Crop domestication and improvement can be described as a process of successive rounds of selection that ultimately results in the isolation of genetic diversity valuable to agriculture from ancestral wild species. These successive rounds of selection have resulted in the crops that feed the world today, but at the cost of reducing their genetic variation, leaving them with less allelic diversity than their wild progenitors and other crop wild relatives (CWR) (Ross-Ibarra et al., 2007; van Heerwarden et al., 2011). This is often referred to as “domestication bottleneck” (Hammer, 1984; Tanksley and McCouch, 1997). The reduction in genetic diversity during domestication is exacerbated by the demand for high crop productivity and crop uniformity in the field and the marketplace. The unintended consequence of recurrent selection is that potentially valuable genetic variants and associated phenotypes have been filtered out of crop gene pools. Luckily, however, many of these traits, ranging from disease resistance to drought tolerance and even yield-related traits, are still preserved in CWR. Gene flow during the domestication process...
or adaptive introgression from CWR have been sources of additional genetic diversity in some crops (Hufford et al., 2013; Sawler et al., 2013). Breeders have also worked to recover some of the beneficial genetic diversity lost (or never included) during the domestication and crop improvement process by crossing cultivated varieties with wild species. This “prebreeding” attempts to reset the genetic diversity of crops by reintroducing genetic variation that has been left behind. In fact, prebreeding has also been employed to use genetic diversity that was not previously accessible either due to genetic incompatibilities or nonoverlapping geographic ranges (Cooper et al., 2001; Dwivedi et al., 2008; Ogbonnaya et al., 2013). Cases dating back to the beginning of the 18th century document the use of wild species to transfer resistance to crops, with their use in developing commercial cultivars beginning a century later (Prescott-Allen and Prescott-Allen, 1986). The use of CWR in improvement programs for a wide range of crops did not, however, gain real prominence until the 1970s and 1980s ( Hoyt and Brown, 1988).

Over the past few decades, there have been significant successes in introducing traits from wild species into cultivated crops, mostly for overcoming biotic stresses. Landmark cases include the introduction of late blight [caused by Phytophthora infestans (Mont.) de Bary] resistance from the wild potato Solanum demissum Lindl. and stem rust (caused by Puccinia graminis ssp. graminis Pers.:Pers.) resistances from the wild wheat Aegilops tauschii Coss. ( Prescott-Allen and Prescott-Allen 1986; Kilian et al., 2010). Late blight in potato was responsible for the Irish potato famine, and overcoming rust resistance in wheat was key to the Green Revolution. While the primary strategy for crop improvement today remains recurrent selection among elite modern varieties, scientists and breeders are increasingly looking to wild species as sources of novel material to widen the genetic bases of crops ( Cooper et al., 2001; Hajjar and Hodgkin, 2007; Moore, 2015). Given the predicted impact of climate change, population growth, and changing production and consumption patterns on our agricultural systems, crops need to deliver increased yields under progressively more challenging conditions. The additional sources of allelic diversity that can be found in wild species on the far side of the domestication bottleneck are therefore predicted to become ever more important resources (Reynolds et al., 2004; Stander, 1993; Valkoun, 2001; Haussmann et al., 2004; Sharma et al., 2013). Prebreeding can involve both basic and applied research. The precise extent to which CWR are currently being used in prebreeding is difficult to quantify. Meta-analyses of the research literature and variety release reports are useful ways to gain an overall picture of trends in the use of CWR in crop improvement (Harlan, 1976; Stalker, 1980; Prescott-Allen and Prescott-Allen, 1981, 1986; Hajjar and Hodgkin, 2007; Maxted and Kell, 2009; Hunter and Heywood, 2011). These estimates

THE CROP IMPROVEMENT CONTINUUM

The process of introducing genetic diversity from wild species into cultivars for food security requires a significant amount of time, resources, and human capacity. It is a long process, starting in the wild, where genetic resource experts, botanists, and taxonomists locate, identify, and collect propagating material. This then passes through the hands of genebank curators for conservation and characterization; geneticists, agronomists, and pathologists for further characterization and evaluation, trait and gene discovery, and validation; and finally pre-breeding, or germplasm enhancement programs, and breeders for variety development. These products then feed into seed systems (both formal and informal) and eventually end up in farmers’ fields.

Prebreeding is a key step in linking the valuable traits of CWR to modern variety development by providing breeders with wild genetic diversity in a more immediately usable form (Stander, 1993; Valkoun, 2001; Haussmann et al., 2004; Sharma et al., 2013). Prebreeding can involve both basic and applied research. The precise extent to which CWR are currently being used in prebreeding is difficult to quantify. Meta-analyses of the research literature and variety release reports are useful ways to gain an overall picture of trends in the use of CWR in crop improvement (Harlan, 1976; Stalker, 1980; Prescott-Allen and Prescott-Allen, 1981, 1986; Hajjar and Hodgkin, 2007; Maxted and Kell, 2009; Hunter and Heywood, 2011). These estimates
are, however, limited. Prebreeding, by definition, does not yield varieties ready to be released, a key metric for the success of breeding programs. On the other hand, once a useful trait has been introgressed, it is likely to spread rapidly through breeding programs, but quantifying progress in terms of numbers of varieties released is difficult, since early introgressions into breeding stocks are often not well documented and many modern varieties are released by private companies, which often do not make pedigrees public. While breeders sometimes publish release notes along with new varieties, materials under development in public research institutions are underreported. Frequently, new variety publications include limited genealogy, which ignore or are unaware of historical and ancestral crosses made with CWR. What is in the pipeline of the breeding programs of private companies is, of course, commercially sensitive and is therefore even more difficult to estimate. In the absence of mechanisms for accurately tracing the genetic contribution of CWR to a given variety and its phenotypic effect, it is difficult to estimate economic value, which would be a useful tool for informing policy decisions. Existing valuation exercises (Prescott-Allen and Prescott–Allen, 1986; Pimentel et al., 1997; Hein and Gatzweiler, 2006; Hunter and Heywood, 2011) show a wide spread of estimates of economic value, likely underestimating the potential value of wild species, and do not reflect the breadth of ongoing work with CWR. There is clearly a need to improve mechanisms for monitoring the use of wild species in breeding programs.

There are several ways in which wild diversity can be used, and it is possible to distinguish two main avenues: (i) “Choose first”: wild materials that express a certain trait of interest are chosen on the basis of phenotypic, genotypic, or collection locality data and are used in targeted crossing, then the off-spring are evaluated; or (ii) “Cross first”: a wider range of wild and domesticated materials are crossed and the resulting progeny are screened for traits of interest in the domesticated background. Depending on the trait, the first approach can be done either by targeted pathology screens exploring agronomic performance data (observable traits expressed directly by an individual), genotypic data (alleles known to be involved in a given trait of interest), or a combination of both using the tools of statistical genetics. This concept has been described as predictive characterization in the literature (Thormann et al., 2014). Once traits of interest have been identified in a wild accession or individual, they need to be transferred into crop backgrounds. Alternatively, crosses between wild and domesticated taxa are made first, and their progeny, either as F1, or later-generation materials, are screened for beneficial traits. The latter strategy, though much less focused initially, has the potential to reveal unexpected sources of diversity that only become apparent when introgressed in a domesticated background.

Once alleles of interest have been transferred into a target background, a certain number of backcrosses are needed to dilute the deleterious diversity that is often associated with CWR introgressions. When these undesirable traits are linked to a trait of interest, it is often described as linkage drag (Zamir, 2001). Wild species have many traits associated with poor agronomic performance that have been systematically selected out of their domesticated relatives. These can include, amongst other things, low yields, seed shattering, and smaller seed or fruit size (Salamini et al., 2002). These traits introduce numerous practical issues for breeders, such as difficulty recovering sufficient numbers of seeds, not being able to use standard equipment, or needing to address different agronomic or horticultural requirements. Linkage drag remains a significant deterrent to using wild species in many crop improvement programs, although dense genetic mapping followed by marker-assisted selection (MAS) may be used in some cases to help tackle this difficulty.

Despite these difficulties, optimism is pervasive across the expert communities surveyed that wild species will play an increasingly important role in crop improvement. Our in-depth literature review of the past use of CWR is the most comprehensive of such efforts to date and expands on the 10-yr-old work of Hajjar and Hodgkin (2007), who focused on 19 crops and included extensive breeder interviews, as well as the review by Maxted and Kell (2009) and the earlier, first comprehensive review of CWR use in plant breeding (breeding uses) by Robert and Christine Prescott-Allen in 1986. Our results, which have now been made available as an online resource (http://www.cwrdiversity.org/checklist/), include 4157 potential or confirmed “uses” of CWR in crop improvement, spread across 127 different crops and 970 CWR taxa. They segregate into seven “breeding use classes” as follows: agronomic trait (485), abiotic stress (700), biotic stress (2427), fertility trait (272), morphological trait (20), phenological trait (54), and quality trait (199) (Fig. 1–3, Supplemental Tables S1 and S2, Table 1). As Fig. 1 clearly shows, sunflower (Helianthus annuus L.), wheat (Triticum aestivum L.), and potato (Solanum tuberosum L.) are the crops for which most breeding uses of CWR have been documented. For sunflower, the largest “use” category after biotic stresses are fertility traits, which is explained by the historically important role CWR have played in identifying sources for cytoplasmic-male sterility for the development of hybrid sunflowers. For wheat and potato, the abiotic stress categories appear to be particularly strong, indicating that, unlike for most other crops, CWR of potato and wheat appear to have been exploited quite significantly for abiotic stress resistances already. Overall, the number of “use” references appears to be increasing over time, particularly since the turn of the century (Fig. 3). This indicates a trend towards an increased recognition in
the scientific community of the value of these species. In 2011, the number of cited breeding uses peaked due to the release of the book series *Wild Crop Relatives: Genomic and Breeding Resources* (Kole, 2011).

The extent of CWR use varies widely among crops. Crops with long histories of breeding with their wild relatives continue to benefit the most from wild genetic diversity (Fig. 1). Rice (*Oryza sativa* L.), tomato (*Solanum lycopersicum* L.), and wheat in particular have sizable and well established prebreeding programs that focus specifically on CWR, leveraging advanced genomic tools and diverse characterization and evaluation data (Hajjar and Hodgkin, 2007; Kilian et al., 2011; Nemeth et al., 2015). Relatively neglected crops such as cowpea (*Vigna unguiculata* (L.) Walp.), cassava (*Manihot esculenta* Crantz), sorghum (*Sorghum bicolor* (L.) Moench), sweet potato (*Ipomoea batatas* (L.) Lam.), and pearl (*Pennisetum glaucum* (L.) R. BR.) and finger millets (*Eleusine coracana* (L.) Gaertn.) have yet to benefit from largescale investment in prebreeding programs and are therefore in the earlier stages of developing varieties using CWR. According to our survey, for some of these crops, progress has been made in identifying wild species as sources of traits of interest, but the release of varieties that contain wild material in their pedigrees remains rare.

For some crops, such as alfalfa (*Medicago sativa* L.), cassava, chickpea (*Cicer arietinum* L.), cowpea, finger millet, maize (*Zea mays* L.), and sweet potato, breeders have largely been able to find sufficient sources of resistance and variation for traits of interest within the domesticated gene pool. Wild species in each of these cases have been identified as potential sources for specific traits but are not sought by breeders for the widening of the genetic base of the crop per se. Even in the case of tomato, where the use of wild species in breeding is pervasive and most lines today host alleles from wild species (Hajjar and Hodgkin, 2007), CWR have not yet been systematically explored for heat tolerance (a breeding priority in tomato under predicted climate-change scenarios) because sources have been identified in cultivated material. In maize, *Teosinte* and *Tripsacum* species have been explored to a much lesser extent than wild species of crops with breeding programs of comparable size and investment. This may be the case because of the large amount of genetic diversity in maize landraces, meaning that breeders have less of an incentive to look outside of...
the primary genepool. Another factor, according to our expert consultations, is that the private sector is less likely to consider the use of CWR in crop breeding programs for which the profit margin relies on minor increases of the yield advantage every year, such as maize or soybean [Glycine max (L.) Merr.]. The risk of linkage drag and introducing unwanted variation that could negatively influence the finely tuned “genetic machinery” of these high-yielding varieties is commonly considered too high. However, crops with a relatively narrow genetic base need the genetic variation in wild gene pools to continue to adapt.

Breeders in apple (Malus pumila Borkh.), banana (Musa acuminata Colla and Musa balbisiana Colla), barley (Hordeum vulgare L.), bean (Phaseolus vulgaris L.), bread and durum wheat (Triticum durum Desf.), lentil (Lens culinaris Medik.), pearl millet, potato, rice, sorghum, and soybean in particular report a lack of genetic variation for traits of interest within the domesticated gene pool, necessitating greater exploration of CWR. Barley breeders, for example, have reported in our consultation meetings that they are starting to reach the limit of what they are able to do with the genetic variation that is available in the domesticated gene pool, and many crops remain highly vulnerable to biotic stresses, such as bacterial or fungal pathogens, without identified sources of resistance. Apple, bean, lentil, and soybean experts likewise emphasize large genetic bottleneck effects and insufficient genetic variation in cultivated varieties.

Table 1. An overview of challenges, advancements, bottlenecks, and strategies of crop wild relative (CWR) use, as determined through the consultations with prebreeding experts.

<table>
<thead>
<tr>
<th>Challenges to using CWR</th>
<th>Advancements facilitating use</th>
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<tbody>
<tr>
<td>Interspecific crossability</td>
<td>Tissue culture and embryo rescue</td>
</tr>
<tr>
<td>Missing data on species’ basic biology (ploidy levels, life history traits, taxonomy)</td>
<td>Ongoing research</td>
</tr>
<tr>
<td>Lack of understanding of gene–trait relationships</td>
<td>Ongoing genomics research</td>
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<tr>
<td>Predicting how allelic combinations will be expressed in different cultivated crop backgrounds</td>
<td>Development of introgressed materials</td>
</tr>
<tr>
<td>Transferring gene(s) of interest from wild to cultivated backgrounds with precision</td>
<td>Basic research into epistasis</td>
</tr>
<tr>
<td>Funding constraints</td>
<td>Advanced embryo rescue techniques</td>
</tr>
<tr>
<td>Human capacity limitations</td>
<td>Transgenic technologies</td>
</tr>
<tr>
<td>Bottlenecks in the prebreeding process</td>
<td>Marker-assisted selection (MAS)</td>
</tr>
<tr>
<td>Underrepresentation of wild species diversity in genebank collections</td>
<td>Efforts to communicate the value of CWR, particularly in the context of climate change, will raise the profile of prebreeding programs and help secure resources and build capacity.</td>
</tr>
<tr>
<td>Unidentified and misidentified accessions in genebanks</td>
<td>Increase collection, informed with gap analyses</td>
</tr>
<tr>
<td>Lack of characterization and evaluation data for genebank accessions</td>
<td>Improve documentation of incoming materials (collection of passport data, consistent taxonomy)</td>
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<tr>
<td>Restricted access to materials under development</td>
<td>Compare accessions using genomics data</td>
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<tr>
<td>Disconnect among research communities</td>
<td>Mechanisms to ensure feedback to genebanks from screening</td>
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<tr>
<td>Lack of clarity regarding roles and responsibilities</td>
<td>Systematic and coordinated characterization and evaluation efforts; compilation of multilocation evaluation data</td>
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<td></td>
<td>Bioinformatics initiatives to manage data and make it accessible</td>
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THE INTROGRESSION PIPELINE

Crop experts throughout the consultations point out that hybridization via controlled pollination remains the primary way that novel genes are introduced into cultivated crops. For most crops, there are at least some wild taxa that are readily cross-compatible (see www.cwrdiversity.org/checklist), although successful crossing is often challenging and time-consuming. The crop genepool concept developed by Harlan and de Wet (1971) is used to classify relatedness between crops and related taxa. Species are categorized into primary, secondary, and tertiary gene pools. Species in the primary gene pool typically have no or only limited crossing barriers with the crop, while species in more distant gene pools commonly encounter cross-incompatibility and hybrid sterility. As the evolutionary distance between wild species and the crop increases (i.e., secondary and tertiary gene pools), they become more difficult to use (Harlan and de Wet, 1971). For crops for which a categorization of CWR into gene pools according to relative crossability has not been done, taxonomic classification can be a useful proxy. This method of classification is referred to as taxon group concept (Maxted et al., 2006). Incompatibility barriers can be manifested in several ways; for example, poor pollen growth, embryo formation, hybrid viability, and hybrid fertility can all inhibit CWR use (Andersson and de Vicente, 2010; Dempewolf et al., 2012).
In our consultation meetings, it became clear that a wide range of techniques is being used to overcome crossing barriers. In many cases, such as common bean, wide hybridization is performed initially with a large number of accessions, to assess combining ability, and then with a smaller number of carefully selected lines for specific trait-based analyses (Porch et al., 2013). Bridge crosses are another technique that uses an intermediary species, which is crossable with both parents, to indirectly transfer genes into the desired cultivated background (van de Wiel et al., 2010). Often, interspecific hybrids have difficulty forming viable seeds or healthy embryos. These issues can be overcome by removing young embryos from ovules and growing them in an artificial medium, a technique referred to as embryo rescue. Embryo rescue is considered standard practice in lentil prebreeding programs, for example, with very low failure rates. In cases where hybridization does not work due to lack of pollen recognition or otherwise, a technique called somatic hybridization, or protoplast fusion, can be used. This approach has been successfully employed to make interspecific hybrids in many crops (Glimelius et al., 1991; Liu et al., 2005). An additional set of techniques may be required to facilitate wide crosses between species with different ploidy levels. Doubling the number of chromosomes is sometimes necessary to return to the correct ploidy level of the crop. Chemicals, primarily colchicine, can be used to induce chromosome doubling. Synthetic hexaploid wheat lines are derived using these techniques (Dreisigacker et al., 2008; Kilian et al., 2011). Some ploidy barriers can be overcome with carefully planned crossing schemes. Edible banana are sterile triploids. Breeders cross them with either wild or cultivated diploid material to get tetraploids, which have great potential as a source for useful traits but are also sterile. This material is crossed again with a diploid species to create a secondary triploid with novel genes. Conventional breeding logic contends that only edible diploids should be used in crosses; however, banana wild species with undesirable phenotypes have proven to lend valuable hidden traits to hybrids (R. Swennen, personal communication, 2014).

Although progress has been made in overcoming crossing barriers, they remain a substantial hurdle for the increased use of CWR in crop improvement programs, and the number of scientists who have the necessary experience in cytogenetics is dwindling. This decreasing capacity in many crop improvement programs is a growing constraint and needs to be addressed through more dedicated training programs and the inclusion of training in teaching these techniques in relevant higher education programs.

TECHNOLOGICAL ADVANCES IN USING CWR

Once genetic material has been introduced into a domesticated background from CWR, populations are commonly developed to study the introduced genes, map their location in the genome, and further deploy them using MAS. Using MAS increases the efficiency of the breeding process, as large numbers of progeny can be screened in a systematic way (van de Wiel et al., 2010). Before MAS can be performed, the trait must first be genetically mapped and the genotypic effects validated. Genetic mapping correlates genotypes with phenotypes, tagging the alleles responsible for a trait with molecular marker(s). We learned during the consultations that genomewide association studies (GWAS) or quantitative trait locus (QTL) mapping approaches are used most commonly (Takeda and Matsuoka, 2008; Morrell et al., 2011). For example, QTLs have been identified in wild bean associated with seed size and yield, and genetic analyses are being conducted on domesticated bean varieties to advance understandings of drought and heat tolerance and to shed light on key genomic regions worth exploring in wild species (Wright and Kelly, 2011). Additional mapping methods are currently being employed to increase mapping accuracy: for example, multiparent advance generation intercross (MAGIC) (Cavanagh et al., 2008) populations and nested association mapping (NAM) populations (Yu et al., 2008; Nice et al., 2016).

Some population development schemes aim to generate specific types of recombinants. Chromosome segment substitution lines (CSSLs) are series of introgression lines, each with one or more chromosome segments from the donor species in a common recipient background, which together represent the entire donor species’ genome. These CSSLs allow the detection and mapping of QTLs across the whole genome and the study of interactions among QTLs (Xu et al., 2010). They have the added benefit of being discrete and approachable genetic resources for breeders. Other types of introgression lines are useful for isolating the effect of a single gene from a wild species in a crop background. For example, tomato breeders at the World Vegetable Centre (AVRDC) annually distribute hundreds of introgression lines with genes from wild tomatoes to breeders in national agricultural research institutes, who then develop and release varieties suited to local conditions (P. Hanson, personal communication, 2014). Desired recombinants can be produced in early generations given a large enough population. For example, marker-assisted backcrossing (MABC) has been used in lowland rice to introduce an important QTL into a new background in just two rounds of backcrossing (Iftekharuddaula et al., 2011).

With genomic tools, it is also becoming increasingly possible to directly assess the contribution of CWR in modern varieties (Baute et al., 2015), and different software tools are emerging to facilitate such analyses (Milne et al., 2010; Shaw et al., 2014). Genome scans can also reveal candidate domestication and improvement loci, as
well as postdomestication introgression with wild relatives (Lin et al., 2014; Russell et al., 2016).

The systematic investigation of diversity from CWR and landraces—often called “native trait research”—is also experiencing a revival in some parts of private industry. This is sometimes preferred as an alternative to the use of transgenic technologies associated with high regulatory costs and widespread consumer concern. However, transgenic approaches continue to prove useful as an experimental method in understanding the function of candidate alleles from CWR. Once the function of an allele is confirmed, MAS can be used to incorporate allele(s) without incurring the regulatory costs associated with genetically modified organisms. Transgenic technologies are mostly employed by the private sector (Brunner et al., 2011), and since we lack access to such commercially sensitive information, their actual use is likely underestimated here.

Genomics has revolutionized the rate of marker development and deployment. Several crops now have dense single-nucleotide polymorphism (SNP) chip platforms (biallelic SNP arrays) (Comadran et al., 2012; Wang et al., 2014), and the cost of DNA sequencing has continued to drop significantly with time. Among the most recent SNP platforms developed are those for *Hordeum* species (Bayer et al., unpublished data, 2017), *Brassica napus* L. (Clarke et al., 2016), *Capsicum annuum* L. (Hulse-Kemp et al., 2016), and *Helianthus annuus* L. (Livaja et al., 2016). Crops lacking SNP genotyping platforms, or in cases where new, diverse material is being used (such as CWR), high-throughput sequencing offers a cheap and rapid way to deploy thousands to millions of markers for mapping studies (Kilian and Graner, 2012). Reduced representation methods, such as restriction-site-associated DNA sequencing (RAD-seq) (Baxter et al., 2011), genotyping-by-sequencing (GBS) (Elshire et al., 2011), or even NimbleGen Exome capture (Warr et al., 2015) have been used to this effect in several CWR species already (He et al., 2014; Wendler et al., 2015; Russell et al., 2016). The marker density offered by these technologies allows for rapid fine mapping and can saturate mapping populations in terms of detecting all of the recombination events. For association panels, which contain a great deal more recombination events than biparental populations, ultra-dense markers may be especially important.

The availability of a (draft) “reference” genome sequence greatly facilitates the use of high-throughput sequence data. Although much of the early sequencing efforts focused primarily on cultivated material, largescale CWR genomic (re)sequencing efforts are now well underway (The 100 Tomato Genome Sequencing Consortium et al., 2014; Brozynska et al., 2016; www.africanorphancrops.org), often with reduced representation methods (Russell et al., 2016). Whole-genome shotgun sequence (WGS) approaches can also be used to characterize CWR germplasm. Rice, for example, has a relatively small genome at 430 Mbp, and several wild genomes have been resequenced using WGS (Xu et al., 2011; Li et al., 2014; Alexandrov et al., 2015). Many complete germplasm collections are already being genotyped or even sequenced, including maize (Romay et al., 2013), chickpea (Varshney 2016), rice (Li et al., 2014), soybean (Song et al., 2015), wheat (Sehgal et al., 2015), and others; this will vastly increase our knowledge and understanding of these genepools.

Landscape genomics and environmental association analysis are also ways to detect putatively valuable alleles in CWR (Anderson et al., 2016). Genomics may thus be able to support the collecting and conservation of CWR, as well as their use. Unlike collection data such as environment or local ecology, genomics data can be generated for every single plant. Sequence data truly represent the diversity in accessions and could be used as a common currency with which to compare accessions. It must be noted, however, that the development and application of genomics resources is still not a priority for all crop communities. For example, experts from the pea (*Pisum sativum* L.) and alfalfa crop communities acknowledged that, although genomics approaches are useful, making morphological characterization data available together with germplasm is currently more urgent and is an essential prerequisite for genomics to be used effectively.

The advent of genome editing is predicted to revolutionize plant breeding (Voytas 2013; Bortesi and Fischer, 2015; Khatodia et al., 2016). These technologies enable scientists to alter the genome of an organism with unprecedented precision and without the introduction of DNA from another organism. Although these methods are still in their infancy, their increased precision will likely result in substantial efficiency gains in crop improvement processes (Hu et al., 2016; Wang et al., 2016). However, as the regulatory landscape continues to evolve, it remains to be seen whether they will prove a viable alternative to transgenic and traditional breeding approaches (EFSA Panel on Genetically Modified Organisms, 2012; Araki and Ishii, 2015). In any case, genome editing relies on a thorough understanding of the genetic basis of a given trait that is still often lacking for the majority of traits of interest, which are commonly complex and quantitative. However, alterations of larger pieces of sequence information and of different genes simultaneously (e.g., haplotype blocks), as well as of structural variants (e.g., chromosomal fragments), are becoming increasingly feasible (Lowder et al., 2015; Ma et al., 2015). Genome-editing tools may well provide a further boost to the use of CWR in the near term. Crop wild relatives and the sequence information contained within them may serve as a reference library for all kinds of allelic diversity. Information on allelic diversity and its phenotypic effects is an essential requirement for many of the genome-editing techniques that are now emerging. In fact, these
approaches will enable the use of sequence information from more distantly related, cross-incompatible CWR (and indeed non-CWR) for crop improvement (Nogué et al., 2016; Spindel and McCouch 2016).

**BARRIERS TO INCREASED USE OF CWR AND WAYS TO OVERCOME THEM**

According to the experts that we surveyed, the lack of data on CWR, both phenotypic and genotypic, is the most important barrier to their increased use (Fig. 4). Approximately 7 million crop accessions are currently stored ex situ in genebanks, of which an estimated 2 million are biologically unique (Commission on Genetic Resources for Food and Agriculture, 2010). Much critical information is lacking for a lot of this material. Without accurate passport, characterization, and evaluation data, both genebank curators and potential users of materials are left with unanswered questions regarding how much diversity is in each accession, how similar accessions are, how much duplication exists within and among collections, how to capture the most diversity across the genepool, and how to find alleles for specific traits. This limits the usefulness of collections, and experts from many crop communities warn against further collecting without first improving the existing system of managing collections and their associated data, both of CWR and derived genetic stocks. Using standardized systems for identifying accessions and agreed taxonomies are essential measures for improving documentation and will facilitate the development of standardized protocols for managing collections. The reported lack of data on crop wild relatives could, in some cases, indicate actual gaps in collections with regards to physical CWR accessions. Genebank collections are often incomplete, and the diversity of the CWR for many crops is not captured to an adequate extent (Commission on Genetic Resources for Food and Agriculture, 2010). Collection and conservation of this diversity is critical, because the future of materials that are still in the wild is precarious. Priorities moving forward are to fill these gaps and to ensure that maximum diversity is represented within collections before it is too late (Castañeda-Álvarez et al., 2016). One strategy for targeting underrepresented diversity of the greatest potential value is to focus collection in areas of extreme climates to mine for specific, rare traits. Linking collection location information with genomic data to map allelic variation and predict the usefulness of specific wild populations, or even alleles, is a novel approach (Thormann et al., 2014; Anderson et al., 2016; Russell et al., 2016). The collection of at least the most basic passport data along with all new samples is essential, and the FAO/Bioversity Multi-Crop Passport Descriptors V.2.1 are a useful minimum requirement (Alercia et al., 2015). Data recorded at the time of collection is crucially important, but collection data should ideally move beyond just collection locality and associated environmental factors. Soil type, disease presences, and the local ecology are all important factors in the evolutionary history of a particular CWR accession, and this information may turn out to be of key importance to someone interested in the potential usefulness of a particular accession. In our consultations, many crop communities highlighted the need for common information resources to be developed that make germplasm available together with all relevant information (McCouch et al., 2013). Comprehensive online databases exist for some crops already, but efforts have been insufficient for bridging the basic research and the applied breeding communities, and there is still little data available for breeding materials under development.

Other major challenges to an increased use of CWR include: biological barriers to crossing, a lack of basic characterization information available on wild species, linkage drag, perceptions of wild species’ inferiority relative to elite material, a lack of understanding of the genetic base of complex traits, poor management and maintenance of CWR in ex situ collections, lack of institutional support, and funding limitations. Identifying traits of interest is complicated by the difficulty in predicting how alleles from wild species will be expressed once transferred into crop backgrounds and grown in the field. Wild species can carry beneficial allelic variation for traits without expressing them directly. This “hidden” or “cryptic” variation can sometimes be revealed during the crossing process in transgressive segregation (deVicente and Tanksley, 1993; Xu et al., 1998; Gibson and Dworkin, 2004). It is difficult to predict potential yield gains from nondomesticated plants with small seeds and pods that shatter, for example, or to identify...
interesting alleles when their measured effects are masked by inferior wild background traits. Experts in wheat and banana emphasized how cryptic variation can result in significant, and often unexpected, superior performance of crosses between wilds and their domesticated crops. The perception of wild species’ inferiority on the basis of their agronomic performance at face value can deter breeders from exploring them for useful traits.

Experts across the crop communities call for a more systematic evaluation and screening of trait variability within CWR ex situ collections. Potato, pea, and sorghum experts emphasized that existing accessions remain underexploited, that the screening of materials is far from exhaustive, and that funds are more appropriately concentrated on maintaining existing collections, rather than developing prebreeding materials or expanding collections. These recommendations, however, are consistently qualified with warnings about genetic erosion of wild populations in situ and the continued importance of targeted collection based on gap analyses. This is a real dilemma in many crop expert communities, given limited financial resources. Coordinated phenotypic evaluation of prebreeding material among researchers is recognized as a valuable means of assessing genotype–environment interaction. Common introgression lines developed from agreed sets of cultivated backgrounds could be exchanged and used to develop regionally adapted materials. However, phenotypic evaluations of CWR of clonal crops—especially those of long-lived perennials—can be challenging, due to resource limitations and practical considerations associated with their cultivation.

The conservation and sharing of an “advanced germplasm” that has undergone some level of development is considered important by many of the expert communities. Many of these materials carry beneficial traits introgressed from CWR, yet they are not appropriately maintained or made available for further use. The International Treaty of Plant Genetic Resources for Food and Agriculture (ITPGRFA) provides an access and benefit-sharing framework that also considers such “materials under development.” The adoption and implementation of this internationally agreed framework should be more strongly advocated and followed.

An additional hurdle that is often overlooked is inadequate human resource capacity to make use of wild accessions stored in genebanks. Several crop communities, including banana, wheat, and pea, reported an aging skill base and a decline in available expertise in botany, cytogenetics, taxonomy, and curation of genebank collections of wild material. Our capacity to employ methods to better maintain and use CWR is predicated on reestablishing this skill base and investing in strong breeding capacity.

Resource constraints underpin the majority of the challenges discussed here. Funding is identified as a limiting factor to accessing CWR diversity across all crop communities surveyed. Identifying, isolating, and transferring traits of interest from wild species into crop backgrounds, and then evaluating the resulting material, is a time-consuming and risky endeavor that requires significant funding and long-term commitment. However, since CWR are at the nexus between food security, climate change adaptation, and biodiversity conservation issues, donors can be presented with an opportunity to allocate resources in a way that contributes to all of these ends at once. The establishment of long-term funding for prebreeding programs through Public Private Partnerships (PPPs) is one potential strategy, and pioneering success stories are underway: for example, on wheat, LOLA-WISP (http://www.wheatisp.org/) and WHEALBI (http://www.whealbi.eu/), and apples (https://sites.google.com/a/nordgen.org/ppp-apples/).

CONCLUSIONS

Table 1 summarizes the findings presented in this review. Significant advances have been made towards overcoming the challenges associated with using CWR in crop improvement. Ongoing basic and applied research will indubitably further facilitate their use in coming years.

At the same time, the numerous challenges in the prebreeding process identified in this report need to be addressed for progress to be achieved. In particular, strategies aimed at increasing coordination among actors along the prebreeding continuum will facilitate sharing of characterization and evaluation data, as well as raw genetic material stored in genebanks and more advanced materials under development. Important elements of a strategy for improving the prebreeding process are likely to include the selection of common parents to be used in introgression lines, the systematic and coordinated evaluation of prebreeding materials in multiple locations, the development of feedback mechanisms for screening data, and data management and sharing initiatives.

Many accessions of CWR currently held in genebanks lack important information, including where they were collected, what their basic characteristics are, what they may be useful for, and whether they are a unique sample or a duplicate of another. Extensive consultation with experts in 24 crop communities identified this lack of passport, characterization, and evaluation information on both wild germplasm and intermediary materials as the biggest constraint to increasing the use of CWR in breeding. Lack of clarity surrounding the specific roles of actors along the prebreeding continuum and coordination among basic and applied research communities, along with funding and capacity limitations, have inhibited the systematic use of the breadth of wild species diversity that is available today.
In conclusion, some key requirements for the more effective use of CWR in crop improvement include:

1. Collection of underrepresented diversity, informed by gap analyses based on taxonomic designations and ecogeographic information, along with detailed passport data to both conserve the breadth of diversity available in the wild and facilitate predictive trait mining based on ecogeographic data;
2. Coordinated evaluation and sharing of prebreeding products across environmental conditions to better understand genotype–environment interactions;
3. Improved information sharing and feedback, especially with regards to genotypic and phenotypic data—and the way they are linked—between genetic resource conservationists, pre-breeders, breeders, and end users; and
4. Enhanced coordination between basic and applied research communities.

As the need to explore wild genetic diversity grows along with threats of climate change and the narrowing genetic base of crops, it is increasingly important that we understand how wild species are being used to improve crops and where efforts should best be concentrated to overcome constraints to use. A closer look at the current state of CWR use reveals persisting challenges, as well as significant opportunities for harnessing their value. Our capacity to overcome these challenges and harness the adaptive capacity stored in CWR is predicated on increased coordination, information sharing, and investment in prebreeding programs and human capacity development. Gains have already been made and momentum is building. With a clearer picture of where bottlenecks remain, the value of CWR for increasing the adaptive capacity of agricultural crops can be realized.

Conflict of Interest
The authors declare there to be no conflict of interest.

Supplemental Material Available
Supplemental material for this article is available online.

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