8 Barley Genetic Resources for Climate-Change Adaptation

Searching for Heat-Tolerant Traits through Rapid Evaluation of Subsets

A. Jilal, H. Ouabbou, and M. Maatougui

CONTENTS

Barley (*Hordeum vulgare* L.) has been cultivated over millennia, making it one of the oldest domesticated crops (Salamini et al. 2002). Ranking fourth in global cereal-crop production, it is used for animal feed, brewing malts, and human consumption (von Bothmer et al. 2003). Given barley's long history as a crop involving migration and selection leading to adaptation to different environments and agroecology gradients, leading to a plethora of uses, it has the potential of being an excellent model for further elucidation of agricultural responses to changing and evolving climates (Dawson et al. 2015). Barley landraces are genetically heterogeneous populations comprising inbreeding lines and hybrid segregates generated by a low level of random outcrossing in each generation (Nevo 1992). The genetic structure of landraces may be considered as an evolutionary approach to survival and performance under arid and semiarid conditions (Schulze 1988). They are composed of several genotypes reported for both cultivated and wild barley (Brown 1978, 1979, Asfaw 1989). Natural selection accompanied by human selection during centuries of cultivation resulted in landraces that are genetically variable for qualitative and quantitative characters, have good adaptation to specific

environmental conditions, and give dependable yields (Harlan 1992). Barley landraces have developed abundant patterns of variation and represent a largely untapped reservoir of useful genes for adaptation to biotic and abiotic stresses (Brush 1995, Tarekegn and Weibull 2011) to contribute to the improvement of modern cultivars (Hadjichristodoulou 1995).

The escalating size of ex-situ collections of plant genetic resources (e.g., 31,000 barley accessions held in ICARDA genebanks) and the limited funds available to assess data were earlier identified as limitations to the use of germplasm collections (Frankel and Brown 1984). Exploiting accessions for useful gene discovery, the focused identification of germplasm strategy (FIGS) has been developed by Mackay and Street (2004) to improve the use of genebank collections by increasing the chances of finding useful traits in relatively small subsets of germplasm reported for stem rust resistance in a FIGS wheat subset (Bari et al. 2012). Reducing time, space, and money consumption and assessing a large genetic resource are considered real challenges for genetic resource managers and breeders.

To exploit genetic resources collections for useful genes, partitioning techniques were proposed, among which is the core-collection strategy, which aims to capture diversity and be amenable to the FIGS trait-based approach. The development of the core-collection concept and theory was by Frankel and Brown (1984, Brown 1989a,b). A core collection consists of a representative sample of a base collection and accounts for the genetic variability of a crop and its related species, with minimum redundancy (Frankel and Brown 1984). The common practice to develop a core is based on setting up a hierarchical structure that will capture the alleles present in the base collection, within a conservation perspective. The core should capture most alleles that contribute to adaptation to specific environments (Brown 1989a, Allard 1992) and the accessions are selected based on stratification. The two main factors to be considered in the stratified random sampling procedure are geographic distribution and genotypic composition (Brown 1989a, Crossa et al. 1994, van Hintum 2000). This chapter addresses the issue of space allocated for collection evaluation and the reduction of collection size based on partitioning using both the core concept and trait-based concepts.

METHODOLOGY

The approach was a combination of a partitioning strategy and a field-evaluation strategy to reduce, on the one hand, the number of accessions to be screened and, on the other hand, the area where evaluation was conducted (Figure 8.1).

PARTITIONING USING PRIOR INFORMATION

Partitioning was conducted using environmental data (*a priori* information) to develop one subset that is likely to contain climate-change–related traits and another subset representative of the different environments where the barley accessions were originally sampled across Morocco. Each subset contains 100 accessions, of which 30 accessions were selected at random (Figure 8.2). Both subsets were grown in the same field for comparison based on *a posteriori* evaluation. The environmental
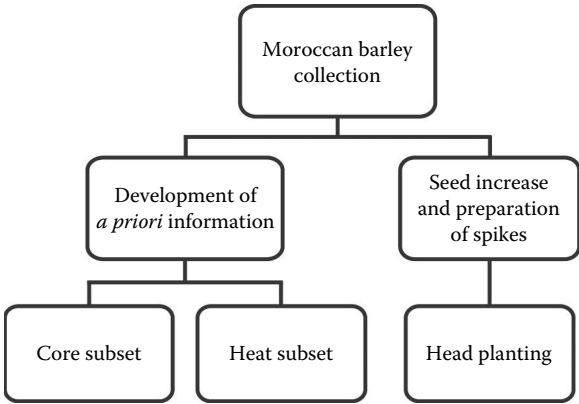
FIGURE 8.1   Partitioning and evaluation scheme.
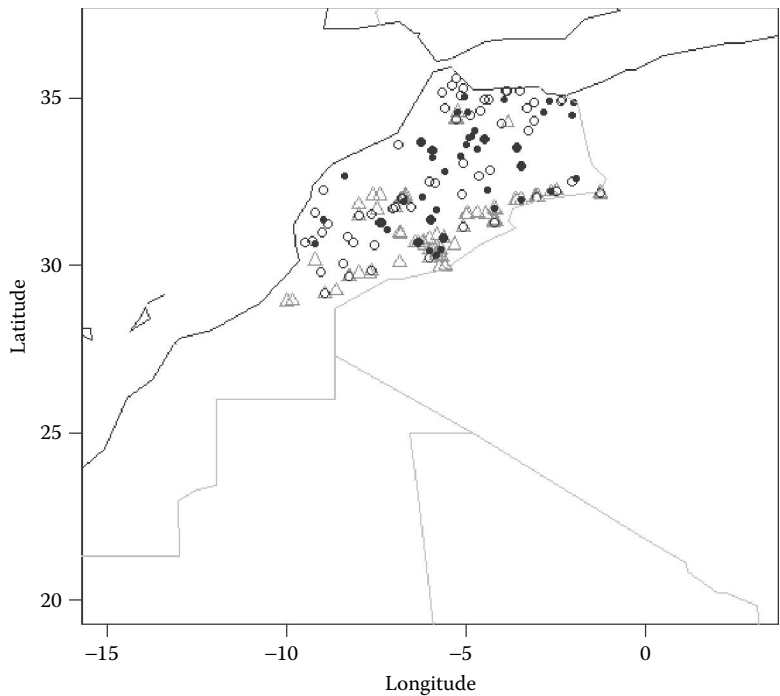
FIGURE 8.2   Barley landraces site distribution map for the heat subset in triangles and the representative subset in circles; the random set in dots is part of each of the two subsets as controls.

data used in the partitioning consisted of 19 climate variables extracted from world climate data (Table 8.1).

Climate data for each of the variables in Table 8.1 were extracted from bioclim surface data, which is used in the development of Hutchinson's environmental niche model as an *n-dimensional hyperspace* of climatic variables where a species can

TABLE 8.1
Climate Data Used in the Development of Subsets

Variable	Description
BIOCLIM1	Annual mean temperature
BIOCLIM2	Mean diurnal range (Mean of monthly [max temp–min temp])
BIOCLIM3	Isothermality (BIO2/BIO7 × 100)
BIOCLIM4	Temperature seasonality (standard deviation × 100)
BIOCLIM5	Maximum temperature of warmest month
BIOCLIM6	Minimum temperature of coldest month
BIOCLIM7	Temperature annual range (BIO5–BIO6)
BIOCLIM8	Mean temperature of wettest quarter
BIOCLIM9	Mean temperature of driest quarter
BIOCLIM10	Mean temperature of warmest quarter
BIOCLIM11	Mean temperature of coldest quarter
BIOCLIM12	Annual precipitation
BIOCLIM13	Precipitation of wettest month
BIOCLIM14	Precipitation of driest month
BIOCLIM15	Precipitation seasonality (coefficient of variation)
BIOCLIM16	Precipitation of wettest quarter
BIOCLIM17	Precipitation of driest quarter
BIOCLIM18	Precipitation of warmest quarter
BIOCLIM19	Precipitation of coldest quarter

Source: World climate data at http://www.worldclim.org/.

occur (Booth et al. 2014). This encompasses other important elements supporting the contention that variations in climate exert a strong influence on the distribution of a species (Booth et al. 2014). These bio-climatic variables (bioclim data) were derived from monthly surface data as synthetic variables to represent biologically meaningful variables coded as BIOCLIM1 through BIOCLIM19. They represent trends, seasonality, and extreme or limiting environmental factors that are detrimental to plant development (phenology) (Hijmans et al. 2005). Monthly data (*wtmin*, *wtmax*, and *wprec*) were also extracted and used in the analysis of data, especially to superimpose these data with the crop-growing period as per the crop-growing day requirements in terms of temperature. Under heat stress, these requirements will be expected to increase inversely proportional to the range for the crop stages.

This part of the partitioning process is to identify traits that breeders have long sought in order to combine an optimized grain-filling period with maturity rather than with earliness alone. As depicted in Figure 8.2, partitioning has been carried out for both the core subset and the trait-based subsets, in particular, to assess genetic resources of barley for heat traits based on agro-morphological data and climate data.

A Posteriori Evaluation

To compare and validate the partitioning based on climate data, the accessions were compared based on their evaluation attributes. Both subsets were grown among 697 entries at Merchouch experimental station (33°.6049N; 06°.71600W; 410 masl) in one 5-m row for each accession. Observations were taken on several agro-morphological and physiological traits. After harvesting, all the accessions were analyzed with near-infrared spectroscopy (Infraneo machine) and the absorbance data (in the range from 850 to 1048 nm) and protein content have been determined.

Further evaluation was carried out based on a honeycomb design of hill plots with the aim to also reduce the area needed for evaluation (Figure 8.3). Subsequent evaluation was also carried out focusing mostly on the two subsets and heat-related traits, among which were canopy temperature and phenology traits, specifically grain-filling period.

RESULTS

The subsets were classified based on their environment and their evaluation scores. Based on the monthly average maximum temperature (*wtmax*) of March, the successful heat-subset accessions are all from areas where temperatures are high, while those of the core or representative samples are more from areas with mild to low temperatures. When the classification is based on April *wtmax*, the distinction in origin between the heat subset and the random and core subsets is even more apparent (Figure 8.4).

Similarly, when the partitioning is based on evaluation data (*a posteriori*), there is also a difference among the subsets, although it is not as clear as when environmental data is used. For example, the histograms of Figure 8.5 for evaluation of accessions with absorption spectroscopy and for protein content show that the heat subset

FIGURE 8.3 Barley head planting using the honeycomb planting design.
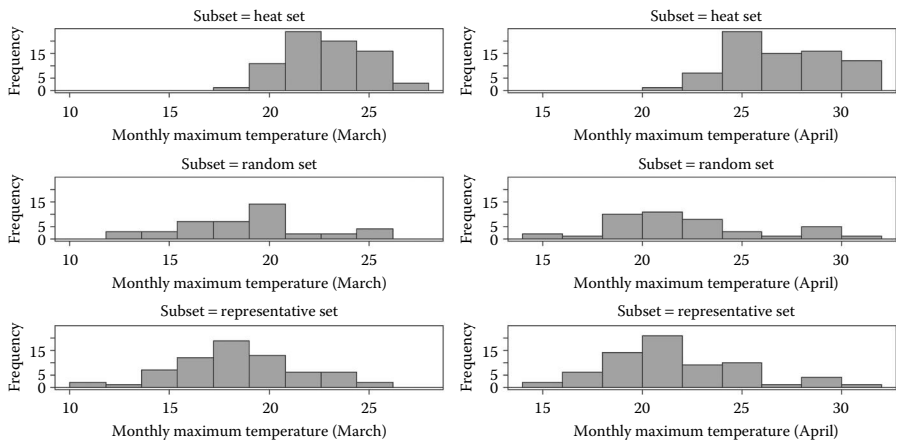
FIGURE 8.4 Distribution of accessions per climate data (monthly average maximum temperatures) for March (*left*) and April (*right*).
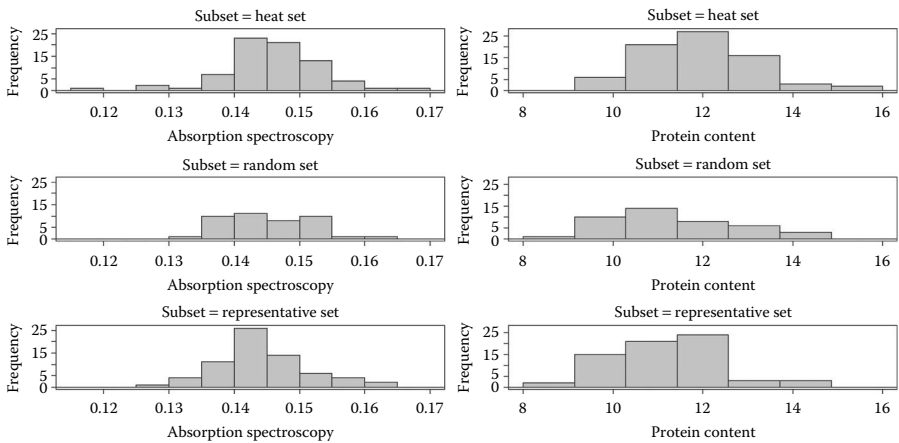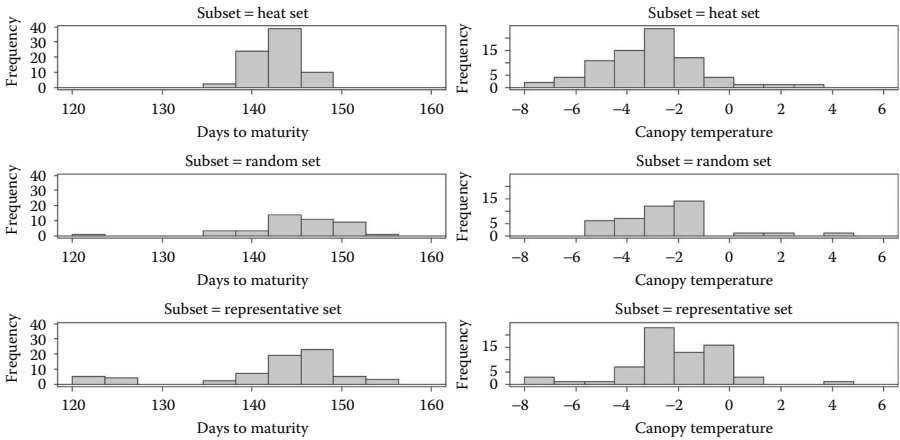

FIGURE 8.5 Distribution of accessions per two examples of evaluation data: absorption spectroscopy (*left*) and protein content (*right*).

may have traits that are different when compared to the core or random subsets, with the heat subset more likely to yield heat-related traits.

The subsequent evaluation involving heat-related traits such as days to maturity (a measure of phenology) and canopy temperature, especially in the grain-filling period, has shown that the two subsets are different (Figure 8.6). The heat subsets are most likely to yield heat tolerance when compared to the representative and random subsets.

These results indicate that there is potential not only to identify useful traits but also to combine traits, such as the desired grain-filling period with the optimum time of reaching maturity. The identification of a genotype with a grain-filling period that

FIGURE 8.6 Distribution of accessions based on two climate-related traits: days to maturity (*left*) and canopy temperature (*right*); the range of days to maturity for most accessions in the heat subset fall within a tight range when compared to the random and representative sets (*left*).

allows the plant to achieve optimum grain filling while adjusting its time to maturity when conditions are not favorable could be more interesting than a genotype with simply increased earliness.

DISCUSSION

The rejuvenation of barley accessions in ex-situ conservation is time, space, and money consuming, in addition to the constraint of limited screening capacity for traits of interest of the massive genebank collection. The barley spike represents a good source of information on the accession since it gives feedback on the grain and spike characters (spike density, spike width, row type, spike length, spike shape, number of spikelet/spike, grain weight/spike, grain plumpness, etc.). It has been used traditionally for seed storing as a bouquet for a long time. Head planting is used for increasing pre-basic seed in some countries such as Algeria and for testing heat tolerance during off-season for cereals. Combining this important source of information on the accession with an adequate experimental design, such as the honeycomb design, which compares the middle plant to the encircled ones and perform the evaluation in the absence of interplant (Fasoulas and Fasoula 1995), could be an effective way to screen, increase, and disseminate genetic accessions throughout the world (Figure 8.1).

Targeted partitioning is likely to yield the desired traits including climate-change-related traits, such as heat tolerance. The environmental data can be used as surrogate information when evaluating collections. Crops such as barley, which has been grown over different environmental gradients, may harbor genetic variation with the potential to adapt to changing climate conditions. Recent research by Dawson et al. (2015) showed that barley, in particular, can be an excellent model to elucidate the adaptation of crops to climate change. Barley genetic resources are

thus of great relevance to the future as stated at the 2014 Lillehammer International Conference on *Genetic Resources for Food and Agriculture in a Changing Climate*, emphasizing the value of genetic resources for farming in the future (Præbel and Groeneveld 2014).
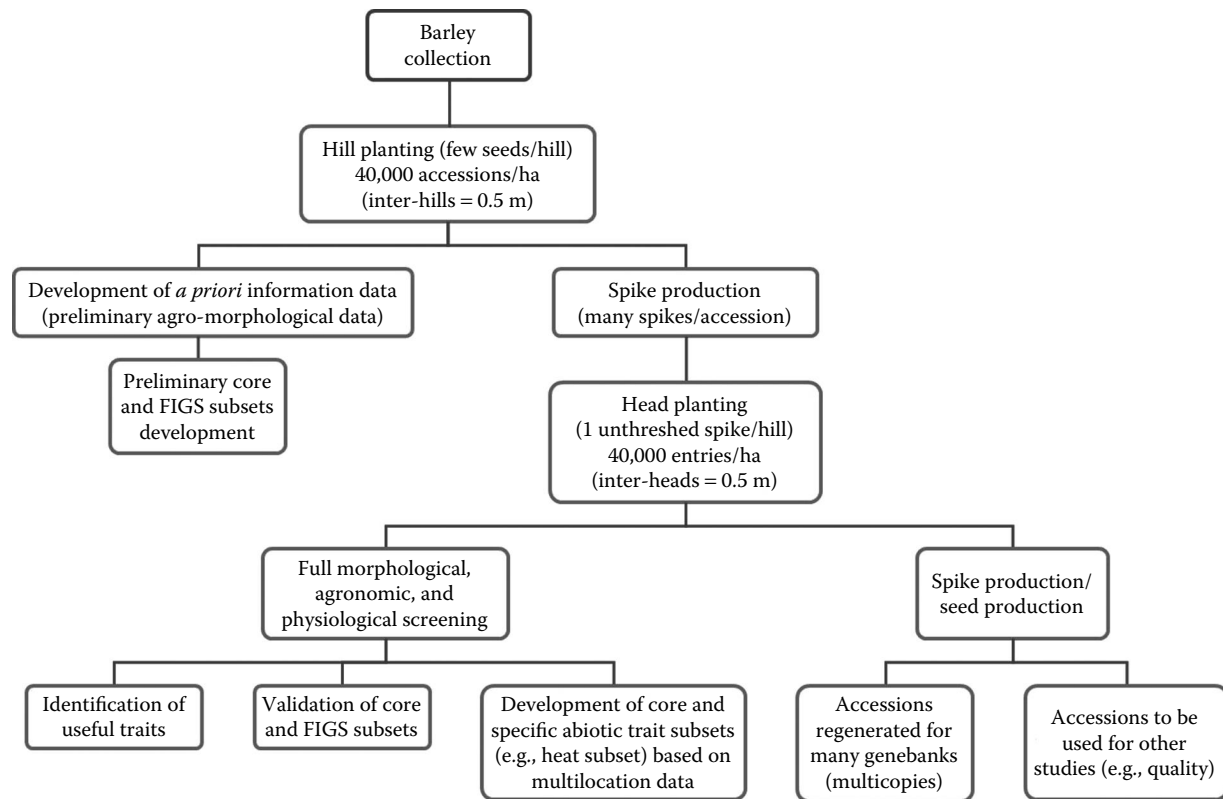
Genetic resources overall have helped in providing traits for crop improvement. A combination of partitioning strategies along with careful seed increase, taking into account optimal times for evaluation and space availability can help to address the limited screening capacity for traits in the face of the large size of genebank collections.

This new procedure has many advantages, such as the following:

- Large numbers of accession can be evaluated (10,000 accession/ha).
- Intraplant competition is possible.
- Artificial inoculation is facilitated for disease and pest screening.
- Tolerance for abiotic stresses (drought, salinity, and cold) can be easily screened.
- Biotic and abiotic stresses are easily scored.
- Samples and sample integrity can be tracked.
- Regeneration of accessions takes place in a reduced area.
- Fragile seeds, such as naked barley, can be easily handled, avoiding embryo damage during threshing.
- Duplication of accessions is facilitated, since they are stored as spikes.
- Reduced threshing.
- Easy planting and harvesting.
- Seed loss while threshing is avoided.
- Overall, time, money, and space are conserved.

Lack of evaluation of genetic resources has been reported by many authors as the main factor preventing the utilization of genetic resources. To evaluate all the genetic resources of barley, for example, for climate-change-related traits, would require large field areas, assessing large number of accessions, a considerable outlay of funds, and a great amount of time. The research reported in this chapter can help to address both the timing and the space requirement for evaluation by proposing new ways of planting and assessing the large collections that are presently conserved at genebanks. The scheme in Figure 8.7 outlines our proposal; however, the issue of accession integrity, given that a genebank landrace accession is usually a mixture, has yet to be amply addressed. In order to screen high numbers of accessions in a small space (40,000 accessions/ha) within two years, from the few seed per accession of barley in the genebanks, we can conduct a hill-plot trial, in order to evaluate the agro-morphological traits (by development of core and FIGS subsets) and to increase seed and spikes (developing information on the stored germplasm). The honeycomb design using head planting in multilocation tests will allow an efficient screening of germplasm for traits of interests (even physiological traits), always in a small space. After two years of screening, useful traits will be identified and subsets will be validated in addition to the regeneration of the accessions for future study and storage (Figure 8.7).

```
                        ┌─────────────┐
                        │   Barley    │
                        │ collection  │
                        └──────┬──────┘
                               │
                   ┌───────────────────────┐
                   │ Hill planting (few     │
                   │ seeds/hill)            │
                   │ 40,000 accessions/ha   │
                   │ (inter-hills = 0.5 m)  │
                   └───────────┬───────────┘
            ┌──────────────────┴──────────────────┐
┌─────────────────────────────┐        ┌──────────────────────┐
│ Development of a priori      │        │ Spike production     │
│ information data             │        │ (many spikes/        │
│ (preliminary agro-           │        │ accession)           │
│ morphological data)          │        └──────────┬───────────┘
└──────────────┬───────────────┘                   │
        ┌──────────────┐              ┌─────────────────────────┐
        │ Preliminary  │              │ Head planting            │
        │ core and     │              │ (1 unthreshed spike/hill)│
        │ FIGS subsets │              │ 40,000 entries/ha        │
        │ development  │              │ (inter-heads = 0.5 m)    │
        └──────────────┘              └────────────┬─────────────┘
```

FUTURE WORK

The search for climate-related traits continues with further evaluation, along with analysis of evaluation data to compare morphological and physiological data with results at three other stations. The data will also include absorbance data (linked to all molecules with C, H, O, and N atoms, e.g., NDVI, $\Delta^{13}C$, $\Delta^{18}O$, photosynthesis, and quality parameters).

Other comparison will be also conducted using other partitioning tools. In terms of core collections, we will be using PowerMarker to select accessions from the Moroccan barley collection based on the accumulated data from the three stations (three contrasting environments). For trait-based partitions, different mathematical modeling techniques will be carried out using both linear and nonlinear approaches. These analyses can be performed with Unscrambler (as well as with MATLAB® or R program). The analysis of the data may also involve a moving grid adjustment, which is a spatial method to adjust for environmental variation in field trials common in unreplicated plant breeding field trials (Technow 2011).

REFERENCES

Allard, R.W. 1992. Reproductive systems and dynamic management of genetic resources. In *Reproductive biology and plant breeding*, eds. Y. Dattee, C. Dumas, and A. Gallais, 325–334. Berlin, Germany: Springer-Verlag.

Asfaw, Z. 1989. Variation in hordein polypeptide pattern within Ethiopian barley *Hordeum vulgare* L. (Poaceae). *Hereditas* 110:185–191.

Bari, A., K. Street, M. Mackay, D.T.F. Endresen, E. De Pauw, and A. Amri. 2012. Focused identification of germplasm strategy (FIGS) detects wheat stem rust resistance linked to environmental variables. *Genet Resour Crop Ev* 59:1465–1481.

Booth, T.H., H.A. Nix, J.R. Busby, and M.F. Hutchinson. 2014. Bioclim: The first species distribution modelling package, its early applications and relevance to most current MaxEnt studies. *Diversity Distrib* 20:1–9. doi:10.1111/ddi.12144.

Brown, A.H.D. 1978. Isozymes, plant population genetic structure, and genetic conservation. *Theor Appl Genet* 52:145–157.

Brown, A.H.D. 1979. Enzyme polymorphism in plant populations. *Theor Popul Biol* 15:1–42.

Brown, A.H.D. 1989a. The case for core collections. In *The use of plant genetic resources*, eds. A.H.D. Brown, O.H. Frankel, D.R. Marshall, and J.T. Williams, 136–156. New York: Cambridge University Press.

Brown, A.H.D. 1989b. Core collections: A practical approach to genetic resources management. *Genome* 31:818–824.

Brush, S.B. 1995. In situ conservation of landraces in centers of crop diversity. *Crop Sci* 35:346–354.

Crossa, J., S. Taba, S.A. Eberhart et al. 1994. Practical considerations for maintaining germplasm in maize. *Theor Appl Genet* 89:89–95.

Dawson, I.K., J. Russell, W. Powell, B. Steffenson, W.T. Thomas, and R. Waugh. 2015. Barley: A translational model for adaptation to climate change. *New Phytol* 206(3):913–931.

Fasoulas, A.C. and V.A. Fasoula. 1995. Honeycomb selection designs. *Plant Breed Rev* 13:87–139.

Frankel, O.H. and A.H.D. Brown. 1984. Current plant genetic resources: A critical appraisal. In *Genetics: New frontiers, Vol. 4. Applied genetics Proceedings of the International Congress of Genetics, 15th,* December 12–21 1983, eds. V.L. Chopra, B.C. Joshi, R.P. Sharma, and H.C. Bansal, 1–11. New Delhi, India: Oxford and IBH.

Hadjichristodoulou, A. 1995. Evaluation of barley landraces and selections from natural outcrosses of *H. vulgare* ssp. *spontaneum* with ssp. *vulgare* for breeding in semi-arid areas. *Genet Resour Crop Ev* 42:83–89.

Harlan, J.R. 1992. *Crops and man*. Madison, WI: American Society of Agronomy.

Hijmans, R.J., S.E. Cameron, J.L. Parra, P.G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* 25:1965–1978.

Mackay, M. and K. Street. 2004. Focused identification of germplasm strategy—FIGS. In *Proceedings of the 54th Australian Cereal Chemistry Conference and the 11th Wheat Breeders' Assembly*, September 21–24, Canberra, ACT, Australia, eds. C.K. Black, J.F. Panozzo, and G.J. Rebetzke, 138–141. Melbourne, Victoria: Cereal Chemistry Division, Royal Australian Chemical Institute (RACI).

Nevo, E. 1992. Origin, evolution, population genetics and resources of wild barley, *Hordeum spontaneum*, in the fertile crescent. In *Barley: Genetics, biochemistry, molecular biology and biotechnology*, ed. P.R. Shewry, 19–43. Wallingford, CT: CABI.

Præbel, A. and L.F. Groeneveld (eds.). 2014. *Book of abstracts: Genetic resources for food and agriculture in a changing climate*, January 27–29, Lillehammer, Norway. Ås, Norway: The Nordic Genetic Resource Center.

Salamini, F., H. Ozkan, A. Brandolini, R. Schäfer-Pregl, and W. Martin. 2002. Genetics and geography of wild cereal domestication in the near east. *Nat Rev Genet* 3:429–441.

Schulze, E.D. 1988. Adaptation mechanisms of noncultivated arid-zone plants: Useful lesson for agriculture? In *Drought research priorities for the dryland tropics*, eds. E.R. Bidinger and C. Johansen, 159–177. Patancheru, India: ICRISAT.

Tarekegn, K. and J. Weibull. 2011. *Adaptation of Ethiopian barley landraces to drought stress conditions*. Saarbrücken, Germany: Lambert Academic Publishing.

Technow, F. 2011. R Package mvngGrAd: Moving grid adjustment in plant breeding field trials. R package version 0.1. University of Hohenheim, Institute of Plant Breeding, Seed Science and Population Genetics, Stuttgart, Germany.

van Hintum, Th.J.L., A.H.D. Brown, C. Spillane, and T. Hodgkin. 2000. *Core collections of plant genetic resources*. IPGRI Technical Bulletin No. 3. Rome, Italy: IPGRI.

von Bothmer, R., Th.J.L. van Hintum, H. Knüpffer, and K. Sato (eds.). 2003. *Diversity in barley* (Hordeum vulgare). (*Developments in plant genetics and breeding, Vol. 7.*) Amsterdam, The Netherlands: Elsevier Science.