Willcox G, Qualset CO (eds.) The origins of agriculture and crop domestication. ICARDA, Aleppo, Syria. Pp. 51–64.

Davari A, Khoshbakht K, Ghalegolab Behbahani A, Veisi H (2013) A qualitative assessment of diversity and factors leading to genetic erosion of vegetables: a case study of Varamin (Iran). International Journal of AgriScience 3(3):198–212.

Davis MB, Shaw RG, Etterson JR (2005) Evolutionary responses to changing climate. Ecology 86(7):1704–1714.

Dawson IK, Chalmers KH, Waugh R, Powell W (1993) Detection and analysis of genetic variation in *Hordeum spontaneum* populations from Israel using RAPD markers. Molecular Ecology 2:151–159.

De Oliveira LO, Martins ER (2002) A quantitative assessment of genetic erosion in ipecac (*Psychotria ipecacuanha*). Genetic Resources and Crop Evolution 49:607–617.

Del Rio AH, Bamberg JB, Huaman Z, Salas A, Vega SE (1997) Assessing changes in the genetic diversity of potato gene banks. 2. *In situ* vs *ex situ*. Theoretical and Applied Genetics 95:199–204.

Deu M, Sagnard F, Chantereau J, Calatayud C, Hérault D, Mariac C, Pham JL, Vigouroux Y, Kapran I, Traoré PS, Mamadou A, Gérard B, Ndjeunga J, Bezançon G (2008) Niger-wide assessment of *in situ* sorghum genetic diversity with microsatellite markers. Theoretical and Applied Genetics 116:903–913.

Deu M, Sagnard F, Chantereau J, Calatayud C, Vigouroux Y, Pham JL, Mariac C, Kapran I, Mamadou A, Gérard B, Ndjeunga J, Bezançon G (2010) Spatio-temporal dynamics of genetic diversity in *Sorghum bicolor* in Niger. Theoretical and Applied Genetics 120(7):1301–1313.

Dosmann M, Groover A (2012) The importance of living botanical collections for plant biology and the 'next generation' of evo-devo research. Frontiers in Plant Science 3:1–5.

Dreiseitl A, Bockelman HE (2003) Sources of powdery mildew resistance in a wild barley collection. Genetic Resources and Crop Evolution 50:345–350.

Dulloo ME, Hanson J, Jorge MA, Thormann I (2008a) Regeneration guidelines: general guiding principles, in: Dulloo ME, Thormann I, Jorge MA, Hanson J (eds) Crop specific regeneration guidelines [CD-ROM]. CGIAR System-wide Genetic Resource Programme (SGRP), Rome, Italy. 6 pp.

Dulloo ME, Labokas J, Iriondo JM, Maxted N, Lane A, Laguna E, Jarvis A, Kell SP (2008b) Genetic reserve location and design. In: Iriondo JM, Maxted N, Dulloo ME (eds) Conserving plant genetic diversity in protected areas. CAB International. Pp 23–64.

Dulloo ME, Fiorino E, Thormann I (2015) Research on conservation and use of crop wild relatives. In: Yadav S, Redden R, Maxted N, Dulloo ME, Guarino L, Smith P (eds) Crop wild relatives and climate change. Wiley-Blackwell 400 pages. Pp 108–129.

Ellstrand NC (2003) Dangerous liaisons? When cultivated plants mate with their wild relatives. Johns Hopkins University Press, Baltimore, USA.

Engels JMM (2001) Home gardens—a genetic resource perspective. In: Watson JW, Eyzaguirre PB (eds) Proceedings of the second international home garden workshop. Bioversity International, Rome, Italy. Pp 3–9.

Erkkila MJ, Leah R, Ahokas H, Cameron-Mills V (1998) Allele-dependent barley grain β-Amylase activity. Plant Physiology 117:679–685.

Esquinas-Alcazar JT (1993) Plant genetic resources. In: Hayward MD, Bosemark NO, Romagosa I (eds) Plant Breeding: Principles and Prospects, Chapman & Hall, London, UK. Pp 33–51.

Etterson JR, Franks SJ, Mazer SJ, Shaw RG, Soper Gorden NL, Schneider HE, Weber JJ, Winkler KJ, Weis AE (2016) Project Baseline, an unprecedented resource to study evolution across space and time. American Journal of Botany 103(1):164–173.

FAO (2006) Country Pasture/Forage Resource Profiles. Jordan. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO (2009) International Treaty on Plant Genetic Resources for Food and Agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO (2010) The second report on the state of the world's plant genetic resources for food and agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO (2013) Report of the Fourteenth Regular Session of the Commission on Genetic Resources for Food and Agriculture. CGRFA 14/13/report. Available at: http://www.fao.org/nr/cgrfa/cgrfa-meetings/cgrfa-comm/fourteenth-reg/en/.

FAOSTAT (2015) http://faostat3.fao.org/home/E Statistical Division of the Food and Agriculture Organization of the United Nations, Rome, Italy.

Franks SJ, Sim S, Weis AE (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. PNAS 104:1278–1282.

Franks SJ, Avise JC, Bradshaw WE, Conner JK, Etterson JR, Mazer SJ, Shaw RG, Weis AE (2008) The resurrection initiative: storing ancestral genotypes to capture evolution in action. BioScience 58(9):870–873.

Franks SJ, Weber JJ, Aitken SN (2014) Evolutionary and plastic responses to climate change in terrestrial plant populations. Evolutionary Applications 7:123–139.

Freiwan M, Kadioglu M (2008) Climate variability in Jordan. International Journal of Climatology 28:69–89.

Fu YB, Horbach C (2012) Genetic diversity in a core subset of wild barley germplasm. Diversity 4:239–257.

Fuller DQ, Willcox G and Allaby RG (2012) Early agricultural pathways: moving outside the 'core area' hypothesis in Southwest. Journal of Experimental Botany 63(2):617–633.

Gallagher RV, Hughes L, Leishman MR (2009) Phenological trends among Australian alpine species: using herbarium records to identify climate-change indicators. Australian Journal of Botany 57:1–9.

Galluzzi G, Eyzaguirre P, Negri V (2010) Home gardens: neglected hotspots of agrobiodiversity and cultural diversity. Biodiversity and Conservation 19:3635–3654.

Gao L, Chen W, Jiang W, Ge S, Hong D, Wang X (2000) Genetic erosion in northern marginal population of the common wild rice *Oryza rufipogon* Griff. and its conservation, revealed by the change of population genetic structure. Hereditas 133(1):47–53.

Gao LZ (2003) The conservation of Chinese rice biodiversity: genetic erosion, ethnobotany and prospects. Genetic Resources and Crop Evolution 50:17–32.

Genger RK, Williams KJ, Raman H, Read BJ, Wallwork H, Burdon JJ, Brown AHD (2003) Leaf scald resistance genes in *Hordeum vulgare* and *Hordeum vulgare* ssp. *spontaneum*: parallels between cultivated and wild barley. Australian Journal of Agricultural Research 54(12):1335–1342.

Ghazanfar SA, Taifour H, El-Oqlah A, Borosova R, Osborne J (2015) Checklist of the vascular plants of Jordan. Kew Publishing, London, UK.

Gomaa NH, Montesinos-Navarro A, Alonso-Blanco C, Pico FX (2011) Temporal variation in genetic diversity and effective population size of Mediterranean and subalpine *Arabidopsis thaliana* populations. Molecular Ecology 20:3540–3554.

Goodrich WJ (1987) Monitoring genetic erosion: detection and assessment. IBPGR report no. 87/33. International Board for Plant Genetic Resources, Rome, Italy.

Greene SL, Kisha TJ, Yu L-X, Parra-Quijano M (2014) Conserving plants in gene banks and nature: Investigating complementarity with *Trifolium thompsonii* Morton. PLoS ONE 9(8): e105145.

Guarino L (1995) Assessing the threat of genetic erosion. In: Guarino L, Ramanatha Rao V, Reid R (eds) Collecting plant genetic diversity: Technical guidelines. International Plant Genetic Resources Institute (IPGRI), Rome, Italy; Plant Production and Protection Division, FAO, Rome, Italy; World Conservation Union (IUCN), Gland, Switzerland; CABI Publishing, Wallingford, UK. Pp. 67–74.

Gupta PK, Sharma PK, Balyan HS, Roy JK, Sharma S, Beharav A, Nevo E (2002) Polymorphism at rDNA loci in barley and its relation with climatic variables. Theoretical and Applied Genetics 104:473–481.

Gustafsson M, Claësson L (1988) Resistance to powdery mildew in wild species of barley. Hereditas 108:231–237.

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

Hammer K, Laghetti G (2005) Genetic erosion: examples from Italy. Genetic Resources and Crop Evolution 52:629–634.

Hansen MM, Olivieri I, Waller DM, Nielsen EE, the GeM working group (2012) Monitoring adaptive genetic responses to environmental change. Molecular Ecology 21(6):1311–1329.

Harlan JR (1995) Barley, *Hordeum vulgare* (Gramineae-Triticeae). In: Smartt J, Simmonds NW (eds) Evolution of crop plants. Longman Group, Harlow, Essex. Pp. 140–147.

Harlan JR, de Wet JMJ (1971) Toward a rational classification of cultivated plants. Taxon 20:509–517.

Haseneyer G, Stracke S, Paul C, Einfeldt C, Broda A, Piepho H-P, Graner A, Geiger HH (2010) Population structure and phenotypic variation of a spring barley world collection set up for association studies. Plant Breeding 129:271–279.

Hoban S, Arntzen JA, Bruford MW, Godoy JA, Hoelzel R, Segelbacher G, Vila C, Bertorelle G (2014) Comparative evaluation of potential indicators and temporal sampling protocols for monitoring genetic erosion. Evolutionary Applications 7(9):984–998.

Hoban S, Schlarbaum S (2014) Optimal sampling of seeds from plant populations for ex-situ conservation of genetic biodiversity, considering realistic population structure. Biological Conservation 177:90–9.

Hoyt E (1988) Conserving the wild relatives of crops. IBPGR, IUCN, WWF, Rome and Gland.

Hübner S, Hüffken M, Oren E, Haseneyer G, Stein N, Graner A, Schmid K, Fridman E (2009) Strong correlation of wild barley (*Hordeum spontaneum*) population structure with temperature and precipitation variation. Molecular Ecology 18:1523–1536.

Hübner S, Günther T, Flavell A, Fridman E, Graner A, Korol A, Schmid KJ (2012) Islands and streams: clusters and gene flow in wild barley populations from the Levant. Molecular Ecology 21:1115–1129.

Hughes CE, Govindarajulu R, Robertson A, Filer DL, Harris SA, Bailey CD (2007) Serendipitous backyard hybridization and the origin of crops. PNAS 104:14389–14394.

Ivandic V, Walther U, Graner A (1998) Molecular mapping of a new gene in wild barley conferring complete resistance to leaf rust (*Puccinia hordei* Otth). Theoretical and Applied Genetics 97:1235–1239.

Ivandic V, Hackett CA, Nevo E, Keith R, Thomas WTB, Foster BP (2002) analysis of simple sequence repeats (SSRs) in wild barley form the Fertile Crescent: associations with ecology, geography and flowering time. Plant Molecular Biology 48:511–527.

Jain SK (1975) Genetic reserves. In: Frankel OH and Hawkes JG (eds.) Crop genetic resources for today and tomorrow. Cambridge University Press, UK. Pp. 379–396.

Jakob SS, Rödder D, Engler JO, Shaaf S, Özkan H, Blattner FR, Kilian B (2014) Evolutionary history of wild barley (*Hordeum vulgare* subsp. *spontaneum*) analyzed using multilocus sequence data and paleodistribution modeling. Genome Biology and Evolution 6(3):685–702.

Jana S, Pietrzak LN (1988) Comparative assessment of genetic diversity in wild and primitive cultivated barley in a center of diversity. Genetics 119:981–990.

Jaradat AA (1989a) Diversity within and between populations of two sympatrically distributed *Hordeum* species in Jordan. Theoretical and Applied Genetics 78:653–656.

Jaradat AA (1989b) Ecotypes and genetic divergence among sympatrically distributed *Hordeum vulgare* and *Hordeum spontaneum* from the xeric region of Jordan. Theoretical and Applied Genetics 78:857–862.

Jaradat AA (1991) Grain protein variability among populations of wild barley (*Hordeum spontaneum* C. Koch.) from Jordan. Theoretical and Applied Genetics 83:164–168.

Jaradat AA (1992) Genetic diversity of four esterase loci in natural populations of *Hordeum spontaneum* C. Koch from Jordan. Theoretical and Applied Genetics 84:725–729.

Jarvis A, Ferguson ME, Williams DE, Guarino L, Jones PG, Stalker HT, Valls JFM, R. N. Pittman RN, Simpson CE, Bramel P (2003) Biogeography of wild *Arachis*: Assessing conservation status and setting future priorities. Crop Science 43:1100–1108.

Jarvis A, Lane A, Hijmans RJ (2008) The effect of climate change on crop wild relatives. Agriculture Ecosystems & Environment 126:13–23.

Jarvis S, Fielder H, Hopkins J, Maxted N, Smart S (2015) Distribution of crop wild relatives of conservation priority in the UK landscape. Biological Conservation 191:444–451.

Jones IT, Pickering RA (1978) The mildew resistance in *Hordeum bulbosum* and its transference into *H. vulgare* genotypes. Annals of Applied Biology 88:295–298.

Jump AS, Hunt JM, Martinez-Izquierdo JA, Penuelas J (2006) Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*. Molecular Ecology 15:3469–3480.

Keiša A, Maxted N, Ford-Lloyd B (2008) The assessment of biodiversity loss over time: wild legumes in Syria. Genetic Resources and Crop Evolution 55(4):603–612.

Kew (2016) The State of the world's plants report – 2016. Royal Botanic Gardens, Kew, UK.

Khazaei H, Street K, Bari A, Mackay M, Stoddard FL (2013) The FIGS (focused identification of germplasm strategy) approach identifies traits related to drought adaptation in *Vicia faba* genetic resources. PLoS ONE 8(5):e63107.

Khlestkina EK, Huang XQ, Quenum FJ-B, Chebotar S, Roeder MS, Boerner A (2004) Genetic diversity in cultivated plants—loss or stability? Theoretical and Applied Genetics 108:1466–1472.

Khlestkina EK, Varshney RK, Röder MS, Graner A, Börner A (2006) A comparative assessment of genetic diversity in cultivated barley collected in different decades of the last century in Austria, Albania and India by using genomic and genic simple sequence repeat (SSR) markers. Plant Genetic Resources: Characterization and Utilization 4:125–133.

Kiambi D, Ford-Lloyd B, Jackson MT, Guarino L, Maxted N, Newbury HJ (2005) Collection of wild rice (*Oryza* L.) in east and southern Africa in response to genetic erosion. Plant Genetic Resources Newsletter 142:10–20.

Kiang YT, Antonovics J, Wu L (1979) The extinction of wild rice (*Oryza perennis formosana*) in Taiwan. Journal of Asian Ecology 1:1–9.

Kilian B, Ozkan H, Kohl J, von Haeseler A, Barale F, Deusch O, Brandolini A, Yucel C, Martin W, Salamini F (2006) Haplotype structure at seven barley genes: relevance to genepool bottlenecks, phylogeny of ear type and site of barley domestication. Molecular Genetics and Genomics 276:230–241.

Kind OD, Masel J (2007) The evolution of bet-hedging adaptations to rare scenarios. Theoretical Population Biology 72(4):560–575.

Kindler SD, Springer TL (1991) Resistance to Russian wheat aphid in wild *Hordeum* species. Crop Science 31(1):94–97.

Lakew B, Henry RJ, Eglinton J, Baum M, Ceccarelli S, Grando S (2013) SSR analysis of introgression of drought tolerance from the genome of *Hordeum spontaneum* into cultivated barley (*Hordeum vulgare* ssp *vulgare*). Euphytica 191(2):231–243.

Lauterbach D, Burkart M, Gemeinholzer B (2012) Rapid genetic differentiation between *ex situ* and their *in situ* source populations an example of the endangered *Silene otites*. Botanical Journal of the Linnean Society 168:64–75.

Li YC, Krugman T, Fahima T, Beiles A, Korol AB, Nevo E (2001) Spatiotemporal allozyme divergence caused by aridity stress in a natural population of wild wheat, *Triticum dicoccoides*, at the Ammiad microsite, Israel. Theoretical and Applied Genetics 102:853–864.

Li Z, Wu N, Gao X, Wu Y, Oli KP (2013) Species-level phonological responses to 'global warming' as evidenced by herbarium collections in the Tibetan Autonomous Region. Biodiversity and Conservation 22:141–152.

Lin JZ, Brown AHD, Clegg MT (2001) Heterogeneous geographic patterns of nucleotide sequence diversity between two alcohol dehydrogenase genes in wild barley (*Hordeum vulgare* subsp. *spontaneum*). PNAS 98:531–536.

Liu F, Sun GL, Salomon B, von Bothmer R (2002) Characterization of genetic diversity in core collection accessions of wild barley, *Hordeum vulgare* ssp. *spontaneum*. Hereditas 136:67–73.

Liviero L, Maestri E, Gulli M, Nevo E, Marmiroli N (2002) Ecogeographic adaptation and genetic variation in wild barley, application of molecular markers targeted to environmentally regulated genes. Genetic Resources and Crop Evolution 49:133–144.

Magos Brehm J, Saifan S, Taifour H, Abu Laila K, Al-Assaf A, Al-Oqlah A, Al-Sheyab F, Bani-Hani R, Ghazanfar S, Haddad N, Shibli R, Abu Taleb T, bint Ali B, Maxted N (2016) Crop wild relatives, a priority in Jordan? Developing a national strategy for the conservation of plant diversity in Jordan using a participatory approach. In: Maxted N, Dulloo ME and Ford-Lloyd BV (eds) Enhancing crop genepool use: Capturing wild relative and landrace diversity for crop improvement. CAB International, Wallingford, UK. Pp. 172–188.

Mariac C, Robert T, Allinne C, Remigereau MS, Luxereau A, Tidjani M, Seyni O, Bezancon G, Pham JL, Sarr A (2006) Genetic diversity and gene flow among pearl millet crop/weed complex: a case study. Theoretical and Applied Genetics 113(6):1003–1014.

Marsh DM, Trenham PC (2008) Current trends in plant and animal population monitoring. Conservation Biology 22(3):647–55.

Matus IA, Hayes PM (2002) Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. Genome 45(6):1095–106.

Maurer A, Draba V, Jiang Y, Schnaithmann F, Sharma R, Schumann E, Kilian B, Reif JC, Pillen K (2015) Modeling the genetic architecture of flowering time control in barley through nested association mapping. BMC Genomics 16:290.

Maurer A, Draba V, Pillen K (2016) Genomic dissection of plant development and its impact on thousand grain weight in barley through nested association mapping. Journal of Experimental Botany 67(8):2507–2518.

Maxted N, Ford-Lloyd BV, Jury SL, Kell SP, Scholten MA (2006) Towards a definition of a crop wild relative. Biodiversity & Conservation 15:2673–2685.

Maxted N, Guarino L (2006) Genetic erosion and genetic pollution of crop wild relatives In: Ford-Lloyd BV, Dias SR, Bettencourt E (eds) Genetic erosion and pollution assessment methodologies. Proceedings of PGR Forum Workshop 5, Terceira Island, Autonomous Region of the Azores, Portugal, 8–11 September 2004. Published on behalf of the European Crop Wild Relative Diversity Assessment and Conservation Forum, by Bioversity International, Rome, Italy. Pp. 35–46.

Maxted N, Kell SP (2009) Establishment of a global network for the *in situ* conservation of crop wild relatives: Status and needs. FAO Commission on Genetic Resources for Food and Agriculture, Rome, Italy. 266 pp.

Maxted N, Magos Brehm J, Kell S (2013) Resource book for preparation of national conservation plans for crop wild relatives and landraces. Food and Agriculture Organization of the United Nations, Rome, Italy.

McCouch S, Baute GJ, Bradeen J, Bramel P, Bretting PK, Buckler E, et al. (2013) Feeding the future. Nature 499:23–24.

Megersa G (2014) Genetic erosion of barley in North Shewa Zone of Oromiya Region, Ethiopia. International Journal of Biodiversity and Conservation 6(3):280–289.

Miller-Rushing AJ, Primack RB, Primack D, Mukunda S (2006) Photographs and herbarium specimens as tools to document phonological changes in response to global warming. American Journal of Botany 93:1667–1674.

Molina-Cano JL, Russell JR, Moralejo MA, Excacena JL, Arias G, Powell W (2005) Chloroplast DNA microsatellite analysis supports a polyphyletic origin for barley. Theoretical and Applied Genetics 110:613–619.

Moore G, Williams KA (2011) Legal issues in plant germplasm collecting. In: Guarino L, Ramanatha Rao V, Goldberg E (eds) Collecting plant genetic diversity: Technical guidelines. 2011 update. Bioversity International, Rome. Available online from: http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=669 Accessed 19 June 2016.

Morishima H, Oka HI (1995) Genetic erosion in wild and cultivated rice species. Rice genetics newsletter 12:168–170. National Institute of Genetics, Japan.

Morrell PL, Clegg MT (2007) Genetic evidence for a second domestication of barley (*Hordeum vulgare*) east of the Fertile Crescent. PNAS 104:3289–3294.

Morrell PL, Lundy KE, Clegg MT (2003) Distinct geographic patterns of genetic diversity are maintained in wild barley (*Hordeum vulgare* ssp. *spontaneum*) despite migration. PNAS 100:10812–10817.

Morrell PL, Toleno DM, Lundy KE, Clegg MT (2005) Low levels of linkage disequilibrium in wild barley (*Hordeum vulgare* ssp. *spontaneum*) despite high rates of self-fertilization. PNAS 102(7):2442-2447.

Naz AA, Arifuzzaman M, Muzammil S, Pillen K, Jeon J (2014) Wild barley introgression lines revealed novel QTL alleles for root and related shoot traits in the cultivated barley (*Hordeum vulgare* L.). BMC Genetics 15:107.

NCARTT (2007) The second country report on the state of the plant genetic resources for food and agriculture. The Hashemite Kingdom of Jordan. National Center for Agricultural Research and Technology Transfer NCARTT, Amman, Jordan.

Neale DB, Saghai-Maroof MA, Allard RW, Zhang Q, Jorgensen RA (1986) Chloroplast DNA diversity in populations of wild and cultivated barley. Genetics 120:1105–1110.

Nevo E (1986) Genetic diversity and environmental associations of wild barley, *Hordeum spontaneum* in Turkey. Genetica 68:203–203.

Nevo E (2014) Evolution of wild barley at "Evolution Canyon": adaptation, speciation, pre-agricultural collection, and barley improvement. Israel Journal of Plant Science 62(1–2):22–32.

Nevo E, Zohary D, Brown AHD, Haber M (1979) Genetic diversity and environmental associations of wild barley, *Hordeum spontaneum*, in Israel. Evolution 33(3):815–833.

Nevo E, Beiles A, Gutterman Y, Storch N, Kaplan D (1984) Genetic resources of wild cereals in Israel and vicinity. II. Phenotypic variation within and between populations of wild barley, *Hordeum spontaneum*. Euphytica 33(3):737–756.

Nevo E, Beiles A, Zohary D (1986a) Genetic resources of wild barley in the Near East: structure, evolution and application in breeding. Biological Journal of the Linnean Society 27:355–380.

Nevo E, Beiles A, Kaplan D, Golenberg EM, Olswig-Whittaker L, Naveh Z (1986b) Natural selection of allozyme polymorphism: a microsite test revealing ecological genetic differentiation in wild barley. Evolution 40:13–20.

Nevo E, Baum B, Beiles A, Johnson DA (1998) Ecological correlates of RAPD DNA diversity of wild barley, *Hordeum spontaneum*, in the Fertile Crescent. Genetic Resources and Crop Evolution 45:151–159.

Nevo E, Fu YB, Pavlicek T, Khalifa S, Tavasi M, Beiles A (2012) Evolution of wild cereals during 28 years of global warming in Israel. PNAS 109(9):3412–3415.

Olofsson H, Ripa J, Jonzén N (2009) Bet-hedging as an evolutionary game: the trade-off between egg size and number. Proceedings of the Royal Society B 276:2963–2969.

Orabi J, Backes G, Wolday A, Yahyaoui A, Jahoor A (2007) The Horn of Africa as a center of barley diversification and a potential domestication site. Theoretical and Applied Genetics 114(6):1117–27.

Ozkan H, Kafkas S, Ozer MS, Brandolini (2005) Genetic relationships among South-east Turkey wild barley populations and sampling strategies of *Hordeum spontaneum*. Theoretical and Applied Genetics 112:12–20.

Pakniyat H, Powell W, Baird E, Handley LL, Robinson D, Scrimgeour CM, Nevo E, Hackett CA, Caligari PDS, Forster BP (1997) AFLP variation in wild barley (*Hordeum spontaneum* C. Koch) with reference to salt tolerance and associated ecogeography. Genome 40:332–341.

Parra-Quijano M, Iriondo JM, de la Cruz M, Torres E (2011) Strategies for the development of core collections based on ecogeographical data. Crop Science 51:656–666.

Parra-Quijano M, Iriondo JM, Frese L, Torres E (2012) Spatial and ecogeographical approaches for selecting genetic reserves in Europe. In: Maxted N, Dulloo ME, Ford-Lloyd BV, Frese L, Iriondo JM, Pinheiro de Carvalho MAA (eds) Agrobiodiversity Conservation: securing the diversity of crop wild relatives and landraces. CAB International, Wallingford, UK. Pp. 20–28.

Patto MCV, Aardse A, Buntjer J, Rubiales D, Martín A, Niks RE (2001) Morphology and AFLP markers suggest three *Hordeum chilense* ecotypes that differ in avoidance to rust fungi. Canadian Journal of Botany 79(2):204–213.

Peroni N, Hanazaki N (2002) Current and lost diversity of cultivated varieties, especially cassava, under swidden cultivation systems in the Brazilian Atlantic Forest. Agriculture, Ecosystems and Environment 92:171–183.

Pickering RA, Hill AM, Michel M, Timmerman-Vaughan GM (1995) The transfer of a powdery mildew resistance gene from *Hordeum bulbosum* L. to barley (*H. vulgare* L.) chromosome 2 (2I). Theoretical and Applied Genetics 91:1288–1292.

Pickering R, Johnston PA (2005) Recent progress in barley improvement using wild species of *Hordeum*. Cytogenetic and Genome Research 109:344–349.

Poets AM, Fang Z, Clegg MT, Morell PL (2015) Barley landraces are characterized by geographically heterogeneous genomic origins. Genome Biology 16:173.

Primack D, Imbres C, Primack RB, Miller-Rushing AJ, Tredici PD (2004) Herbarium specimens demonstrate earlier flowering times in response to warming in Boston. American Journal of Botany 91:1260–1264.

Rao NK, Hanson J, Dulloo ME, Ghosh K, Nowell D, Larinde M (2006) Manual of seed handling in genebanks. Handbooks for Genebanks No. 8. Bioversity International, Rome, Italy.

Repkova J, Dreiseitl A, Lizal P, Kyjovska Z, Teturova K, Psotkova R, Jahoor A (2006) Identification of resistance genes against powdery mildew in four accessions of *Hordeum vulgare* ssp. *spontaneum*. Euphytica 151:23–30.

Rogers DL (2004) Genetic erosion no longer just an agricultural issue. Native Plants Journal Fall 2004:112–122.

Rucinska A, Puchalski J (2011) Comparative molecular studies on the genetic diversity of an *ex situ* garden collection and its source population of the critically endangered polish endemic plant *Cochlearia polonica* E. Froehlich. Biodiversity & Conservation 20:401–413.

Ruge B, Linz A, Pickering R, Proeseler G, Greif P, Wehling P (2003) Mapping of Rym14HB, a gene introgressed from *Hordeum bulbosum* and conferring resistance to BaMMV and BaYMV in barley. Theoretical and Applied Genetics 107:965–971.

Ruge-Wehling B, Linz A, Habeku A, Wehling P (2006) Mapping of RYMl6Hb, the second soilborne virus resistance gene introgressed from *Hordeum bulbosum*. Theoretical and Applied Genetics 113:867–673.

Russell J, Dawson IK, Flaell AJ, Steffenson B, Weltzien E, Booth A, Ceccarelli S, Grando S, Waugh R (2011) Analysis of > 1000 single nucleotide polymorphisms in geographically matched samples of landrace and wild barley indicates secondary contact and chromosome-level differences in diversity around domestication genes. New Phytologist 191:564–578.

Russell J, van Zonneveld M, Dawson IK, Booth A, Waugh R, Steffenson B (2014) Genetic Diversity and Ecological Niche Modelling of Wild Barley: Refugia, Large-Scale Post-LGM Range Expansion and Limited Mid-Future Climate Threats? PLoS ONE 9(2): e86021.

Saghai-Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allard RW (1994) Extraordinarily polymorphic microsatellite DNA in barley: Species diversity, chromosomal locations, and population dynamics. PNAS 91:5466–5470.

Schmalenbach I, Körber N, Pillen K (2008) Selecting a set of wild barley introgression lines and verification of QTL effects for resistance to powdery mildew and leaf rust. Theoretical and Applied Genetics 117:1093–1106.

Schmalenbach I, Léon J, Pillen K (2009) Identification and verification of QTLs for agronomic traits in wild barley introgression lines. Theoretical and Applied Genetics 118:483–497.

Schmalenbach I, Pillen K (2009) Detection and verification of malting quality QTLs using wild barley introgression lines. Theoretical and Applied Genetics 118:1411–1427.

Serwinski J, Faberova I (eds) (1999) Proceedings of the technical meeting on the methodology of the FAO World Information and Early Warning System on Plant Genetic Resources, held at the Research Institute of Crop Production, Prague, Czech Republic 21 – 23 June 1999. Food and Agriculture Organization of the United Nations, Rome, Italy.

Shakhatreh Y, Haddad N, Alrababah M, Grando S, Ceccarelli S (2010) Phenotypic diversity in wild barley (*Hordeum vulgare* L. ssp. *spontaneum* (C. Koch) Thell.) accessions collected in Jordan. Genetic Resources and Crop Evolution 57:131–146.

Shakhatreh Y, Baum M, Haddad N, Alrababah M, Ceccarelli S (2016) Assessment of genetic diversity among Jordanian wild barley (*Hordeum spontaneum*) genotypes revealed by SSR markers. Genetic Resources and Crop Evolution 63(5):813–822.

Sharma S, Beharav A, Balyan HS, Nevo E, Gupta PK (2004) Ribosomal DNA polymorphism and its association with geographical and climatic variables in 27 wild barley populations from Jordan. Plant Science 166:467–477.

Shaw RG, Shaw FH (2014) Quantitative genetic study of the adaptive process. Heredity 112:13–20.

Siol M, Bonnin I, Olivieri I, Prosperi JM, Ronfort J (2007) Effective population size associated with self-fertilization: lessons from temporal changes in allele frequencies in the selfing annual *Medicago truncatula*. Journal of Evolutionary Biology 20:2349–2360.

Steffenson BJ, Olivera P, Roy JK, Jin Y, Smith KP, Muehlbauer GJ (2007) A walk on the wild side: mining wild wheat and barley collections for rust resistance genes. Australian Journal of Agricultural Research 58:532–544.

Szigat G, Pohler W (1982) *Hordeum bulbosum* x *H. vulgare* hybrids and their back-crosses with cultivated barley. Cereal Research Communications 10:73–78.

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277(5329):1063–1066.

Tarawneh Q, Kadioglu M (2003) An analysis of precipitation climatology in Jordan. Theoretical and Applied Climatology 74:123–136.

Thormann I, Engels JMM (2015) Genetic diversity and erosion – a global perspective. Chapter 10. In: Ahuja MR, Jain SM (eds) Genetic Diversity and Erosion in Plants – Indicators and Prevention, Volume 1, Springer, Berlin, Germany. Pp 263–294.

Thormann I, Gaisberger H, Mattei F, Snook L, Arnaud E (2012) Digitization and online availability of original collecting mission data to improve data quality and enhance the conservation and use of plant genetic resources. Genetic Resources and Crop Evolution 59(5):635–644.

Thormann I, Parra-Quijano M, Endresen DTF, Rubio-Teso ML, Iriondo MJ, Maxted N (2014) Predictive characterization of crop wild relatives and landraces. Technical guidelines version 1. Bioversity International, Rome, Italy.

Thormann I, Fiorino E, Halewood M, Engels JMM (2015) Plant genetic resources collections and associated information as baseline resource for genetic diversity studies – an assessment of the IBPGR supported collections. Genetic Resources and Crop Evolution 62(8):1279–1293.

Thormann I, Reeves P, Reilley A, JMM Engels, Lohwasser U, Börner A, Pillen K, Richards CM (2016a) Geography of genetic structure in barley wild relative *Hordeum vulgare* subsp. *spontaneum* in Jordan. PLoS ONE 11(8): e0160745. DOI:10.1371/journal.pone.0160745.

Thormann I, Reeves P, Thumm S, Reilley A, JMM Engels, Lohwasser U, Börner A, Pillen K, Richards CM (2016b) Genotypic and phenotypic changes in wild barley (*Hordeum vulgare* subsp. *spontaneum*) during a period of climate change in Jordan. Genetic Resources and Crop Evolution. DOI: 10.1007/s10722-016-0437-5.

Thuiller W, Lavorel S, Araújo MB, Sykes MT, Prentice IC (2005) Climate change threats to plant diversity in Europe. PNAS 102(23):8245–8250.

Toubia-Rahme H, Johnston PA, Pickering RA, Steffenson BJ (2003) Inheritance and chromosomal location of *Septoria passerinii* resistance introgressed from *Hordeum bulbosum* into *Hordeum vulgare*. Plant Breeding 122:405–409.

Turpeinen T, Tenhola T, Manninen O, Nevo E, Nissilä E (2001) Microsatellite diversity associated with ecological factors in *Hordeum spontaneum* populations in Israel. Molecular Ecology 10(6):1577–1591.

Turpeinen T, Vanhala T, Nevo E, Nissilä E (2003) AFLP genetic polymorphism in wild barley (*Hordeum spontaneum*) populations in Israel. Theoretical and Applied Genetics 106:1333–1339.

Tyagi K, Park MR, Lee HJ, Lee CA, Rehman S, Steffenson B, Lee KJ, Yun SJ (2011) Diversity for seedling vigor in wild barley (*Hordeum vulgare* L. subsp. *spontaneum*) germplasm. Pakistan Journal of Botany 43(4):2167–2173.

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: Characterization and Utilization 8(1):1–15.

Vanhala TK, van Rijn CPE, Buntjer J, Stam P, Nevo E, Poorter H, van Eeuwijk FA (2004) Environmental, phenotypic and genetic variation of wild barley (*Hordeum spontaneum*) from Israel. Euphytica 137:297–309.

Vavilov NI (1926) Studies in the origin of cultivated plants. Institute of Applied Botany and Plant Breeding, Leningrad, Russia.

Vellend M, Brown CD, Kharouba HM, Mccune JI, Myers-Smith IH (2013) Historical ecology: using unconventional data sources to test for effects of global environmental change. American Journal of Botany 100(7):1294–1305.

Vigouroux Y, Mariac C, De Mita S, Pham JL, Gerard B, Kapran I, Sagnard F, Deu M, Chantereau J, Ali A, Ndjeunga J, Luong V, Thuillet A, Saidou A, Bezancon G (2011) Selection for earlier flowering crop associated with climatic variations in the Sahel. PLoS ONE 6(5):1–9.

Vincent H, von Bothmer R, Knüpffer H, Amri A, Konopka J, Maxted N (2013) Genetic gap analysis of wild *Hordeum* taxa. Plant Genetic Resources: Characterization and Utilization 10(3):242–253.

Volis S, Mendlinger S, Orlovsky N (2000) Variability in phenotypic traits in core and peripheral populations of wild barley *Hordeum spontaneum* Koch. Hereditas 133:235–247.

Volis S, Mendlinger S, Turuspekov Y, Esnazarov U, Abugalieva S, Orlovsky N (2001) Allozyme variation in Turkmenian populations of wild barley, *Hordeum spontaneum* Koch. Annals of Botany 87:435–446.

Volis S, Mendlinger S, Ward D (2002a) Differentiation in populations of *Hordeum spontaneum* along a gradient of environmental productivity and predictability: life history and local adaptation. Biological Journal of the Linnean Society 77:479–490.

Volis S, Mendlinger S, Ward D (2002b) Adaptive traits of wild barley plants of Mediterranean and desert origin. Oecologia 133:131–138.

von Bothmer R (1992) The wild species of *Hordeum*: relationships and potential use for improvement of cultivated barley, in: Shewry PR (ed) Barley: genetics, biochemistry, molecular biology and biotechnology. CAB International, Wallingford, UK. Pp 3–18.

von Bothmer R, Jacobsen N, Baden C, Jørgensen RB, Linde-Laursen I (1995) An ecogeographical study of the genus *Hordeum*. 2nd edition. Systematic and ecogeographic studies on crop genepools 7. International Plant Genetic Resources Institute, Rome, Italy.

von Bothmer R, Sato K, Komatsuda T, Yasuda S, Fischbeck G (2003) The domestication of cultivated barley. In: von Bothmer R, Sato K, Knüpffer H, van Hintum T (eds) Diversity in barley (*Hordeum vulgare* L.). Elsevier Science B.V., Amsterdam, The Netherlands. Pp 9–27.

von Korff M, Wang H, Léon J, Pillen K (2005) AB-QTL analysis in spring barley. I. Detection of resistance genes against powdery mildew, leaf rust and scald introgressed from wild barley. Theoretical and Applied Genetics 111(3):583–90.

von Korff M, Wang H, Leon J, Pillen K (2006) AB-QTL analysis in spring barley. II. Detection of favourable exotic alleles for agronomic traits introgressed from wild barley (*Hordeum vulgare* ssp. *spontaneum*). Theoretical and Applied Genetics 112:1221–1231.

von Korff M, Wang H, Léon J, Pillen K (2008) AB-QTL analysis in spring barley: III. Identification of exotic alleles for the improvement of malting quality in spring barley (*H. vulgare* ssp. *spontaneum*). Molecular Breeding 21(1):81–93.

Walther U, Rapke H, Proeseler G, Szigat G (2000) *Hordeum bulbosum*: A new source of disease resistance-transfer of resistance to leaf rust and mosaic viruses from *H. bulbosum* into winter barley. Plant Breeding 119:215–218.

Wandeler P, Hoeck PEA, Keller KF (2007) Back to the future: museum specimens in population genetics. Trends in Ecology and Evolution 22(12):634–642.

Wang G, Schmalenbach I, von Korff M, Léon J, Kilian B, Rode J, Pillen K (2010) Association of barley photoperiod and vernalization genes with QTLs for flowering time and agronomic traits in a BC2DH population and a set of wild barley introgression lines. Theoretical and Applied Genetics 120:1559–1574.

Warschefsky E, Penmetsa RV, Cook DR, von Wettberg EJ (2014) Back to the wilds: tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. American Journal of Botany 101(10):1791–800.

Willemen L, Scheldeman X, Soto Cabellos V, Salazar SR, Luigi Guarino L (2007) Spatial patterns of diversity and genetic erosion of traditional cassava (*Manihot esculenta* Crantz) in the Peruvian Amazon: An evaluation of socio-economic and environmental indicators. Genetic Resources and Crop Evolution 54:1599–1612.

Williams SE, Shoo LP, Isaac JL, Hoffmann AA, Langham G (2008) Towards an integrated framework for assessing the vulnerability of species to climate change. PLoS Biology 6(12):e325

Witcombe JR, Bourgois J, Rifaie R (1982) Germplasm collections from Syria & Jordan. Plant Genetic Resources Newsletter 50:2–8.

Xu J, Snape JW (1988) The cytology of hybrids between *Hordeum vulgare* and *H. bulbosum* revisited. Genome 30:486–494.

Xu J, Kasha KJ (1992) Transfer of a dominant gene for powdery mildew resistance and DNA from *Hordeum bulbosum* into cultivated barley (*H. vulgare*). Theoretical and Applied Genetics 84:771–777.

Yang Z, Zhang T, Bolshoy A, Beharav A, Nevo E (2009) Adaptive microclimatic structural and expressional dehydrin 1 evolution in wild barley, *Hordeum spontaneum*, at 'Evolution Canyon', Mount Carmel, Israel. Molecular Ecology 18:2063–2075.

Zhang Q, Saghai-Maroof MA, Kleinhofs A (1993) Comparative diversity analysis of RFLPs and isozymes within and among populations of *Hordeum vulgare* ssp. *spontaneum*. Genetics 134:909–916.

Zohary D (1999) Monophyletic vs. polyphyletic origin of the crops on which agriculture was founded in the Near East. Genetic Resources and Crop Evolution 46:133–142.

Zohary D, Hopf M (2000) Domestication of plants in the old world. Oxford University Press, New York, USA.

7 ABBREVIATIONS

Abbreviation	Explanation
AFLP	Amplified Fragment Length Polymorphism
BCD	Bioversity Collecting Mission Database
CBD	Convention on Biological Diversity
CWR	Crop Wild Relative(s)
FAO	Food and Agriculture Organization of the United Nations
GIS	Geographic Information System
GPA2	Second Global Plan of Action for PGRFA
GSPC	Global Strategy for Plant Conservation
IBD	Isolation by Distance
IBPGR	International Board for Plant Genetic Resources
ICARDA	International Center for Agricultural Research in Dry Areas
IPGRI	International Plant Genetic Resources Institute
IPK	Leibniz Institute of Plant Genetics and Crop Plant Research, Germany
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
LOA	Letter of Agreement
NCARE	National Center for Agricultural Research and Extension, Jordan
NordGen	Nordic Genetic Resource Center
PGRFA	Plant Genetic Resources for Food and Agriculture
PIC	Prior informed consent
RAPD	Random Amplified Polymorphic DNA
rDNA	Ribosomal Deoxyribonucleic Acid
RFLP	Restriction Fragment Length Polymorphism
SINGER	System-wide Information Network for Genetic Resources of the CGIAR
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
WIEWS	World Information and Early Warning System on Plant Genetic Resources

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Thormann I, Reeves P, Thumm S, Reilley A, Biradar CM, Engels JMM, Lohwasser U, Börner A, Pillen K, Richards CM (2016) Genotypic and phenotypic changes in wild barley (*Hordeum vulgare* subsp. *spontaneum*) during a period of climate change in Jordan. Genetic Resources and Crop Evolution DOI: 10.1007/s10722-016-0437-5.

Thormann I, Reeves P, Reilley A, Engels JMM, Lohwasser U, Börner A, Pillen K, Richards CM (2016) Geography of genetic structure in barley wild relative *Hordeum vulgare* subsp. *spontaneum* in Jordan. PLoS ONE 11(8): e0160745.

Thormann I, Parra-Quijano M, Rubio-Teso ML, Endresen DTF, Dias S, Iriondo JM, Maxted M (2016) Predictive characterization methods for accessing and using CWR diversity. Chapter 8. In: Maxted N, Dulloo ME, Ford-Lloyd BV (eds.) Enhancing Crop Genepool Use: Capturing Wild Relative and Landrace Diversity for Crop Improvement. CAB International UK. Pp 64-77.

Thormann I, Fiorino E, Halewood M, Engels JMM (2015) Plant genetic resources collections and associated information as baseline resource for genetic diversity studies – an assessment of the IBPGR supported collections. Genetic Resources and Crop Evolution 62(8):1279-1293.

Thormann I, Engels JMM (2015) Genetic diversity and erosion – a global perspective. Chapter 10. *In*: Ahuja MR, Jain SM (eds.) Genetic Diversity and Erosion – Indicators and Prevention, Volume 1, Springer. Pp 263-294.

Dulloo ME, Fiorino E, **Thormann** I (2015) Research on Conservation and Use of Crop Wild Relatives. In: Redden R, Yadav SS, Maxted N, Dulloo ME, Guarino L, Smith P (eds.) Crop Wild Relatives and Climate Change. Wiley-Blackwell 400 pages. ISBN: 978-1-118-85433-4. Chapter 7:108-129.

Thormann I, Parra-Quijano M, Endresen DTF, Rubio-Teso ML, Iriondo JM, Maxted M (2014) Predictive characterization of crop wild relatives and landraces. Technical guidelines version 1. Bioversity International, Rome, Italy.

Dulloo ME, **Thormann** I, Fiorino E, De Felice S, Rao VR, Snook L (2013) Trends in research using plant genetic resources from germplasm collections: from 1996 to 2006. Crop Science 53:1-11.

Thormann I, Alercia A, Dulloo ME (2013) Core descriptors for *in situ* conservation of crop wild relatives v.1. Bioversity International, Rome, Italy.

Thormann I, Qiu Yang, Allender C, Bas N, Campbell G, Dulloo E, Ebert AW, Lohwasser U, Pandey C, Robertson LD, Spellman O (2013) Development of best practices for *ex situ* conservation of radish germplasm in the context of the Crop Genebank Knowledge Base. Genetic Resources and Crop Evolution. 60(4):1251-1262.

Thormann I, Gaisberger H, Mattei F, Snook L, Arnaud E (2012) Digitization and online availability of original collecting mission data to improve data quality and enhance the conservation and use of plant genetic resources. Genetic Resources and Crop Evolution. 59:5 635-644.

Thormann I (2011) Published sources of information on wild plant species. In: Guarino L, Ramanatha Rao V, Goldberg E, editors. Collecting Plant Genetic Diversity: Technical Guidelines. 2011 update. Bioversity International, Rome. ISBN 978-92-9043-922-6.

Dulloo ME, Jarvis DI, **Thormann** I, Scheldeman X, Salcedo J, Hunter D, Hodgkin T (2010) The state of *in situ* management. In: FAO. The second report on the State of the World's Plant Genetic Resources for Food and Agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy. Pp 30-51. ISBN 978-92-5-106534-1.

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Eidesstattliche Erklärung/ *Declaration under Oath*

Ich erkläre an Eides statt, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word or content.

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