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Introgressiomics: a new approach for using crop wild relatives in breeding for adaptation to climate change

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Abstract

The need to boost agricultural production in the coming decades in a climate change scenario requires new approaches for the development of new crop varieties that are more resilient and more efficient in the use of resources. Crop wild relatives (CWRs) are a source of variation for many traits of interest in breeding, in particular tolerance to abiotic and biotic stresses. However, their potential in plant breeding has largely remained unexploited. CWRs can make an effective contribution to broadening the genetic base of crops and to introgressing traits of interest, but their direct use by breeders in breeding programs is usually not feasible due to the presence of undesirable traits in CWRs (linkage drag) and frequent breeding barriers with the crop. Here we call for a new approach, which we tentatively call 'introgressiomics', which consists of mass scale development of plant materials and populations with introgressions from CWRs into the genetic background of crops. Introgressiomics is a form of

pre-emptive breeding and can be focused, when looking for specific phenotypes, or un-focused, when it is aimed at creating highly diverse introgressed populations. Exploring germplasm collections and identifying adequate species and accessions from different genepools encompassing a high diversity, using different strategies like the creation of germplasm diversity sets, Focused Identification of Germplasm Strategy (FIGS) or gap analysis, is a first step in introgressiomics. Interspecific hybridization and backcrossing is often a major barrier for introgressiomics, but a number of techniques can be used to potentially overcome these and produce introgression populations. The generation of chromosome substitution lines (CSLs), introgression lines (ILs), or multi-parent advanced inter-cross (MAGIC) populations by means of marker-assisted selection allows not only the genetic analysis of traits present in CWRs, but also developing genetically characterized elite materials that can be easily incorporated in breeding programs. Genomic tools, in particular high-throughput molecular markers, facilitate the characterization and development of introgressiomics populations, while new plant breeding techniques (NPBTs) can enhance the introgression and use of genes from CWRs in the genetic background of crops. An efficient use of introgressiomics populations requires moving the materials into breeding pipelines. In this respect public-private partnerships (PPPs) can contribute to an increased use of introgressed materials by breeders. We hope that the introgressiomics approach will contribute to the development of a new generation of cultivars with dramatically improved yield and performance that may allow coping with the environmental changes caused by climate change while at the same time contributing to a more efficient and sustainable agriculture.

Keywords: crop wild relatives, plant genetic resources, introgression breeding, hybridization, backcrossing, genomics

1. Introduction

The expected increasing demand of plant products in the coming decades, with an estimated need of the doubling in relation to 2005 levels of agricultural production by 2050 (Tilman et al., 2011) in a climate change scenario, represents a formidable challenge for plant breeders. The current yearly rate of yield increase for major crops is clearly insufficient to meet this goal (Ray et al., 2013). Forecasts of agricultural productivity indicate that agriculture will be one of the sectors most affected by climate change (Rosenzweig et al., 2014). The negative

effects of climate change on agricultural productivity will probably be greatest in tropical and subtropical areas (Knox et al., 2012; Rosenzweig et al., 2014), where a large part of the human population lives in marginal conditions in developing countries. Climate change will undoubtedly increase both permanent and occasional abiotic stresses (drought, high temperatures, salinity) in a large part of the global agricultural land and has the potential to drive abandonment of cultivation and desertification in some regions of the world. In addition, climate change is expected to result in increased biotic stress, as many pests and diseases will potentially migrate to areas where they were not present due to environmental limitations (Bebber et al., 2013).

Although many improvements have been made in increasing the efficiency of inputs used in agriculture (energy, water, agrochemicals) in the near future higher yields will have to be obtained with less input (Godfray et al., 2010; Ray et al., 2013). Also, many agricultural lands have become salinized or have lost fertility due to mismanagement (Fita et al., 2015). The availability of new arable land is not only limited but it is undesirable to increase the agricultural land area on a global scale given that conservation of biodiversity is important for maintenance of functioning ecosystems and genetic input to agriculture in the longer term.

Meeting the challenges of increasing agricultural production in the face of climate change will require new strategies to develop new crop varieties with increased resilience to climate change-related stresses (Fita et al., 2015). Significant advances have been made in developing varieties tolerant to abiotic or biotic stresses but mostly for monogenic or oligogenic traits. The genetic variation available in cultivated species for adaptation to climate change is generally limited and it may be impossible to achieve major advances by resorting only to alleles present in the cultivated genepool (Hajjar and Hodgkin, 2007; Vincent et al., 2013; Dempewolf et al., 2014; Warschefsky et al., 2014).

Breeders have long recognized the value of crop wild relatives (CWRs) as sources of novel beneficial variation for resistance or tolerance to stresses (Bessey, 1906; Harlan and de Wet, 1971; Hajjar and Hodgkin, 2007). In this respect, most of the modern varieties of several important crops, like wheat or tomato, carry introgressions from wild relatives (Menda et al., 2014; Wulff and Moscou, 2014). CWRs are wild species that are able to exchange genes with the cultivated taxa through sexual or somatic hybridization (Harlan and de Wet, 1971, Maxted et al., 2006). CWRs are phylogenetically closely related to the crop and often encompass great genetic diversity. Some CWRs develop well under marginal conditions (low rainfall, high temperatures, high pressure of pests and diseases) that would be highly stressful for elite varieties of crops (Dwivedi et al., 2008; Dempewolf et al., 2014; Warschefsky et al., 2014). The

enormous potential of crop wild relatives for breeding new varieties adapted to climate change has largely remained unexploited, however, and the use of CWRs in breeding has largely focused on introgressing loci for disease resistance, while tolerance to abiotic stress has been little explored (Hajjar and Hodgkin, 2007; Maxted and Kell, 2009).

Recently an initiative has been launched aiming at adapting agriculture to climate change through the use of crop wild relatives (CWRs) for improving the cultivated gene pool of 29 crops included in the Annex 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) (Dempewolf et al., 2014). This project is aiming at collecting, preserving, evaluating, and preparing the use of CWRs with the ultimate aim to broaden the genetic base and thereby adapt our crops to climate change. Other initiatives, like Diversity Seek (DivSeek) are aimed at unlocking the potential of the diversity of crops and wild relatives present in genebanks (Meyer, 2015)

In this paper we present a novel strategy, tentatively named 'introgressiomics', for the systematic exploitation of the native variation present in CWRs for an efficient adaptation of our crops to climate change.

2. Crop wild relatives for broadening the genetic base of crops

Plant domestication is a unique and complex evolutionary process in which natural and artificial selection resulted in new plants adapted to human needs (Meyer and Purugannan, 2013). Domesticated plants generally present a syndrome of distinctive morphological, physiological and reproductive features compared to their wild ancestors (Hammer, 1984). For example in cereals, non-shattering phenotypes, with larger seeds, synchronized ripening, change in the plant architecture and loss of seed dormancy are characteristic traits of domesticates (Salamini et al., 2002). In other crops, such as those used for their fruits, tubers or leaves, domestication involved loss of bitterness and anti-nutritional or toxic compounds, removal or reduction of physical defence mechanisms like prickles, and gigantism of the organs used by humans (Meyer et al., 2012). One extreme example is that of giant pumpkins (*Cucurbita maxima*) with record weights of over a ton for a single fruit (Savage et al., 2015). Many crops also experienced changes in the reproductive system that isolated them from the CWRs (Meyer and Purugannan, 2013). In other cases crossability has been maintained within the genepool (Jarvis and Hodgkin, 1999; Meyer and Purugganan, 2013). Autogamy, which allows fixation and maintenance of selected characteristics, has been favoured in a number of crops (Meyer et al., 2012). Increased yield potential is also a general feature of domestication.

On many occasions domestication has involved genetic bottlenecks, resulting from a founder effect (domestication from a limited number of individuals), reproductive isolation and/or from strong selection pressures during domestication or crop improvement (Dempewolf et al., 2012; Meyer and Purugannan, 2013). This resulted in a narrow genetic base for most crops as compared to their closest wild progenitors and CWRs (Dwivedi et al., 2008; Hajjar and Hodgkin, 2008; Meyer and Purugannan, 2013; Dempewolf et al., 2014). Modern plant breeding has generally led to a further reduction of genetic diversity in comparison to modern elite varieties and landraces (Tanksley and Nelson, 1996; de Wouw et al., 2010). Notable exceptions to this trend are crops where modern varieties carry introgressions from CWRs. For example, in tomato, modern varieties are genetically more diverse than local landraces due to the multiple introgressions, usually for resistance to diseases, from CWRs (Sim et al., 2012). Nonetheless, the diversity of modern tomato is much lower than that present in its CWRs (Aflitos et al., 2014; Dodsworth et al., 2016).

A narrow toolbox of alleles in the elite varieties limits options for to better adapt crops to climate change. CWRs are often adapted to environments that are more stressful than artificial agricultural conditions. The large genetic diversity present in CWRs thus may constitute a major source of variation for improving crops with higher resilience (Warschefsy et al., 2014).

From a breeder's point of view, the utilization of CWRs present some significant challenges (Hajjar and Hodgkin, 2007; Dwivedi et al., 2008; Meyer and Purugganan, 2013). For example, crossing barriers and low hybrid fertility or sterility represent major barriers for the use of some species of CWRs in breeding programs. Although well-adapted to their natural environment, CWRs often contain a range of undesirable traits to agricultural conditions (low yield, undesirable physical and chemical defence systems, unpleasant flavour, lack of adaptation to cultivated conditions, etc.) (Meyer and Purugganan, 2013). In many cases, these "wild" traits are dominant and polygenic, and thus challenging to select against in the breeding programs. Linkage drag due to reduced recombination is another issue that is frequently observed in introgression programs. Once introgressed into the cultivated genetic background, the CWR chromosomal fragments are challenging to break into smaller components. These fragments often contain genes that confer undesirable phenotypes – and often these are linked to the gene/s controlling the traits of interest (Tanksley and Nelson, 1996; Wendler et al., 2015). In addition, phenotypes of interest in a CWR may not be expressed in a cultivated genetic background. Nonetheless, use of CWRs in breeding has allowed significant improvements in a number of crops (Hajjar and Hodgkin, 2007). Maxted and Kell (2009) list 183 CWR taxa of a

171 total of 29 crops that have been used for the transfer of traits of interest to the crop, while
172 Dempewolf et al. (2017) lists 4,175 potential or confirmed uses of CWR taxa in crop
173 improvement research, spread across 127 crops and 970 CWR taxa. In several cases the use of
174 CWRs in breeding research has not resulted in their use in the development of cultivated
175 varieties (Kilian et al., 2011). Maxted and Kell (2009) list only 39 CWR taxa that have been
176 utilized for the development of advanced cultivars in nine major cereal and legume crops.
177 Furthermore, most uses of CWRs for improvement of these nine crops are related to resistance
178 to diseases and pests (61%), while their use for the improvement of tolerance to abiotic stresses
179 (16%) or yield (7%) has been much lower. Other uses (16%) mostly involve quality traits. In
180 tomato, most of the introgressions from wild taxa have been aimed at disease resistance, with
181 18 resistances having been introduced from seven different species (Díez and Nuez, 2008). In
182 sum, the utilization of CWRs has mostly been restricted to resistance or tolerance to pests and
183 diseases, while other potential uses, like adaptation to abiotic stresses, have been largely
184 neglected. With their adaptations to challenging environments, the utilization of CWRs
185 represents a largely untapped opportunity for breeders to improve the adaptation of crops to
186 abiotic stresses such as drought, salinity and high temperatures (Dwivedi et al., 2008;
187 Dempewolf et al., 2014; Fita et al, 2015).
188 The 'genepool' concept was established by Harlan and de Wet (1971) to denote
189 differences between CWR taxa with regards to the ease of exchanging genes with the crop.
190 Typically, the primary genepool includes CWRs that can be easily crossed with the crop and
191 the offspring is fertile. Secondary genepool CWRs are those that can be crossed with the
192 cultivated species, although sometimes the degree of success is low, and/or the offspring may
193 present low fertility. Finally, the tertiary genepool is composed of CWRs which have strong
194 reproductive barriers with the crop and obtaining hybrids may require specific techniques such
195 as embryo rescue, use of bridge species, stigma excision, or the use of pollen mixtures.
196 Furthermore, hybrids between the crop and tertiary genepool species are often sterile and
197 polyploidization may be needed to restore fertility in some cases. Thus the use of tertiary
198 genepool CWRs for crop improvement is more challenging (Harlan and de Wet, 1971; Khush
199 and Brar, 1992; Dwivedi et al., 2008). Given that crossabilities have not been sufficiently
200 studied for numerous CWRs, phylogenetic relationships are sometimes used to assign CWRs
201 to the respective genepool (Maxted et al., 2016).
202 CWRs are not only of interest for their use in breeding, but can also be used directly.
203 For example, CWRs with high vigour, resistance to diseases or with higher tolerance to abiotic
204 stresses can be used as rootstocks in fruit and vegetable crops (Schwarz et al., 2010). The wild

eggplant *Solanum torvum* is commonly used for eggplant grafting due to its resistance to multiple diseases and high vigour (Ranil et al., 2015). CWRs may also be of direct use for creating new cultivated species, such as for tritordeum, which is an amphiploid hybrid between durum wheat and the wild *Hordeum chilense* (Martín et al., 1999). Tritordeum has good tolerance to drought and high temperatures (Villegas et al., 2010).

Although many CWRs are of interest for adapting our crops to climate change, many CWRs are also threatened by climate change and human impact, as demonstrated for CWRs of cowpea, peanut and potato, among others (Jarvis et al., 2008). The collection and conservation of the CWR diversity continues to be an urgent priority (Maxted and Kell, 2009; Dempewolf et al., 2014).

3. The introgressiomics approach

As CWRs cannot be directly incorporated into commercial breeding programs, pre-breeding activities have to be initiated as a first step for the utilization of CWRs (Longin and Reif, 2014). Most pre-breeding works use CWRs as donors of novel genetic diversity and is based on the following rationale:

(1) *ad hoc*, whenever an urgent breeding need appears (most commonly the occurrence of a new disease or pest), sources of useful variation are being explored. Screening usually starts within the primary genepool, mostly among the cultivars and landraces, sometimes including the closest CWRs. If unsuccessful, then screening is expanded to secondary and tertiary genepools.

(2) Once a suitable source of variation is detected, a crossing (and backcrossing) program is initiated to introgress the gene(s) conferring the desirable trait into the crop. Typically a single CWR donor species and most commonly just a single accession (Hajjar and Hodgkin, 2007) is considered.

Depending on the trait and introgressed fragment, this conventional approach can be time consuming and can require several cycles of backcrossing and selection, followed by selfing and then again further cycles of selection. Examples can be found in tomato, where most modern commercial varieties of tomato harbor resistances to nematodes or viral diseases caused by *Tomato mosaic virus* (ToMV) or *Tomato yellow leaf curl virus* (TYLCV) (Díez and Nuez, 2008). Resistance to nematodes conferred by the *Mi* locus was introduced in the 1940s from *Solanum peruvianum* accession PI128657 (Smith, 1944), while resistance to ToMV conferred by *Tm2*^{2} was introduced in the 1960s from *S. peruvianum* accession number

PI128650 (Alexander, 1963), and later the resistance to TYLCV conferred by *Ty-1* was provided by *S. chilense* accession LA1969 in the 1990s (Zamir et al., 1994). Another successful example of alien gene introgression was the introduction of the *Lr19* leaf rust resistance allele, from *Thinopyrum ponticum* to wheat (Sharma and Knott, 1966). This gene not only confers rust resistance to wheat, but also increases yield, biomass and grain number (Bedő and Láng, 2015).

Alternative approaches have been proposed. McIntosh (1992), for example, suggested the development of 'pre-emptive' breeding populations to introgress resistance loci to wheat rusts with the aim of having promising materials ready for the rapid generation of resistant cultivars – in case the predominant rust races changed or a major resistance locus break down. In this way breeders could quickly develop new resistant varieties. However, this procedure bears significant risks and is expensive. Breeders need a clear strategy, a long time horizon and significant human and financial resources. A modification to this original pre-emptive breeding approach is the creation of 'pre-breeding populations' by crossing the crop with one or several CWRs. Valkoun et al. (2001) created pre-breeding populations of wheat containing introgressions from five different CWRs from genera *Triticum* and *Aegilops*. Some of these carry beneficial traits under certain environments, such as earliness, short plant stature, drought tolerance or resistance to several rusts. Introgression lines (ILs) contain one or a few introgressed genome fragments from a CWR (Zamir, 2001; Gur and Zamir, 2004). Sets of ILs with overlapping fragments of different size can be exploited for different purposes, including for the analysis of the genetic basis for traits of (Zamir, 2001; Alseekh et al., 2013; Guerrero et al., 2016).

Here we suggest a novel approach for the development of pre-breeding materials, tentatively called 'introgressiomics' (Figure 1). 'Introgressiomics' consists of a *mass scale systematic development of plant materials and populations carrying introgressions of genome fragments obtained from (mostly wild) crop relatives into the genetic background of crops that may allow developing new generations of cultivars with improved properties*. 'Introgressiomics' is aiming at the massive generation of introgression materials for future (foreseen and unforeseen) needs and therefore may be considered as an advanced form of pre-emptive breeding. The ultimate aim of introgressiomics is to provide breeders with a significantly enlarged genetic pool from which new generations of cultivars adapted to future challenges in crop production can be rapidly obtained. Importantly, the materials developed through an introgressiomics approach are ready to be directly incorporated into breeding

pipelines. Such materials will facilitate the work of breeders, especially with respect to the challenge of breeding for complex traits (Cattivelli et al., 2008; Dempewolf et al., 2014).

The first steps introgressiomics program are as follows: 1) identification of CWRs to be used in the program; 2) hybridization and backcrossing of the crop with a number (as large as possible) of CWRs from different genepools using special techniques when needed; 3) development of multiple special introgression populations containing introgressed fragments from one or several CWRs using genomic tools; 4) creating repositories of the introgressiomics populations and materials and databases with phenotypic and genomic information; and lastly, 5) moving the materials into breeding pipelines (Figure 1).

An important aspect of introgressiomics is that populations may be created consisting of multiple genomic fragments obtained from different CWR sources. This approach of 'pyramiding of genomic regions of interest' would be beneficial for some breeding programs aiming at improving complex traits such as yield-related parameters.

Introgressiomics is similar, although more ambitious, than the approach proposed by Warschefsky et al. (2014), who pointed to the "*need for systematic efforts to introgress broad subsets of wild relative diversity to incorporate the range of useful adaptations for disease resistance, abiotic stress tolerance, and other agronomic challenges that are required in order to increase the resiliency and productivity of agriculture in the 21st century*". Warschefsky et al. (2014) suggested a five-step approach similar to that of introgressiomics consisting of: 1) build comprehensive collections of CWRs, 2) sequence them, 3) create sets of purpose-driven hybrid populations and characterize them phenotypically, 4) develop a predictive network of genotype-phenotype associations, and 5) deploy the identified phenotypes into crop breeding pipelines. Their proposal presents substantial differences in its conception than the introgressiomics approach, as can be observed by comparing our Figure 1 with the Figure 1 of Warschefsky et al. (2014). The SCREAMing (Systematic Creation of Really Exotic Abnormal Material) approach has been proposed by Bert Vandenberg (pers. comm.) to develop pre-breeding populations using CWRs, but a comprehensive description of the approach has not been published yet.

Introgressiomics, as proposed here can be: i) focused and directed at specific phenotypes, which will determine what CWRs and types of populations are required, or ii) un-focused, in which the objective is the generation of introgression materials encompassing the maximum genetic diversity possible.

4. Identification of CWRs for introgressiomics

306

307 The identification of CWRs to be utilized for introgression in the genetic background of
308 the crop is the first step in introgressiomics (Figure 1). This largely depends on the strategy to
309 be used ("focused" vs. "un-focused") and also on the availability of CWRs (Vincent et al., 2013;
310 Castañeda-Álvarez et al., 2016). In general, maximizing genetic diversity to the extent possible
311 is a priority, as this increases the likelihood of capturing beneficial allelic diversity for breeding
312 (Tanksley and McCouch, 1997; Porch et al., 2013). The number of CWR accessions to be used
313 for introgressiomics depends on the resources available, the scale and time horizon, and
314 objectives of the introgressiomics program. In order to select and identify CWRs, the 'Harlan
315 and de Wet' Crop Wild Relatives Inventory (http://www.cwrdiversity.org/checklist/; Vincent
316 et al., 2013) is a helpful resource. Currently, 173 crop genepools are described. Previous and
317 potential use cases of CWRs are also presented (Dempewolf et al., 2017).
318 One option for the identification of CWRs for 'un-focused' introgressiomics is the
319 creation of germplasm diversity sets. This type of germplasm sets allows for a more rational
320 use of accessions, while adjusting the number of accessions that can be realistically used
321 (McKhann et al., 2004). Ideally, these germplasm subsets should include CWR representatives
322 of all genepools. However, germplasm diversity sets of CWRs merely based on diversity
323 (genetic, phenotypic, origins, environmental, or a combination of them) might result in
324 underrepresentation of CWRs from the primary genepool, which generally are typically less
325 variable than CWRs from the secondary or tertiary genepools. Adjustments may be made in the
326 germplasm diversity sets to ensure a good representation of primary genepool CWRs, which
327 are the easiest to use for introgression breeding (Harlan and de Wet, 1971).
328 For 'focused' introgressiomics, germplasm sets based only on diversity are not
329 appropriate, as for this approach it is important that the germplasm sets are enriched for material
330 which is harbouring alleles that may contribute to improving the target trait/s. For example, in
331 creating introgressiomics populations aimed at improving drought tolerance, emphasis should
332 be given to include CWRs known to be tolerant to drought. In this case, strategies, like the
333 Focused Identification of Germplasm Strategy (FIGS) could help selecting potentially
334 beneficial material. FIGS is based on the assumption that accessions carry adaptive traits that
335 reflect the selection pressures to which these were subjected in the environment during
336 evolution (Street et al., 2016). Therefore, the retrieval of climatic and environmental data from
337 databases such as WorldClim (http://www.worldclim.org; Hijmans et al., 2005), WorldGrids
338 (http://worldgrids.org), or the CGIAR Consortium for Spatial Information (http://www.cgiar-
339 sci.org), using the geographical coordinates may help in identifying promising CWR

accessions. The FIGS strategy has been successfully applied for crops like wheat and beans (Bari et al., 2012; Khazaei et al., 2013). However, the potential of FIGS for selecting CWRs for introgression breeding is yet to be fully exploited (Street et al., 2016). A similar approach consisting of the use of eco-geographical data and expert assessment has allowed the identification of CWRs of sunflower to improve tolerance to various abiotic stresses (Kantar et al., 2015). Also, the utilization of historic characterization and evaluation data obtained *ex situ* may be appropriate to identify CWR accessions of potential interest for breeding.

The creation of CWR sets requires the exploration of publicly available *ex situ* germplasm repositories as well as private working collections, when possible. Unlike accessions of the cultivated species, CWRs are often underrepresented in *ex situ* genebanks (Maxted and Kell, 2009; Castañeda-Álvarez et al., 2016), which means that relevant diversity may not be immediately available for introgressiomics. This clearly reduces the potential for introgressing genes of interest from CWRs. The detection of potential collection gaps for CWRs is a first order step in order to fully exploit CWRs (Dempewolf et al., 2014). Gap analysis of germplasm collections is a strategy that was proposed by Maxted et al. (2008) and consists of identifying the taxa and geographical areas underrepresented in *ex situ* collections using taxonomic, genetic, eco-geographical, and threatened status data and other assessments. Additionally, comparison of georeferenced herbarium specimen data with the geographical coordinates of collection of the accessions conserved in *ex situ* collections allows identification of priority CWR taxa for collection as well as conservation areas as has been demonstrated in beans (Ramírez-Villegas et al., 2010) or eggplant (Syfert et al., 2016). In this respect, the Crop Wild Relative Occurrence Database (Centro Internacional de Agricultura Tropical, 2017) contains over five million records of germplasm accessions and herbarium databases on CWR and is of great interest for identification of gaps in the present germplasm collections.

Combining genomics and phenomics information might further assist the selection of CWRs for introgressiomics, as shown by Vosman et al. (2016) for CWRs of cabbage resistant to the cabbage aphid. The combination of phenotypic and genomics data from introgressiomics populations can be used for 'introgressiomics by design', which is an extension of the Breeding by Design™ approach (Peleman and van der Voort, 2003). The 'introgressiomics by design' approach would consist in mapping loci of agronomic interest from different donor CWR and using crossing and pyramiding schemes (Gur and Zamir, 2015) to introduce several favourable introgressions from different CWRs in a single line with the crop genetic background (Peleman and van der Voort, 2003).

5. Interspecific hybridization and backcrossing

A central step for introgressiomics is the hybridization between the crop and the CWR to create introgression populations. Theoretically, the crossability between the crop and all taxa within the primary genepool, which often comprise also the genome donors of the crop, should not present more difficulties than for intra-specific hybridizations (Harlan and de Wet, 1971, Jones, 2003, Maxted et al., 2006; Vincent et al., 2013). Although occasionally and depending on the crop, hybridization can be challenging for several reasons, such as non-synchronous flowering, cross-incompatibilities or fertility issues (Rieseberg and Carney, 1998; Dwivedi et al., 2008; Jones, 2003; Vincent et al., 2013).

Interspecific hybridization between the crop and CWR taxa from the secondary and tertiary genepools (wide or distant crosses) are often more challenging and hampered by pre-zygotic and post-zygotic reproductive barriers (Zenkteler, 1990; Khush and Brar, 1992) (Figure 2). In addition, although it may be possible to obtain hybrids, sterility issues may limite the development of introgressiomics populations. One important pre-zygotic reproductive barrier is pollen-style incompatibility (Dwivedi et al., 2008). As a consequence pollen does not germinate. When 'unilateral' incompatibility exists, hybrids can be obtained using the female parent in which the pollen is able to germinate (Figure 2). Unilateral incompatibility can frequently be observed when crossing self-compatible with self-incompatible species. The cross is successful when using the self-compatible species as a female parent (Dhaliwal, 1992). For example when crossing the cultivated tomato (*Solanum lycopersicum*) with its wild relative *Solanum peruvianum* the cross is unsuccessful when using the self-incompatible *S. peruvianum* species as female parent, but possible when using it as male parent. In the former case the barrier is pre-zygotic and the pollen tube can not penetrate the style. The reciprocal cross is possible, although the embryo must be rescued and cultured *in vitro* to prevent the embryo abortion (Hogenboom, 1984). When 'bilateral' incompatibility exists, several techniques have proven useful to overcome it (Rieseberg and Carney, 1998; Dwivedi et al., 2008). Among others, pollen mixtures of compatible pollen, inactivated or not, stigma exertion, physical or chemical treatments to the stigmas, or in vitro fertilization can be applied (Zenkteler, 1990; Khush and Brar, 1992; Dwivedi et al., 2008). Pollen mixture and treatment of stigmas with H_3BO_3 and GA_3 were used by Picó et al. (2002) to obtain offspring from crosses between the cultivated tomato and the distant wild relatives *S. chilense* and *S. peruvianum*.

If the male gamete is able to reach the ovary and to fertilize the female gamete in the ovule, post-zygotic barriers may prevent obtaining a viable hybrid seed. Most important post-

zygotic barriers are caused by differences in number of chromosomes, ploidy levels, chromosomal alterations (rearrangements, duplications, inversions or translocations), embryo-endosperm incompatibility and hybrid lethality (Khush and Brar, 1992). These post-zygotic barriers (Figure 2) may result in embryo abortion, preventing the development of viable seed. However, different techniques can be applied to try to avoid post-zygotic barriers. For example, when crossing individuals of different ploidy levels, the use of technologies that modify the ploidy level can contribute to the development of viable hybrids. Most frequently used techniques to obtain parental lines with the same level of ploidy are anther culture to produce haploid individuals (chromosome complement reduced to a half), or the duplication of the genome with colchicine (Khush and Brar, 1992; Rieseberg and Carney, 1998; Dwivedi et al., 2008), Also, embryo rescue, at an early stage of development (i.e. before it aborts due to either embryo-endosperm incompatibility or due to abnormal development resulting from genetic imbalance or other alterations), has been a successful tool in producing interspecific hybrids between crops and CWRs (Khush and Brar, 1992; Sharma et al., 1996).

When pre-zygotic and post-zygotic barriers cannot be overcome to obtain hybrid plants, the use of bridge species has to be considered (Shivanna and Bahadur, 2015). These species are compatible with one or both target taxa for interspecific hybridization. Once the interspecific hybrid between one of the taxa and the bridge species has been obtained, F1 plants can then been crossed with the other species - or with the other interspecific hybrid between the bridge species and the other parent. In this way, it has been possible to transfer genes from some CWRs to cultivated species. For example, the wild strawberry *Fragaria vesca* is a potential a bridge species for introgression breeding of strawberry (*Fragaria x ananassa*) from the wild *F. nilgerriensis*, *F. nubicola*, *F. pentaphylla* and *F. viridis* (Bors and Sullivan, 2005). Somatic hybridization may be an alternative to obtain interspecific hybrids, as long as regeneration of plants is possible from hybrid somatic cells (Johnson and Veilleux, 2000). In this case the hybrid plant may be a polyploid having the full genome complements of both parental species. In some cases, like bread wheat, where crossing between elite varieties and CWR is frequently unsuccessful, inhibition of crossing is under the control of a few genes, so that wheat varieties or stocks homozygous for the crossability alleles *kr1* and *kr2* have increased crossability with CWR (Alfares et al., 2009).

Once interspecific hybrids have been obtained, backcross generations are produced (Zamir, 2001; Gur and Zamir, 2004). Although interspecific hybrids may be viable, they may be partially or completely sterile due to irregular chromosome pairing due to different ploidy levels or a low degree of synteny that results in non-viable gametes (De Storme and Mason,

2014). Some approaches can be used to increase the success rate of obtaining viable progeny from hybrids with reduced or low sterility. One strategy is to use the low-fertility hybrid as a female parent, since for the fertilization the pollen must have a high vigour to germinate and reach the ovule - a requirement not needed for the female gamete. This applies also to other plants from subsequent backcross generations. One way to recover fertility of the hybrid is duplicating its genome (Khush and Brar, 1992; Rieseberg and Carney, 1998; Shivanna and Bahadur, 2015). In this way normal chromosome pairing can be restored, although the hybrid plant will be polyploid, which may be an obstacle for obtaining subsequent generations due to different ploidy levels. To prevent this, a common technique is to duplicate also the genome of the recurrent cultivated parent to obtain backcross progeny and to restore the diploid status at a later stage. For example, Toppino et al. (2008) introgressed of resistance to *Fusarium* wilt from *S. aethiopicum* into the genetic background of eggplant (*S. melongena*) by backcrossing the tetraploid somatic hybrid to tetraploid eggplant to obtain the first backcross generation, which was subsequently returned to the diploid stage by anther culture. Generally, the loss of fertility in interspecific hybrids can be recovered in backcross generations, with increasing levels of fertility as the genome of the recurrent cultivated parent is being recovered (Wall, 1970). However, occasionally the phenomenon of selective chromosome elimination of the donor parent may occur, complicating the introgression of fragments of CWR (Dwivedi et al., 2008). In other cases, recombination in the hybrids between chromosomes of the cultivated species and the CWR is supressed or reduced, which makes introgression more difficult (Bedő and Láng, 2015). In this way, in wheat the gene *Ph1* suppresses pairing and recombination of wheat and alien chromosomes; however, in plants that are nullisomic for the *Ph1* gene, or in *ph1b* mutant stocks, homoelogous wheat and alien chromosomes can pair and recombine, which facilitates introgression from CWR in the cultivated wheat (Friebe et al., 2012).

Introgressiomics generally is conceived as the introgression of nuclear genes from wild species into a cultivated genetic background. However, introgression of cytoplasmic genes is also possible to produce alloplasmic hybrids with the nuclear genome of the cultivated species and the cytoplasm of the wild species (Khan et al., 2015). In this case, the cultivated species has to be used as male parent to ensure that the wild cytoplasm is maintained during the process. Nevertheless, it has to be taken into account that the interaction between the nuclear genome of the recurrent parent (cultivated species) and the cytoplasm of the donor (wild species) may result in cytoplasmic sterility (Prakash et al., 2001; Dwivedi et al., 2008; Khan et al., 2015), which may not be desirable if fertile plants of the cultivated species are needed. In that case,

475 the cultivated species should be used as female parent to recover their cytoplasm , at some point
476 during the backcross program.
477
478 **6. Development of introgressiomics populations**
479
480 Once interspecific hybrids have been obtained, introgressiomics populations (Figure 3),
481 have to be developed. Some of the most commonly used populations, which contain genome
482 fragments from CWR, are chromosome substitution lines (CSLs) and introgression lines (ILs).
483 These are considered 'immortal' populations as they can be maintained by selfing, based on
484 backcrossing the hybrid to the recurrent cultivated parent for several generations (Zamir, 2001;
485 Lippman et al., 2007). CSLs consist of lines harboring the full genome complement of the crop
486 except for one chromosome pair that corresponds to the wild donor parent (Cavanagh et al.,
487 2008). CSLs have been extensively used in wheat breeding (Kilian et al. 2011: Khlestkina,
488 2014) and allow ascribing genes and traits to specific chromosomes. Although deleterious genes
489 may be present in the introgressed pair of chromosomes, in contrast to CSLs ILs harbour the
490 full genome of the crop, except for a small chromosomal segment of a donor parent, typically
491 a CWR (Zamir, 2001). The development of collections of ILs, can be of great utility for
492 breeders, because given that the introgression represents only a part of a chromosome, it may
493 contain less deleterious alleles than CSLs (Gur and Zamir, 2004; Lippman et al., 2007). Both
494 CSLs and ILs can be obtained through repeated backcrossing of the hybrid to the recurrent
495 parent. Molecular markers and/or complementary cytogenetic techniques such as genome *in*
496 *situ* hybridization (GISH) help tracking the introgressed fragments and thus support the
497 selection of beneficial materials for subsequent backcross cycles (Gupta et al., 2016). A final
498 step in obtaining ILs is selfing or obtaining doubled haploids to fix the introgressed fragment
499 in a homozygous state (Herzog et al., 2014). Also, ILs can be obtained from CSLs by crossing
500 with the recurrent parent and subsequent selection of individuals in which recombination has
501 taken place (Cavanagh et al., 2008). Similarly sub-ILs (Figure 3) can be obtained from ILs to
502 shorten the introgressed fragment to reduce linkage drag (Alkeesh et al., 2013). A further
503 advantage of ILs is the ability to intercross favourable traits that are present in different ILs for
504 pyramiding favourable alleles (Gur and Zamir 2015). Advanced backcross populations, in
505 which no marker assisted selection has been carried out during population development, may
506 also be of interest as introgressiomics populations (Tanksley and Nelson, 1996; Cowling et al.,
507 2009). In these populations the percentage of the donor genome will be reduced on average by

half in each backcross cycle. Typically, these advanced backcross populations contain several fragments of the donor in several parts of the genome.

Other types of populations can be utilized to obtain 'introgressiomics populations'. For example, recombinant inbred lines (RILs) obtained after crossing one cultivated species and a CWR followed by several generations of selfing have been very useful to dissect traits of interest present in CWR (Peleg et al., 2009; Salinas et al., 2013). However, RILs carry on average 50% of the wild parent, which in most cases are unsuitable for commercial breeding programs without a pre-breeding pipeline in place. An alternative to the backcross method is to develop multi-parental populations, such as multi-parent advanced generation inter-cross (MAGIC), which consist of multi-parent RIL populations (Cavanagh et al., 2008; Pascual et al., 2016).

A more simplistic approach for the development of introgressiomics populations involves the development of several populations, each of which contains introgressions from a single donor CWR parent. Introgressiomics populations may contain introgressions from several CWRs. For example, interspecific hybrids between two CWRs or double hybrids between four CWRs can be used as starting point to develop advanced backcross generations that may contain genome fragments from several CWRs. Also, more than one CWR parent can be included in MAGIC populations to generate RILs that have genomic fragments of several wild species.

One of the main challenges to the development and use of introgressiomics populations is linkage drag, based on reduced recombination at introgressed fragments (Tanksley and Nelson, 1996; Wendler et al., 2015). The use of molecular markers allows selecting individuals carrying recombined introgressed fragments (Alkeesh et al., 2013). Special mating designs, such as sib-mating, can be considered in the backcross scheme to enhance recombination (Wall, 1970; Liu et al., 1996; Rieseberg et al., 1996). Also, the use of congruency backcrossing, where backcrosses towards the recurrent parent are alternating to backcrosses to the donor parent to obtain fertile materials can be used to increase recombination (Haghighi and Ascher, 1988; Muñoz et al., 2004). Recently, Wendler et al. (2015) proposed crossing ILs with overlapping fragments originating from different donors to obtain progenies exhibiting higher levels of recombination within the introgressed fragment. However, in some occasions, due to the lack of synteny, recombination is prevented (Tanksley and Nelson, 1996). For example, in tomato, repeated attempts to reduce the introgression size associated to *Tomato yellow leaf curl virus* (TYLCV) resistance gene *Ty-1* introgressed from *S. chilense* failed due to lack of recombination caused by an chromosomal translocation (inversion) (Verlaan et al., 2011). In these cases

542 reducing the linkage drag is not possible by using standard crossing schemes. One alternative
543 to no or low levels of recombination due to the lack of crossing-over is the development of
544 addition lines, containing an extra chromosome from a donor. In this way it may be possible to
545 recover some plants in which a fragment of the donor is introgressed in the genome of the
546 recipient parent (Friebe et al., 1996; Jacobsen and Schouten, 2007).
547 In polyploid crops, like bread wheat, an alternative to obtain introgressiomics
548 populations using the diversity of CWR is the artificial synthesis of the allopolyploid using
549 genetically diverse materials of the ancestor species as parental lines. For example, hexaploid
550 wheats have been resynthesized through the hybridization of tetraploid wheats and *Aegilops*
551 species (Bedő and Lang, 2015). Some of these materials were backcrossed to breeding lines
552 and were found to be a source of variation for adaptation to several abiotic and biotic stresses
553 (Trethowan and Mujeeb-Kazi, 2008). These synthetic introgression materials revealed a
554 significant increase in genetic diversity (Warburton et al., 2006).
555
556 **7. Genomic tools and new plant breeding techniques for introgressiomics**
557
558 Tremendous advances in next generation sequencing (NGS) technologies have provided
559 breeders with a wide array of genomic tools that facilitate the introgression of CWR fragments
560 into crops (Baute et al., 2015; Kole et al., 2015; Abberton et al., 2016; Brozynska et al., 2016).
561 Some of the most important tools from genomics are the development of molecular markers
562 distributed throughout the genome, the availability of dense genetic maps, the availability of
563 reference genome sequences, transcriptome sequences, the discovery of regulatory elements, as
564 well as gene annotations (Pérez-de-Castro et al., 2012). The use of these tools facilitates the
565 identification of sources of variation and the development and characterization of
566 introgressiomics populations.
567 The availability of molecular markers that can be adapted to high-throughput
568 genotyping platforms is of great utility for identifying those CWRs that may contribute
569 beneficial diversity to the introgressiomics approach. For example, the development of
570 introgressiomics populations in eggplant using wild relatives (Plazas et al., 2016) benefited
571 from molecular marker studies that evaluated the genetic diversity and relationships between
572 eggplant and a wide number of CWRs (Furini and Wunder, 2004; Vorontsova et al., 2013).
573 This facilitated the selection of 15 eggplant CWR accessions from all genepools for the
574 initiation of an introgression breeding program (Plazas et al., 2016).

Molecular markers greatly facilitate the creation and characterization of CSL and IL populations. Marker-assisted foreground and background selection strategies - for those individuals that contain the target chromosomal segments, while at the same time selecting for beneficial alleles in the recipient parental genome - are being applied in several crops (Zamir, 2001, Gur and Zamir, 2004; Pérez-de-Castro et al., 2012). In this way, collections of CSLs and ILs containing introgressed genome fragments of CWRs have been obtained in many crops (Gur and Zamir, 2004; Dwivedi et al., 2008; Khlestkina, 2014). The increased availability of high-density and mapped markers allows the fine mapping of the introgressed fragments, their extent as well as the breaking of ILs into sub-ILs by detecting recombinants (Lippman et al., 2007; Wendler et al., 2015; King et al., 2016).

Apart from providing molecular markers and identifying allelic variants, CWR transcriptome and genome sequences as well as resequencing studies can contribute other relevant information for introgressiomics. For example, synteny studies derived from the comparison of sequences of the crop and its CWRs may help identifying those genomic regions for which major chromosomal rearrangements have taken place during crop evolution and for which recombination will certainly be supressed (Verlaan et al., 2011). Furthermore, sequence information facilitates allele mining in CWR collections in order to detect allelic variants of candidate genes controlling traits of interest (Ramkumar et al. 2016).

The use of the so-called 'new plant breeding techniques' can be useful for introgressiomics (Lusser et al., 2011; Hartung and Schiemann, 2014) as well. In this respect, cis-genesis (Jacobsen and Schouten, 2007) and genome editing, in particular based on CRISPR/Cas9 (Belhaj et al., 2013), are promising. Cis-genesis consists of the genetic transformation of a recipient parent, in the case of introgressiomics the crop, with isolated genes and their promoters from a crossable donor (CWR), without the introduction of reporter or selectable markers from other organisms (Jacobsen and Schouten, 2007). In this way, by using cis-genesis, genes isolated from CWRs can be transferred in the genetic background of the crop without linkage drag (Tardi, 2016). Theoretically, by introducing genes isolated from CWRs from different genepools it would be possible to produce collections of isogenic lines with genes from different donors, as well as to pyramid or to 'stack' genes from different species in a single genotype (Jo et al., 2014). This is particularly interesting in the case of secondary and tertiary genepool species, with strong hybridization barriers (Khush and Brar, 1992; Dwivedi et al., 2008). For example, Jo et al. (2014) introduced two genes for resistance to late blight from the potato CWRs *S. stoloniferum* and *S. venturii* in several potato varieties. Several techniques exist for genome editing in plants (Cardi, 2016), but among them currently the most promising is the

609 CRISPR/Cas9 system (Kole et al., 2015). In this way, it will potentially be possible to introgress
610 traits whose expression depends on one or a few functional SNPs.
611 Cis-genic materials are genetically indistinguishable from those obtained by induced
612 translocation breeding (Jacobsen and Schouten, 2007) and CRISPR/Cas edited genes cannot be
613 distinguished from a natural mutation at the same locus (Belhaj et al., 2013). However, legal
614 uncertainty and the risk of non-acceptance of these technologies by consumers restrict their
615 current usefulness (Lusser et al., 2011; Hartung and Schiemann, 2014).
616
617 **8. Moving the introgressed material into the breeding pipeline**
618
619 The development of introgressiomics materials may require several years depending on
620 the crop and trait, among others. In most occasions, the development of these types of materials
621 cannot been achieved within the time span of a single research project (which is on average 3-
622 5 years). Therefore, on many occasions the public introgressiomics programs may remain
623 unfinished and the materials remain unused by the breeding sector. An important reason could
624 be the lack of characterization and evaluation data, which is essential for breeders. In some
625 cases, materials obtained in the public sector are available. For example, over 300 ILs and subIL
626 of *S. pennellii* introgressed into cultivated tomato are available (Alkeesh et al., 2013). Also, the
627 Tomato Genetic Resources Center (http://tgrc.ucdavis.edu/) maintains a large stock of pre-
628 breeding materials readily usable by breeders.
629 We argue that long-term public-private pre-breeding partnerships (PPPPs) could bridge
630 the gap between the development of introgressiomics materials (pre-breeding) and their
631 utilization in subsequent breeding programs (Lusser, 2014; Dempewolf et al., 2017). These
632 PPPPs, which would involve scientists and breeders, could focus on characterising and
633 evaluating the pre-bred materials in more detail, before breeders are willing to include them in
634 their programs (Lusser, 2014; Warschefsky et al., 2014). Currently, phenotyping is the limiting
635 factor in using introgressiomics populations (Gur and Zamir 2004) and PPPPs could make a
636 great contribution to an increased utilization by conducting more phenotyping experiments in
637 diverse environments. Several successful examples of PPPPs are presented in Lusser (2014) or
638 Moore (2015). For an efficient utilization in breeding, plant materials and related information
639 resulting as an outcome of public-private pre-breeding partnership programs should be
640 publically accessible as a community resource, so that they can contribute to the development
641 of new cultivars (Campi and Nuvolari, 2015). In this respect, global gateways such as Genesys
642 (https://www.genesys-pgr.org) or international inititatives, like DivSeek, can make an

643 important contribution to the sharing of phenotypic and genotypic characterization data of
644 materials stored in genebanks (Meyer, 2015),

645 We propose that introgressiomics materials that clearly belong in the 'pre-competitive
646 domain' should be made available under the terms of the International Treaty for Plant Genetic
647 Resources for Food and Agriculture (ITPGRTA). In this respect, one possibility could be to
648 store introgressiomics materials in germplasm banks for long term conservation (Khoury et al.,
649 2010). Clearly, the development of conservation strategies for pre-breeding materials including
650 mapping populations, cytogenetic stocks and mutant collections would enhance the utilization
651 of CWR and other sources of native diversity.

652

653 **9. Conclusions**

654

655 Introgression breeding from CWRs can make a major contribution to adapting our crops
656 to climate change as well as to the development of new generations of crops with new and
657 improved properties. The new approach we propose, 'introgressiomics', calls for an increased
658 and most efficient use of CWRs in breeding by using the available genetic resources,
659 introgression and population development schemes, phenotyping and genotyping tools and
660 integration of data for obtaining elite materials that can be readily incorporated by breeders in
661 their breeding programs. We hope that by using this holistic approach the practical use of CWRs
662 in developing new commercial varieties with better characteristics will be enhanced. This may
663 result in having more diverse, resilient and resource efficient crops that can contribute to a more
664 sustainable and productive agriculture under the environmental changes resulting from climate
665 change.

666

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References

Abberton M, Batley J, Bentley A, Bryant J, Cai H, Cockram J, Costa de Oliveira, A, Cseke LJ, Dempewolf H, De Pace C, Edwards D, Gepts P, Greenland A, Hall A, Henry J, Hori K, Howe GT, Hughes S, Humphreys M, Lightfoot D, Marshall A, Mayes S, Nguyen HT, Ogbonnaya FC, Ortiz R, Paterson AH, Tuberosa R, Valliyodan B, Varshney RK, Yano M (2016) Global agricultural intensification during climate change: a role for genomics. Plant Biotech J 14:1095-1098. doi: 10.1111/pbi.12467

Aflitos S, Schiljen E, de Jong H, de Ridder D, Smit S, Finkers R , Wang J, Zhang G, Li N, Mao L, Bakker F, Dirks R, Breit T, Gravendeel B, Huits H, Struss D, Swanson-Wagner R, van Leeuwen H, van Ham RCHJ, Fito L, Guignier L, Sevilla M, Ellul P, Ganko E, Kapur A, Reclus M, de Geus B, van de Geest H, te Lintel Hekkert B, van Haarst J, Smits L, Koops A, Sanchez-Perez G, van Heusden AW, Visser R, Quan Z, Min J, Liao L., Wang X, Wang G, Yue Z, Yang X, Xu N, Schranz E, Smets E, Vos R, Rauwerda J, Ursem R, Schuit C, Kerns M, van den Berg J, Vriezen W, Janssen A, Datema E, Jahrman T, Moquet F, Bonnet J, Peters S (2014) Exploring genetic variation in the tomato (*Solanum* section *Lycopersicon*) clade by whole-genome sequencing. Plant J 80:136-148. doi: 10.1111/tpj.12616

Alexander LJ (1963) Transfer of a dominant type of resistance to the four known Ohio pathogenic strains of tobacco mosaic virus (TMV) from *Lycopersicon peruvianum* to *L. esculentum*. Phytopathology 53:869

Alfares W, Bouguennec A, Balfourier F, Gay G, Bergès H, Vautrin S, Sourdille P, Bernard M, Feuillet C (2009) Fine mapping and marker development for the crossability gene *SKr* on chromosome 5BS of hexaploid wheat (*Triticum aestivum* L.). Genetics 183:469-481. doi: 10.1534/genetics.109.107706

Alseekh S, Ofner I, Pleban T, Tripodi P, Di Dato F, Cammareri M, Mohammad A, Grandillo S, Fernie AR, Zamir D (2013) Resolution by recombination: breaking up *Solanum pennellii* introgressions. Trends Plant Sci 18:536-538. doi: 10.1016/j.tplants.2013.08.003

Bari A, Street K, Mackay M, Endresen DTF, De Pauw E, Amri A (2012) Focused Identification of Germplasm Strategy (FIGS) detects wheat stem rust resistance linked to

710 environmental variables. Genet Resour Crop Evol 59:1465-1481. doi: 10.1007/s10722-
711 011-9775-5
712 Baute GJ, Dempewolf H, Rieseberg L (2015) Using genomic approaches to unlock the potential
713 of CWR for crop adaptation to climate change. In: Redden R, Yadav S, Maxted N,
714 Dulloo ME, Guarino L, Smith P (eds) Crop wild relatives and climate change. Wiley,
715 Hoboken, NJ, USA, pp 268-280. doi: 10.1002/9781118854396.ch15
716 Bebber DP, Ramotowski MAT, Gurr SJ (2013) Crop pests and pathogens move polewards in a
717 warming world. Nat Clim Change 3:985-988. doi: 10.1038/nclimate1990
718 Bedő Z, Láng, L. (2015). Wheat breeding: current status and bottlenecks. In: Molnár-Láng M,
719 Ceoloni, C, Doležel J (eds.) Alien introgression in wheat. Springer, Berlin Heidelberg,
720 pp 77-101. doi: 10.1007/978-3-319-23494-6_3
721 Belhaj K, Chaparro-Garcia A, Kamoun S, Nekrasov V (2013) Plant genome editing made easy:
722 targeted mutagenesis in model and crop plants using the CRISPR/Cas system. Plant
723 Meth 9:39. doi: 10.1186/1746-4811-9-39
724 Bessey CE (1906) Crop improvement by utilizing wild species. J Hered os-2:112-118. doi:
725 10.1093/jhered/os-2.1.112
726 Brozynska M, Furtado A, Henry RJ (2016) Genomics of crop wild relatives: expanding the
727 gene pool for crop improvement. Plant Biotech J 14:1070-1085. doi: 10.1111/pbi.12454
728 Campi M, Nuvolari A (2015) Intellectual property protection in plant varieties: A worldwide
729 index (1961-2011). Res Policy 44:951-964. doi: 10.1016/j.respol.2014.11.003
730 Cardi T (2016) Cisgenesis and genome editing: combining concepts and efforts for a smarter
731 use of genetic resources in crop breeding. Plant Breed 135:139-147. doi:
732 10.1111/pbr.12345
733 Castañeda-Alvarez NP, Khoury C, Achicanoy HA, Bernau V, Dempewolf H, Eastwood RJ,
734 Guarino L, Harker RH, Jarvis A, Maxted N, Müller JV, Ramirez-Villegas J, Sosa CC,
735 Struik PC, Vincent H, Toll J (2016) Global conservation priorities for crop wild
736 relatives. Nat Plants 2:16022. doi: 10.1038/nplants.2016.22
737 Cattivelli L, Rizza F, Badeck FW, Mazzucotelli E, Mastrangelo AM, Francia E, Marè C,
738 Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: An
739 integrated view from breeding to genomics. Field Crops Res 105:1-14. doi:
740 10.1016/j.fcr.2007.07.004
741 Cavanagh C, Morell M, Mackay I, Powell W (2008) From mutations to MAGIC: resources for
742 gene discovery, validation and delivery in crop plants. Curr Opinion Plant Biol 11:215-
743 221. doi: 10.1016/j.pbi.2008.01.002

744 Centro Internacional de Agricultura Tropical (2017) A global database for the distributions of
745 crop wild relatives. doi: 10.15468/jyrthk. Accessed via
746 http://www.gbif.org/dataset/07044577-bd82-4089-9f3a-f4a9d2170b2e on 2017-03-03
747 Cowling WA, Buirchell BJ, Falk DE (2009) A model for incorporating novel alleles from the
748 primary gene pool into elite crop breeding programs while reselecting major genes for
749 domestication or adaptation. Crop Pasture Sci 60:1009-1015. doi: 10.1071/CP08223
750 De Storme N, Mason A (2014) Plant speciation through chromosome instability and ploidy
751 change: Cellular mechanisms, molecular factors and evolutionary relevance. Curr Plant
752 Biol 1:10-33. doi: 10.1016/j.cpb.2014.09.002
753 Dempewolf H, Hodgkins KA, Rummell SE, Ellstrand NC, Rieseberg LH (2012) Reproductive
754 isolation during domestication. Plant Cell 24:2710-2717. doi: 10.1105/tpc.112.100115
755 Dempewolf H, Eastwood RJ, Guarino L, Khoury C, Müller, JV, Toll J (2014) Adapting
756 agriculture to climate change: A global initiative to collect, conserve, and use crop wild
757 relatives. Agrocecol Sust Food Syst 38:369-377. doi: 10.1080/21683565.870629
758 Dempewolf H, Baute G, Anderson J, Kilian B, Smith C, Guarino L (2017) Past and future use
759 of wild relatives in crop breeding. Crop Sci: in press. doi: 10.2135/cropsci2016.10.0885
760 Díez MJ, Nuez F (2008) Tomato. In: Prohens J, Nuez F (eds) Vegetables II: Fabaceae,
761 Liliaceae, Solanaceae, and Umbelliferae. Springer, New York, pp 249-323. doi:
762 10.1007/978-0-387-74110-9
763 Dhaliwal HS (1992) Unilateral incompatibility. In: Kalloo G, Chowdhury JB (eds) Distant
764 hybridization of crop plants. Springer, Berlin, pp 32-46. doi: 10.1007/978-3-642-84306-
765 8_3
766 Dodsworth S, Chase MW, Särkinen T, Knapp S, Leitch AR (2016) Using genomic repeats for
767 phylogenomics: a case study in wild tomatoes (*Solanum* section *Lycopersicon*:
768 Solanaceae). Bot J Linn Soc 117:96-105. doi: 10.1111/bij.12612
769 Dwivedi SL, Upadhyaya HD, Stalker HT, Blair MW, Bertioli DJ, Nielen S, Ortiz R (2008)
770 Enhancing crop gene pools with beneficial traits using wild relatives. Plant Breed Rev
771 30:179-230. doi: 10.1002/9780470380130.ch3
772 Fita A, Rodríguez-Burruezo A, Boscaiu M, Prohens J, Vicente O (2015) Breeding and
773 domesticating crops adapted to drought and salinity: a new paradigm for increasing food
774 production. Front Plant Sci 6:978. doi: 10.3389/fpls.2015.00978
775 Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS (1996) Characterization of wheat-alien
776 translocations conferring resistance to diseases and pests. Euphytica 91:59-87. doi:
777 10.1007/BF00035277

778 Friebe B, Qi L, Liu C, Liu W, Gill BS (2012) Registration of a hard red winter wheat genetic
779 stock homozygous for *ph1b* for facilitating alien introgression for crop improvement. J
780 Plant Reg 6:121-123. doi: 10.3198/jpr2011.05.0273crgs
781 Furini A, Wunder J (2004) Analysis of eggplant (*Solanum melongena*)-related germplasm:
782 morphological and AFLP data contribute to phylogenetic interpretations and germplasm
783 utilization. Theor Appl Genet 108:197-208. doi: 10.1007/s00122-003-1439-1
784 Gerstetter C, Görlach B, Neumann K, Schaffrin D (2007) The International Treaty on Plant
785 Genetic Resources for Food and Agriculture within the current legal regime complex on
786 plant genetic resources. J World Intellec Prop 10:259-283. doi: 10.1111/j.1747-
787 1796.2007.00323.x
788 Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson
789 S, Thomas SM, Toulmin C (2010) Food security: The challenge of feeding 9 billion
790 people. Science 327:812-817. doi: 10.1126/science.1185383
791 Guerrero RF, Posto AL, Moyle LC, Hahn MW (2016) Genome-wide patterns of regulatory
792 divergence revealed by introgression lines. Evolution 70:696-706. doi:
793 10.1111/evo.12875
794 Gupta M, Mason AS, Batley J, Bharti S, Banga S, Banga SS (2016) Molecular-cytogenetic
795 characterization of C-genome chromosome substitution lines in *Brassica juncea* (L.)
796 Czern and Coss. Theor Appl Genet 129:1153-1166. doi: 10.1007/s00122-016-2692-4
797 Gur A, Zamir D (2004) Unused natural variation can lift yield barriers in plant breeding. PLOS
798 Biol 2:e245. doi: 10.1371/journal.pbio.0020245
799 Gur A, Zamir D (2015) Mendelizing all components of a pyramid of three yield QTL in tomato.
800 Front Plant Sci 6:1096. doi: 10.3389/fpls.2015.01096
801 Haghighi KR, Ascher PD (1998) Fertile, intermediate hybrids between *Phaseolus vulgaris* and
802 *P. acutifolius* hybrids from congruity backcrossing. Sex Plant Reprod 1:51-58. doi:
803 10.1007/BF00227023
804 Hajjar R, Hodgkin T (2007) The use of crop wild relatives in crop improvement: A survey of
805 developments over the last 20 years. Euphytica 156:1-13. doi: 10.1007/s10681-007-
806 9363-0
807 Hammer K (1984) Das Domestikationssyndrom. Kulturpfl 32:11-34. doi:
808 10.1007/BF02098682
809 Harlan JR, de Wet JMJ (1971) Toward a rational classification of cultivated plants. Taxon
810 20:509-517. doi: 10.2307/1218252

Hartung F, Schiemann J (2014) Precise plant breeding using new genome editing techniques: opportunities, safety and regulation in the EU. Plant J 78:742-752. doi: 10.1111/tpj.12413

Herzog E, Falke KC, Presteri T, Scheuermann, Ouzunova M, Frisch M (2014) Selection strategies for the development of maize introgression populations. PLOS ONE 9:e92429. doi: 10.1371/journal.pone.0092429

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Climat 25:1965-1978. doi: 10.1002/joc.1276

Jacobsen E, Schouten HJ (2007) Cisgenesis strongly improves introgression breeding and induced translocation breeding of plants. Trends Biotech 25:219-223. doi: 10.1016/j.tibtech.2007.03.008

Jarvis DI, Hodgkin T (1999) Wild relatives and crop cultivars: detecting natural introgression and farmer selection of new genetic combinations in agroecosystems. Mol Ecol 8:S159-S173. doi: 10.1046/j.1365-294X.1999.00799.x

Jarvis A, Lane A, Hijmans RI (2008) The effect of climate change on crop wild relatives. Agr Ecosyst Environ 126:13-23. doi: 10.1016/j.agee.2008.01.013

Jo KR, Kim CJ, Kim SJ, Kim TY, Bergervoet M, Jongsma MA, Visser RGF, Jacobsen E, Vossen JH (2014) Development of late blight resistant potatoes by cisgene stacking. BMC Biotech 14:50. doi: 10.1186/1472-6750-14-50

Johnson AAT, Veilleux RE (2000) Somatic hybridization and applications in plant breeding. Plant Breed Rev 20:167-225. doi: 10.1002/9780470650189.ch6

Jones TA (2003) The restoration gene pool concept: beyond the native versus non-native debate. Restor Ecol 11:281-290. doi: 10.1046/j.1526-100X.2003.00064.x

Kantar MB, Sosa CS, Khoury CK, Castañeda-Álvarez NP, Achicanoy HA, Bernau V, Kane NC, Marek L, Seiler G, Rieseberg LH (2015) Ecogeography and utility to plant breeding of the crop wild relatives of sunflower (*Helianthus annuus* L.). Front Plant Sci 6:841. doi: 10.3389/fpls.2015.00841

Khan MMR, Hasnunnahar M, Iwayoshi M, Ogura-Tsujira Y, Isshiki S (2015) Pollen degeneration in three functional male-sterile lines of eggplant with the wild *Solanum* cytoplasms. Hort Environ Biotech 56:350-357. doi: 10.1007/s13580-015-0015-3

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:e63107. doi: 10.1371/journal.pone.0063107

844 Khlestkina EK (2014) Current applications of wheat and wheat-alien precise genetic stocks.
845 Mol Breed 34:273-281. doi: 10.1007/s11032-014-0049-8
846 Khoury C, Laliberté B, Guarino L (2010) Trends in ex situ conservation of plant genetic
847 resources: a review of global crop and regional conservation strategies. Genet Resour
848 Crop Evol 57:625-639. doi: 10.1007/s10722-010-9534-z
849 Khush GS, Brar DS (1992) Overcoming the barriers in hybridization. In: Kalloo G, Chowdhury
850 JB (eds) Distant hybridization of crop plants. Springer, Berlin, pp 47-61. doi:
851 10.1007/978-3-642-84306-8_4
852 Kilian B, Mammen K, Millet E, Sharma R, Graner A, Salamini F, Hammer K, Özkan, H.
853 (2011). *Aegilops*. In: Kole C (ed.) Wild Crop Relatives: Genomic and Breeding
854 Resources, Cereals. Springer, Berlin Heidelberg, pp 1-76. doi: 10.1007/978-3-642-
855 14228-4_1
856 King J, Grewal S, Yang C, Hubbart S, Scholefield D, Ashling S, Edwards KJ, Allen AM,
857 Burridge A, Bloor C, Davassi A, da Silva GJ, Chalmers K, King IP (2016) A step change
858 in the transfer of interspecific variation into wheat from *Amblyopyrum muticum*. Plant
859 Biotech: in press. doi: 10.1111/pbi.12606
860 Kole C, Muthamilarasan M, Henry R, Edwards D, Sharma R, Abberton M, Batley J, Bentley
861 A, Blakeney M, Bryant J, Cai H, Cakir M, Cseke LJ, Cockram J, de Oliveira AC, De
862 Pace C, Dempewolf H, Ellison S, Gepts P, Greenland A, Hall A, Hori K, Highes S,
863 Humphreys MW, Iorizzo M, Ismail AB, Marshall A, Mayes S, Nguyen HT, Ogbonnaya
864 FC, Ortiz R, Paterson AH, Simon PW, Tohme J, Tuberosa R, Valliyodan B, Varshney
865 RK, Wullschleger SD, Yano M, Prasad M (2015) Application of genomics-assisted
866 breeding for generation of climate resilient crops: progress and prospects. Front Plant
867 Sci 6:563. doi: 10.3389/fpls.2015.00563
868 Knox J, Hess T, Daccache A, Wheeler T (2012) Climate change impacts on crop productivity
869 in Africa and South Asia. Environ Res Lett 7:034032. doi: 10.1088/1748-
870 9326/7/3/034032
871 Lippman ZB, Semel Y, Zamir D (2007) An integrated view of quantitative trait variation using
872 tomato interspecific introgression lines. Curr Opin Genet Dev 17:545-552. doi:
873 10.1016/j.gde.2007.07.007
874 Longin CFH, Reif JC (2014) Redesigning the exploitation of wheat genetic resources. Trends
875 Plant Sci 19:631-636. doi: 10.1016/j.tplants.2014.06.012

876 Lusser M (2014) Workshop on public-private partnerships in plant breeding. Science and Policy
877 Report by the Joint Research Centre of the European Commission. Publications Office
878 of the European Union, Luxembourg. doi: 10.2791/80891
879 Lusser M, Parisi C, Plan D, Rodríguez-Cerezo E (2011) New plant breeding techniques: state-
880 of-the –art and prospects for commercial development. Reference Report by the Joint
881 Research Centre of the European Commission. Publications Office of the European
882 Union, Luxembourg. doi: 10.2791/60346
883 Martín A, Alvarez JB, Martín LM, Barro F, Ballesteros J (1999) The development of
884 tritordeum: a novel cereal for food processing. J Cereal Sci 30:85-95. doi:
885 10.1006/jcrs.1998.0235
886 Maxted N, Dulloo E, Ford-Lloyd BV, Iriondo JM, Jarvis A (2008) Gap analysis: a tool for
887 complementary genetic conservation assessment. Diversity Distrib 14:1018-1030. doi:
888 10.1111/j.1472-4642.2008.00512.x
889 Maxted N, Ford-Lloyd BV, Jury S, Kell S, Scholten M (2006) Towards a definition of a crop
890 wild relative. Biodivers Conserv 15:2673-2685. doi: 10.1007/s10531-005-5409-6
891 Maxted N, Kell S (2009) Establishment of a global network for the in situ conservation of crop
892 wild relatives: Status and needs. Commission on Genetic Resources for Food and
893 Agriculture, FAO, Rome.
894 McIntosh RA (1992) Pre-emptive breeding to control wheat rusts. Euphytica 63:103-113. doi:
895 10.1007/BF00023916
896 McKhann HI, Camilleri C, Bérard A, Bataillon T, David JL, Reboud X, Le Corre V, Caloustian
897 C, Gut IG, Brunel D (2004) Nested core collections maximizing genetic diversity in
898 *Arabidopsis thaliana*. Plant J 38:193-202. doi: 10.1011/j.1365-313X.2004.02034.x
899 Menda N, Strickler SR, Edwards JD, Bombarely A, Dunham DM, Martin GB, Mejia L, Hutton
900 SF, Havey MJ, Maxwell DP, Mueller LA (2014) Analysis of wild-species introgressions
901 in tomato inbreds uncover ancestrals origins. BMC Plant Biol 14:287. doi:
902 10.1186/s12870-014-0287-2
903 Meyer RS, DuVal AE, Jensen HR (2012) Patterns and processes in crop domestication: an
904 historical review and quantitative analysis of 203 global food crops. New Phytol 196:29-
905 48. doi: 10.1111/j.1469-8137.2012.04253.x
906 Meyer RS, Purugganan MD (2013) Evolution of crop species: genetics of domestication and
907 diversification. Nature Rev Genet 14:840-852. doi: 10.1038/nrg3605
908 Meyer RS (2015) Encouraging metadata curation in the Diversity Seek initiative. Nat Plants
909 1:15099. http://doi.org/10.1038/nplants.2015.99

Moore G (2015) Strategic pre-breeding for wheat improvement. Nature Plants 1: 15018. http://doi.org/10.1038/nplants.2015.18

Muñoz LC, Blair MW, Duque MC, Tohme J, Roca W (2004) Introgression in common bean × tepary bean interspecific congruity-backcross lines as measured by AFLP markers. Crop Sci 44:637-645. doi: 10.2135/cropsci2004.6370

Pascual L, Albert E, Sauvage C, Duangjit J, Bouchet JP, Bitton F, Desplat N, Brunel D, Le Paslier MC, Ranc N, Bruguier L, Chauchard B, Verschave P, Causse M (2016) Dissecting quantitative trait variation in the resequencing era: complementarity of bi-parental, multi-parental and association panels. Plant Sci 242:120-130. doi: 10.1016/j.plantsci.2015.06.017

Peleg Z, Fahima T, Krugman T, Abbo S, Yakir D, Korol AB, Saranga Y (2009) Genomic dissection of drought resistance in durum wheat × wild emmer wheat recombinant inbreed line population. Plant Cell Environ 32:758-779. doi: 10.1111/j.1365-3040.2009.01956.x

Peleman JD, van der Voort JR (2003) Breeding by Design. Trends Plant Sci 8:330-334. doi: 10.1016/S1360-1385(03)00134-1

Pérez-de-Castro AM, Vilanova S, Cañizares J, Pascual L, Blanca JM, Díez, MJ, Prohens J, Picó B (2012) Application of genomic tools in plant breeding. Curr Genom 13:179-195. doi: 10.2174/138920212800543084

Plazas M, Vilanova S, Gramazio P, Rodríguez-Burruezo A, Fita A, Herraiz, FJ, Ranil R, Fonseka R, Niran L, Fonseka H, Kouassi B, Kouassi A, Kouassi A, Prohens J (2016) Interspecific hybridization between eggplant and wild relatives from different genepools. J Amer Soc Hort Sci 141:34-44.

Porch TG, Beaver JS, Debouck DG, Jackson SA, Kelly JD, Dempewolf H (2013) Use of wild relatives and closely related species to adapt common bean to climate change. Agronomy 3:433-461. doi: 10.3390/agronomy3020433

Prakash S, Ahuja I, Upreti HC, Kumar VD, Bhat SR, Kirti PB, Chopra VL (2001) Expression of male sterility in alloplasmic *Brassica juncea* with *Erucastrum canariense* cytoplasm and the development of a fertility restoration system. Plant Breed 120:479-482. doi: 10.1046/j.1439-0523.2001.00627_x

Ray DK, Mueller ND, West PC, Foley JA (2013) Yield trends are insufficient to double global crop production by 2050. PLOS ONE 8:e66428. doi: 10.1371/journal.pone.0066428

Ramírez-Villegas J, Khoury C, Jarvis A, Debouck DG, Guarino L (2010) A gap analysis methodology for collecting crop genepools: a case study with *Phaseolus* beans. PLOS ONE 5:e13497. doi: 10.1371/journal.pone.0013497

Ramkumar, Madhav MS, Rama Devi SJS, Umakanth B, Pandey MK, Prasad MS, Sundaram RM, Viraktamath BC, Ravindra Babu V (2016) Identification and validation of novel alleles of rice blast resistant gene *Pi54*, and analysis of their nucleotide diversity in landraces and wild *Oryza* species. Euphytica 209:725-737. doi: 10.1007/s10681-016-1666-6

Ranil RHG, Niran HML, Plazas M, Fonseka RM, Fonseka HH, Vilanova S, Andújar I, Gramazio P, Fita A, Prohens J (2015) Improving seed germination of the eggplant rootstock *Solanum torvum* by testing multiple factors using an orthogonal array design. Sci Hort 193:174-181. doi: 10.1016/j. scienta.2015.07.030

Rieseberg LH, Arias DM, Ungerer MC, Linder CR, Sinervo B (1996) The effects of mating designs on introgression between chromosomally divergent sunflower species. Theor Appl Genet 93:633-644. doi: 10.1007/BF00417959

Rieseberg LH, Carney SE (1998) Plant hybridization. New Phytol 140:599-624. doi: 10.1046/j.1469-8137.1998.00315.x

Rosenzweig C, Elliott J, Deryng D., Ruane AC, Müller C, Arneth A, Boote KJ, Folberth C, Glotter M, Khabarov N, Neumann K, Piontek F, Pugh TAM, Schmid E, Stehfest E, Yang H, Jones JW (2014) Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. Proc Natl Acad Sci USA 111:3268-3273. doi: 10.1073/pnas.1222463110

Salamini F, Özkan H, Brandolini A, Schäfer-Pregl R, Martin W (2002) Genetics and geography of wild cereal domestication in the Near East. Nat Rev Genet 3:429–441. doi: 10.1038/nrg817

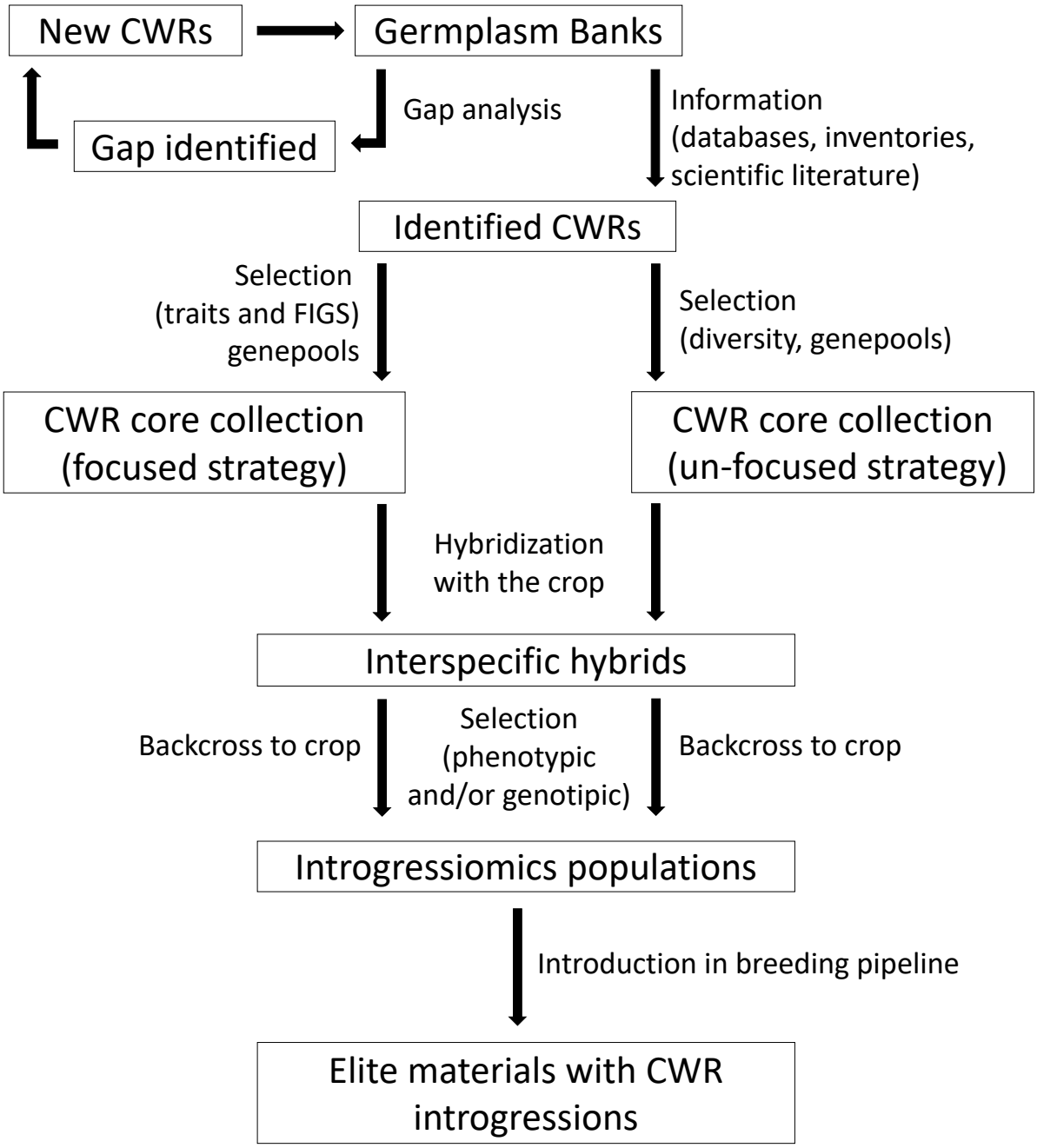
Salinas M, Capel C, Alba JM, Mora B, Cuartero J, Fernández-Muñoz R, Lozano R, Capel J (2013) Genetic mapping of two QTL from the wild tomato *Solanum pimpinellifolium* L. controlling resistance against two-spotted spider mite (T*etranychus urticae* Koch). Theor Appl Genet 126:83-92. doi: 10.1007/s00122-012-1961-0

Savage JA, Haines DF, Holbrook NM (2015) The making of giant pumpkins: how selective breeding changed the phloem of *Cucurbita maxima* from source to sink. Plant Cell Environ 38:1543-1554. doi: 10.1111/pce.12502

974 Schwarz D, Rouphael Y, Colla G, Venema JH (2010) Grafting as a tool to improve tolerance
975 of vegetables to abiotic stresses: Thermal stress, water stress and organic pollutants. Sci
976 Hort 127:162-171. doi: 10.1016/j.scienta.2010.09.016
977 Sharma DR, Kaur R, Kumar K (1996) Embryo rescue in plants – a review. Euphytica 99:325-
978 337. doi: 10.1007/BF00022289
979 Sharma D, Knott DR (1966) The transfer of leaf rust resistance from *Agropyron* to *Triticum* by
980 irradiation. Can J Genet Cytol 8:137-143. doi: 10.1139/g66-018
981 Shivanna KR, Bahadur B (2015) Efficacy of biotechnological approaches to raise wide sexual
982 hybrids. In: Bahadur B, Rajam MV, Sahijram L, Krishnamurthy KV (eds) Plant Biology
983 and Biotechnology, Volume II: Plant genomics and biotechnology. Springer, New
984 Delhi, pp 347-362. doi: 10.1007/978-81-322-2283-5_17
985 Sim SC, Van Deynze A, Stoffel K, Douches DS, Zarka D, Ganal MW, Chetelat RT, Hutton SF,
986 Scott JW, Gardner RG, Panthee DR, Mutschler M, Myers JR, Francis DM (2012) High
987 density SNP genotyping of tomato (*Solanum lycopersicum* L.) reveals patterns of
988 genetic variation due to breeding. PLOS ONE 7:e45520. doi:
989 10.1371/journal.pone.0045520
990 Smith PG (1944) Embryo culture of a tomato species hybrid. Proc Am Soc Hort Sci 44:413-
991 416
992 Street K, Bari A, Mackay M, Amri A (2016) How the Focused Identification of Germplasm
993 Strategy (FIGS) is used to mine plant genetic resources for adaptive traits. In: Maxted
994 N, Dulloo ME, Ford-Lloyd BV (eds) Enhancing crop genepool use: capturing wild
995 relative and landrace diversity for crop improvement. CABI, Wallingford, UK, pp 54-
996 65. doi: 10.1079/9781780646138.0054
997 Syfert M, Castañeda-Álvarez NP, Khoury C, Särkinen T, Sosa CC, Achicanoy HA, Bernau V,
998 Prohens J, Daunay MC, Knapp S (2016) Crop wild relatives of the brinjal eggplant
999 (*Solanum melongena*): poorly represented in genebanks and many species at risk of
1000 extinction. Am J Bot 103:635-651. doi: 10.3732/ajb.1500539
1001 Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential
1002 from the wild. Science 277:1063-1066. doi: 10.1126/science.277.5329.1063
1003 Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the
1004 simultaneous discovery and transfer of valuable QTLs from unadpated germplasm into
1005 elite breeding lines. Theor Appl Genet 92:191-203. doi: 10.1007/BF00223376

1006 Tilman D, Balzer C, Hill J, Befort BL (2011) Global food demand and the sustainable
1007 intensification of agriculture. Proc Natl Acad Sci USA 108:20260-20264. doi:
1008 10.1073/pnas.1116437108
1009 Trethowan RM, Mujeeb-Kazi A (2008) Novel germplasm resources for improving
1010 environmental stress tolerance of hexaploid wheat. Crop Sci 48:1255-1265. doi:
1011 10.2135/cropsci2007.08.0477
1012 Valkoun JJ (2001) Wheat pre-breeding using wild progenitors. Euphytica 119:17-23. doi:
1013 10.1023/A:1017562909881
1014 Van de Wouw, Kik C, van Hintum T, van Treuren R, Visser B (2009) Genetic erosion in crops:
1015 concept, research results and challenges. Plant Genet Resour C 8:1-15. doi:
1016 10.1017/S1479262109990062
1017 Verlaan MG, Szinay D, Hutton SF, de Jong H, Kormelink R, Visser RGF, Scott JW, Bai Y
1018 (2011) Chromosomal rearrangements between tomato and *Solanum chilense* hamper
1019 mapping and breeding of TYLCV resistance gene *Ty-1*. Plant J 68:1093-1103. doi:
1020 10.1111/j.1365-313X.2011.04762.x
1021 Villegas D, Casadesús J, Atienza SG, Martos V, Maalouf F, Karam F, Aranjuelo I, Nogués S
1022 (2010) Tritordeum, wheat and triticale yield components under multi-local
1023 Mediterranean drought conditions. Field Crops Res 116:68-74. doi:
1024 10.1016/j.fcr.2009.11.012
1025 Vincent H, Wiersma J, Kell S, Fielder H, Dobbie S, Castañeda-Álvarez NP, Guarino L,
1026 Eastwood R, León B, Maxted N (2013) A prioritized crop wild relative inventory to
1027 help underpin global food security. Biol Conserv 167:265-275. doi:
1028 10.1016/j.biocon.2013.08.011
1029 Vorontsova MS, Stern S, Bohs L, Knapp S (2013) African spiny *Solanum* (subgenus
1030 *Leptostemonum*, Solanaceae): a thorny phylogenetic tangle. Bot J Linn Soc 173:176-
1031 193. doi: 10.1111/boj.12053
1032 Wall JR (1970) Experimental introgression in the genus *Phaseolus*. I. Effect of mating systems
1033 on interspecific gene flow. Evolution 24:356-366.
1034 Warburton ML, Crossa J, Franco J, Kazi M, Trethowan R, Rajaram S, Pfeiffer W, Zhang P,
1035 Dreisigacker S, van Ginkel M (2006) Bringing wild relatives back into the family:
1036 recovering genetic diversity in CIMMYT improved wheat germplasm. Euphytica
1037 149:289-301. doi: 10.1007/s10681-005-9077-0

1038 Warschefsky E, Penmetsa RV, Cook DR, von Wettberg EJB (2014) Back to the wilds: Tapping
1039 evolutionary adaptations for resilient crops through systematic hybridization with crop
1040 wild relatives. Am J Bot 101:1791-1800. doi: 10.3732/ajb.1400116
1041 Wendler N, Mascher M, Himmelbach A, Johnston P, Pickering R, Stein N (2015) *Bulbosum* to
1042 go: a toolbox to utilize *Hordeum vulgare/bulbosum* introgressions for breeding and
1043 beyond. Mol Plant 8:1507-1519. doi: 10.1016/j.molp.2015.05.004
1044 Wulff BBH, Moscou MJ (2014) Strategies for transferring resistance into wheat: from wide
1045 crosses to GM cassettes. Front Plant Sci 5:692. doi: 10.3389/fpls.2014.00692
1046 Zamir D, Ekstein-Michelson I, Zakay Y, Navot N, Zeidan M, Sarfatti M, Eshed Y, Harel E,
1047 Pleban T, van-Oss H, Kedar N, Rabinowitch HD, Czosnek H (1994) Mapping and
1048 introgression of a tomato yellow leaf curl virus tolerance gene, *TY-1*. Theor Appl Genet
1049 88:141-146. doi: 10.1007/BF00225889
1050 Zamir D (2001) Improving plant breeding with exotic genetic libraries. Nature Rev Genet
1051 2:983-989. doi: 10.1038/35103590
1052 Zenkteler M (1990) *In vitro* fertilization and wide hybridization in higher plants. Crit Rev Plant
1053 Sci 9:267-279. doi: 10.1080/07352689009382290
1054



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1056 Figure 1. Outline of the introgressiomics pre-breeding approach workflow process, from the
1057 use of genetic resources to the development of elite materials with introgressions from crop
1058 wild relatives (CWRs).

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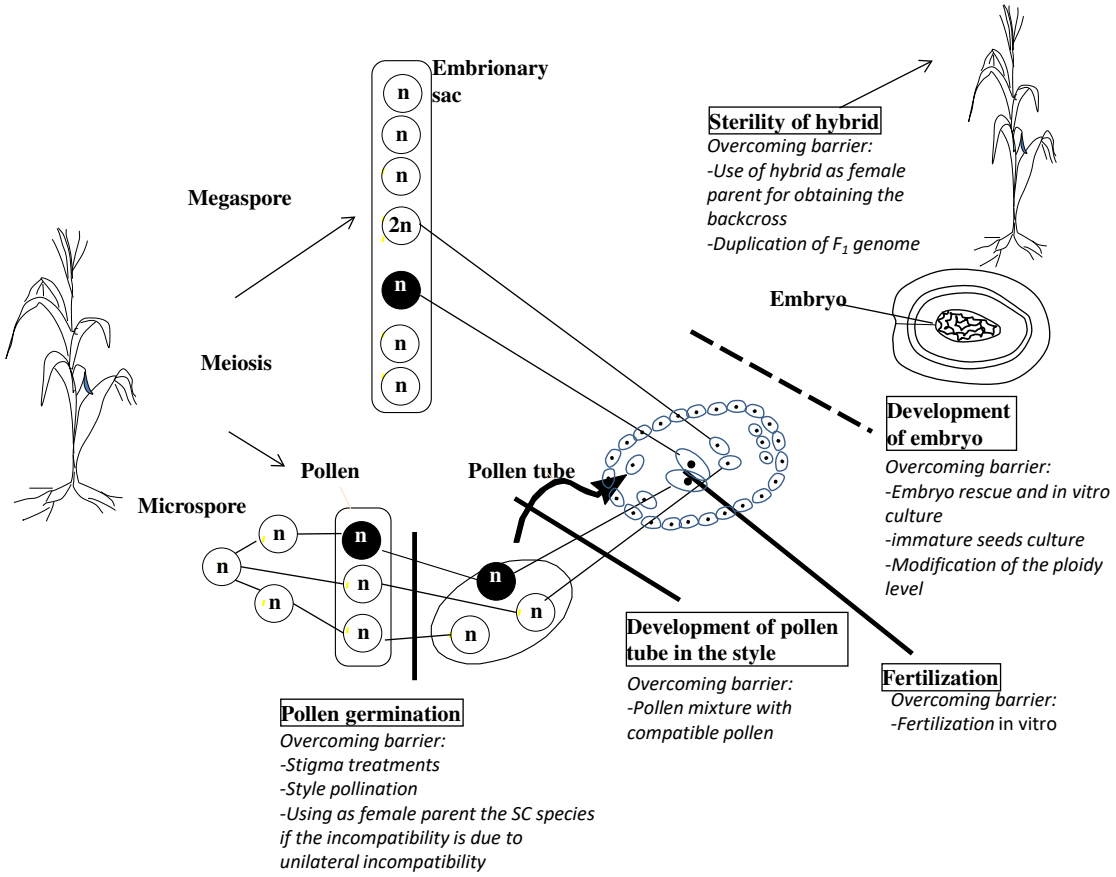

Figure 2. Pre- and post-zygotic barriers difficulting interspecific hybridization and introgression breeding with crop wild relatives (CWRs) and strategies to overcome them.

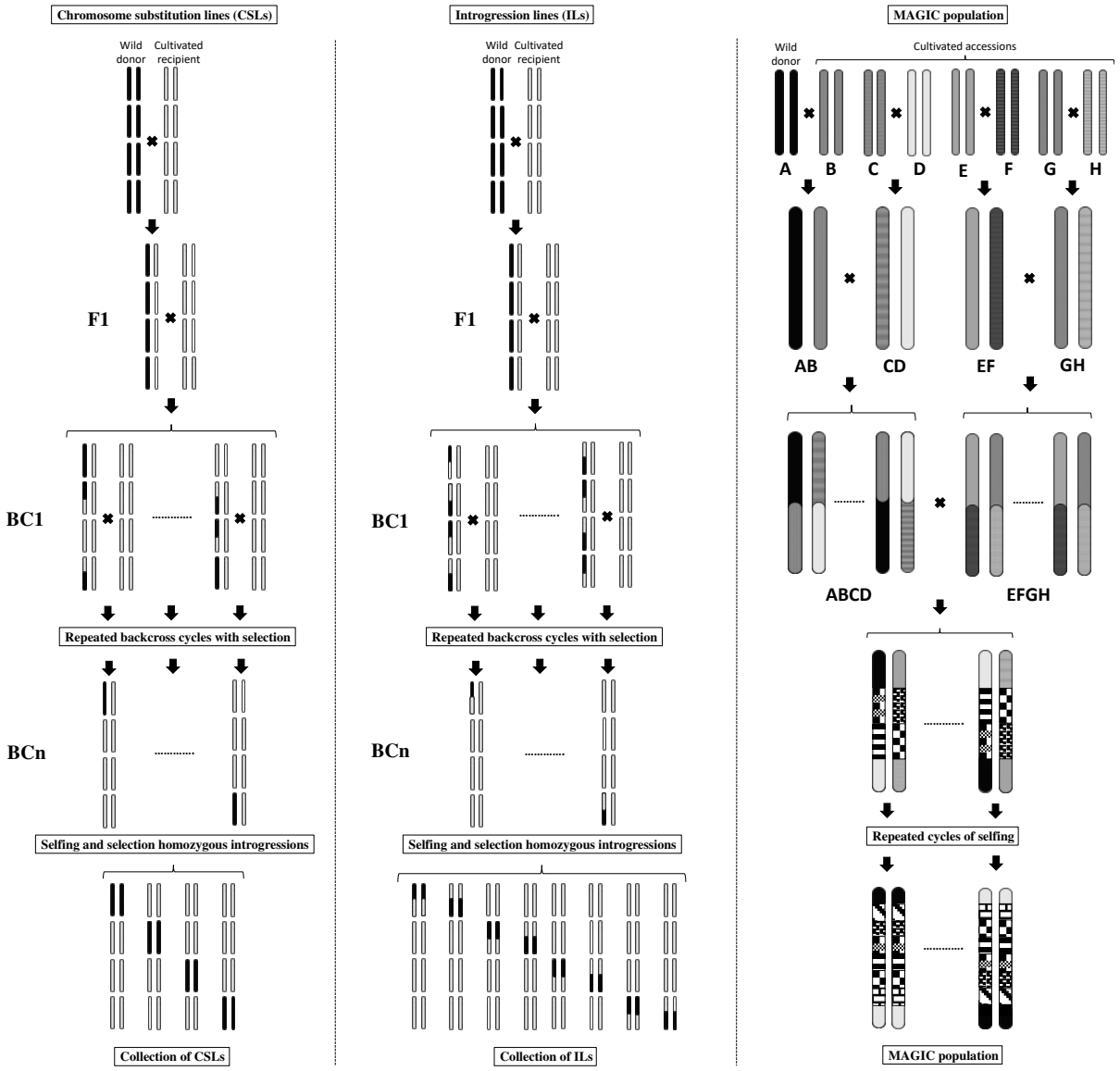

Figure 3. Scheme for the development of several types of introgressiomics populations: chromosome substitution lines (CSLs, left); introgression lines (ILs, center); and, multi-parent advanced inter-cross (MAGIC) lines (right).