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Additional Information

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

3

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21

22 **Abstract**

23

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

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

56

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

59

60

## 61 **1. Introduction**

62

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

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

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

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

93 Breeders have long recognized the value of crop wild relatives (CWRs) as sources of  
94 novel beneficial variation for resistance or tolerance to stresses (Bessey, 1906; Harlan and de  
95 Wet, 1971; Hajjar and Hodgkin, 2007). In this respect, most of the modern varieties of several  
96 important crops, like wheat or tomato, carry introgressions from wild relatives (Menda et al.,  
97 2014; Wulff and Moscou, 2014). CWRs are wild species that are able to exchange genes with  
98 the cultivated taxa through sexual or somatic hybridization (Harlan and de Wet, 1971, Maxted  
99 et al., 2006). CWRs are phylogenetically closely related to the crop and often encompass great  
100 genetic diversity. Some CWRs develop well under marginal conditions (low rainfall, high  
101 temperatures, high pressure of pests and diseases) that would be highly stressful for elite  
102 varieties of crops (Dwivedi et al., 2008; Dempewolf et al., 2014; Warschefsky et al., 2014). The

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

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

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

118

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

120

121 Plant domestication is a unique and complex evolutionary process in which natural and  
122 artificial selection resulted in new plants adapted to human needs (Meyer and Purugganan,  
123 2013). Domesticated plants generally present a syndrome of distinctive morphological,  
124 physiological and reproductive features compared to their wild ancestors (Hammer, 1984). For  
125 example in cereals, non-shattering phenotypes, with larger seeds, synchronized ripening,  
126 change in the plant architecture and loss of seed dormancy are characteristic traits of  
127 domesticates (Salamini et al., 2002). In other crops, such as those used for their fruits, tubers or  
128 leaves, domestication involved loss of bitterness and anti-nutritional or toxic compounds,  
129 removal or reduction of physical defence mechanisms like prickles, and gigantism of the organs  
130 used by humans (Meyer et al., 2012). One extreme example is that of giant pumpkins  
131 (*Cucurbita maxima*) with record weights of over a ton for a single fruit (Savage et al., 2015).  
132 Many crops also experienced changes in the reproductive system that isolated them from the  
133 CWRs (Meyer and Purugganan, 2013). In other cases crossability has been maintained within  
134 the genepool (Jarvis and Hodgkin, 1999; Meyer and Purugganan, 2013). Autogamy, which  
135 allows fixation and maintenance of selected characteristics, has been favoured in a number of  
136 crops (Meyer et al., 2012). Increased yield potential is also a general feature of domestication.

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

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

155 From a breeder's point of view, the utilization of CWRs present some significant  
156 challenges (Hajjar and Hodgkin, 2007; Dwivedi et al., 2008; Meyer and Purugganan, 2013).  
157 For example, crossing barriers and low hybrid fertility or sterility represent major barriers for  
158 the use of some species of CWRs in breeding programs. Although well-adapted to their natural  
159 environment, CWRs often contain a range of undesirable traits to agricultural conditions (low  
160 yield, undesirable physical and chemical defence systems, unpleasant flavour, lack of  
161 adaptation to cultivated conditions, etc.) (Meyer and Purugganan, 2013). In many cases, these  
162 "wild" traits are dominant and polygenic, and thus challenging to select against in the breeding  
163 programs. Linkage drag due to reduced recombination is another issue that is frequently  
164 observed in introgression programs. Once introgressed into the cultivated genetic background,  
165 the CWR chromosomal fragments are challenging to break into smaller components. These  
166 fragments often contain genes that confer undesirable phenotypes – and often these are linked  
167 to the gene/s controlling the traits of interest (Tanksley and Nelson, 1996; Wendler et al., 2015).  
168 In addition, phenotypes of interest in a CWR may not be expressed in a cultivated genetic  
169 background. Nonetheless, use of CWRs in breeding has allowed significant improvements in a  
170 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

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

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

215

### 216 **3. The introgressiomics approach**

217

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

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

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

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



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

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

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

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

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

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

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

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

304

#### 305 **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 gene pools 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 gene pools. 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 gene pool, which generally are typically less  
325 variable than CWRs from the secondary or tertiary gene pools. Adjustments may be made in the  
326 germplasm diversity sets to ensure a good representation of primary gene pool 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-](http://www.cgiar-sci.org)  
339 [sci.org](http://www.cgiar-sci.org)), using the geographical coordinates may help in identifying promising CWR

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

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

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

373

## 374 **5. Interspecific hybridization and backcrossing**

375

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

384 Interspecific hybridization between the crop and CWR taxa from the secondary and  
385 tertiary gene pools (wide or distant crosses) are often more challenging and hampered by pre-  
386 zygotic and post-zygotic reproductive barriers (Zenkteler, 1990; Khush and Brar, 1992) (Figure  
387 2). In addition, although it may be possible to obtain hybrids, sterility issues may limit the  
388 development of introgressomics populations. One important pre-zygotic reproductive barrier  
389 is pollen-style incompatibility (Dwivedi et al., 2008). As a consequence pollen does not  
390 germinate. When ‘unilateral’ incompatibility exists, hybrids can be obtained using the female  
391 parent in which the pollen is able to germinate (Figure 2). Unilateral incompatibility can  
392 frequently be observed when crossing self-compatible with self-incompatible species. The cross  
393 is successful when using the self-compatible species as a female parent (Dhaliwal, 1992). For  
394 example when crossing the cultivated tomato (*Solanum lycopersicum*) with its wild relative  
395 *Solanum peruvianum* the cross is unsuccessful when using the self-incompatible *S. peruvianum*  
396 species as female parent, but possible when using it as male parent. In the former case the barrier  
397 is pre-zygotic and the pollen tube can not penetrate the style. The reciprocal cross is possible,  
398 although the embryo must be rescued and cultured *in vitro* to prevent the embryo abortion  
399 (Hogenboom, 1984). When ‘bilateral’ incompatibility exists, several techniques have proven  
400 useful to overcome it (Rieseberg and Carney, 1998; Dwivedi et al., 2008). Among others, pollen  
401 mixtures of compatible pollen, inactivated or not, stigma exertion, physical or chemical  
402 treatments to the stigmas, or *in vitro* fertilization can be applied (Zenkteler, 1990; Khush and  
403 Brar, 1992; Dwivedi et al., 2008). Pollen mixture and treatment of stigmas with H<sub>3</sub>BO<sub>3</sub> and  
404 GA<sub>3</sub> were used by Picó et al. (2002) to obtain offspring from crosses between the cultivated  
405 tomato and the distant wild relatives *S. chilense* and *S. peruvianum*.

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

408 zygotic barriers are caused by differences in number of chromosomes, ploidy levels,  
409 chromosomal alterations (rearrangements, duplications, inversions or translocations), embryo-  
410 endosperm incompatibility and hybrid lethality (Khush and Brar, 1992). These post-zygotic  
411 barriers (Figure 2) may result in embryo abortion, preventing the development of viable seed.  
412 However, different techniques can be applied to try to avoid post-zygotic barriers. For example,  
413 when crossing individuals of different ploidy levels, the use of technologies that modify the  
414 ploidy level can contribute to the development of viable hybrids. Most frequently used  
415 techniques to obtain parental lines with the same level of ploidy are anther culture to produce  
416 haploid individuals (chromosome complement reduced to a half), or the duplication of the  
417 genome with colchicine (Khush and Brar, 1992; Rieseberg and Carney, 1998; Dwivedi et al.,  
418 2008). Also, embryo rescue, at an early stage of development (i.e. before it aborts due to either  
419 embryo-endosperm incompatibility or due to abnormal development resulting from genetic  
420 imbalance or other alterations), has been a successful tool in producing interspecific hybrids  
421 between crops and CWRs (Khush and Brar, 1992; Sharma et al., 1996).

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

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

442 2014). Some approaches can be used to increase the success rate of obtaining viable progeny  
443 from hybrids with reduced or low sterility. One strategy is to use the low-fertility hybrid as a  
444 female parent, since for the fertilization the pollen must have a high vigour to germinate and  
445 reach the ovule - a requirement not needed for the female gamete. This applies also to other  
446 plants from subsequent backcross generations. One way to recover fertility of the hybrid is  
447 duplicating its genome (Khush and Brar, 1992; Rieseberg and Carney, 1998; Shivanna and  
448 Bahadur, 2015). In this way normal chromosome pairing can be restored, although the hybrid  
449 plant will be polyploid, which may be an obstacle for obtaining subsequent generations due to  
450 different ploidy levels. To prevent this, a common technique is to duplicate also the genome of  
451 the recurrent cultivated parent to obtain backcross progeny and to restore the diploid status at a  
452 later stage. For example, Toppino et al. (2008) introgressed of resistance to *Fusarium* wilt from  
453 *S. aethiopicum* into the genetic background of eggplant (*S. melongena*) by backcrossing the  
454 tetraploid somatic hybrid to tetraploid eggplant to obtain the first backcross generation, which  
455 was subsequently returned to the diploid stage by anther culture. Generally, the loss of fertility  
456 in interspecific hybrids can be recovered in backcross generations, with increasing levels of  
457 fertility as the genome of the recurrent cultivated parent is being recovered (Wall, 1970).  
458 However, occasionally the phenomenon of selective chromosome elimination of the donor  
459 parent may occur, complicating the introgression of fragments of CWR (Dwivedi et al., 2008).  
460 In other cases, recombination in the hybrids between chromosomes of the cultivated species  
461 and the CWR is suppressed or reduced, which makes introgression more difficult (Bedó and  
462 Láng, 2015). In this way, in wheat the gene *Ph1* suppresses pairing and recombination of wheat  
463 and alien chromosomes; however, in plants that are nullisomic for the *Ph1* gene, or in *ph1b*  
464 mutant stocks, homoeologous wheat and alien chromosomes can pair and recombine, which  
465 facilitates introgression from CWR in the cultivated wheat (Friebe et al., 2012).

466 Introgressomics generally is conceived as the introgression of nuclear genes from wild  
467 species into a cultivated genetic background. However, introgression of cytoplasmic genes is  
468 also possible to produce alloplasmic hybrids with the nuclear genome of the cultivated species  
469 and the cytoplasm of the wild species (Khan et al., 2015). In this case, the cultivated species  
470 has to be used as male parent to ensure that the wild cytoplasm is maintained during the process.  
471 Nevertheless, it has to be taken into account that the interaction between the nuclear genome of  
472 the recurrent parent (cultivated species) and the cytoplasm of the donor (wild species) may  
473 result in cytoplasmic sterility (Prakash et al., 2001; Dwivedi et al., 2008; Khan et al., 2015),  
474 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



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

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

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

527 One of the main challenges to the development and use of introgressiomics populations  
528 is linkage drag, based on reduced recombination at introgressed fragments (Tanksley and  
529 Nelson, 1996; Wendler et al., 2015). The use of molecular markers allows selecting individuals  
530 carrying recombined introgressed fragments (Alkeesh et al., 2013). Special mating designs,  
531 such as sib-mating, can be considered in the backcross scheme to enhance recombination (Wall,  
532 1970; Liu et al., 1996; Rieseberg et al., 1996). Also, the use of congruency backcrossing, where  
533 backcrosses towards the recurrent parent are alternating to backcrosses to the donor parent to  
534 obtain fertile materials can be used to increase recombination (Haghighi and Ascher, 1988;  
535 Muñoz et al., 2004). Recently, Wendler et al. (2015) proposed crossing ILs with overlapping  
536 fragments originating from different donors to obtain progenies exhibiting higher levels of  
537 recombination within the introgressed fragment. However, in some occasions, due to the lack  
538 of synteny, recombination is prevented (Tanksley and Nelson, 1996). For example, in tomato,  
539 repeated attempts to reduce the introgression size associated to *Tomato yellow leaf curl virus*  
540 (TYLCV) resistance gene *Ty-1* introgressed from *S. chilense* failed due to lack of recombination  
541 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 (Bedó 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 gene pools for the  
574 initiation of an introgression breeding program (Plazas et al., 2016).

575 Molecular markers greatly facilitate the creation and characterization of CSL and IL  
576 populations. Marker-assisted foreground and background selection strategies - for those  
577 individuals that contain the target chromosomal segments, while at the same time selecting for  
578 beneficial alleles in the recipient parental genome - are being applied in several crops (Zamir,  
579 2001, Gur and Zamir, 2004; Pérez-de-Castro et al., 2012). In this way, collections of CSLs and  
580 ILs containing introgressed genome fragments of CWRs have been obtained in many crops  
581 (Gur and Zamir, 2004; Dwivedi et al., 2008; Khlestkina, 2014). The increased availability of  
582 high-density and mapped markers allows the fine mapping of the introgressed fragments, their  
583 extent as well as the breaking of ILs into sub-ILs by detecting recombinants (Lippman et al.,  
584 2007; Wendler et al., 2015; King et al., 2016).

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

593 The use of the so-called 'new plant breeding techniques' can be useful for  
594 introgressiomics (Lusser et al., 2011; Hartung and Schiemann, 2014) as well. In this respect,  
595 cis-genesis (Jacobsen and Schouten, 2007) and genome editing, in particular based on  
596 CRISPR/Cas9 (Belhaj et al., 2013), are promising. Cis-genesis consists of the genetic  
597 transformation of a recipient parent, in the case of introgressiomics the crop, with isolated genes  
598 and their promoters from a crossable donor (CWR), without the introduction of reporter or  
599 selectable markers from other organisms (Jacobsen and Schouten, 2007). In this way, by using  
600 cis-genesis, genes isolated from CWRs can be transferred in the genetic background of the crop  
601 without linkage drag (Tardi, 2016). Theoretically, by introducing genes isolated from CWRs  
602 from different gene pools it would be possible to produce collections of isogenic lines with genes  
603 from different donors, as well as to pyramid or to 'stack' genes from different species in a single  
604 genotype (Jo et al., 2014). This is particularly interesting in the case of secondary and tertiary  
605 gene pool species, with strong hybridization barriers (Khush and Brar, 1992; Dwivedi et al.,  
606 2008). For example, Jo et al. (2014) introduced two genes for resistance to late blight from the  
607 potato CWRs *S. stoloniferum* and *S. venturii* in several potato varieties. Several techniques exist  
608 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 be 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 initiatives, 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 (ITPGRFA). 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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679

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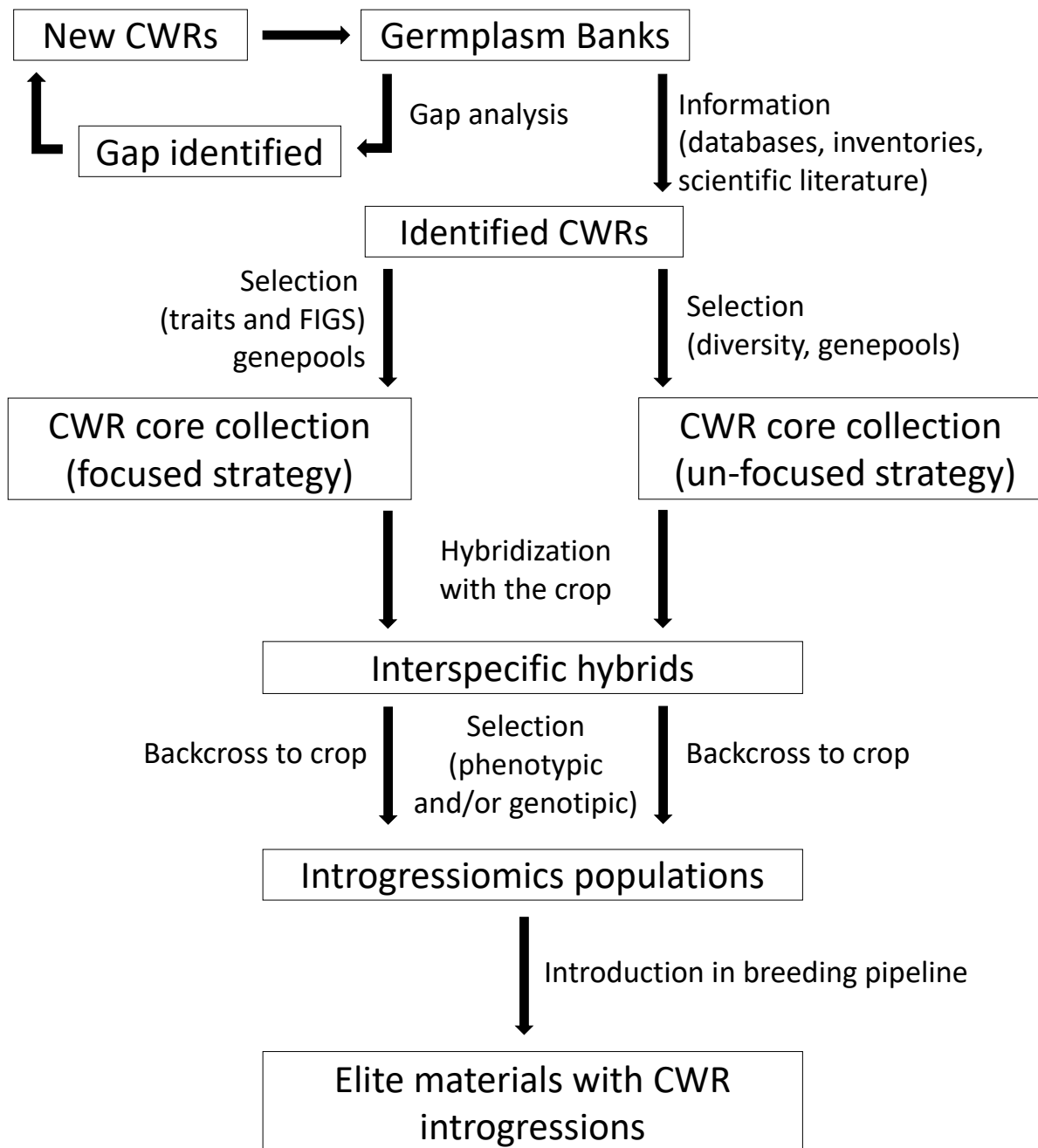
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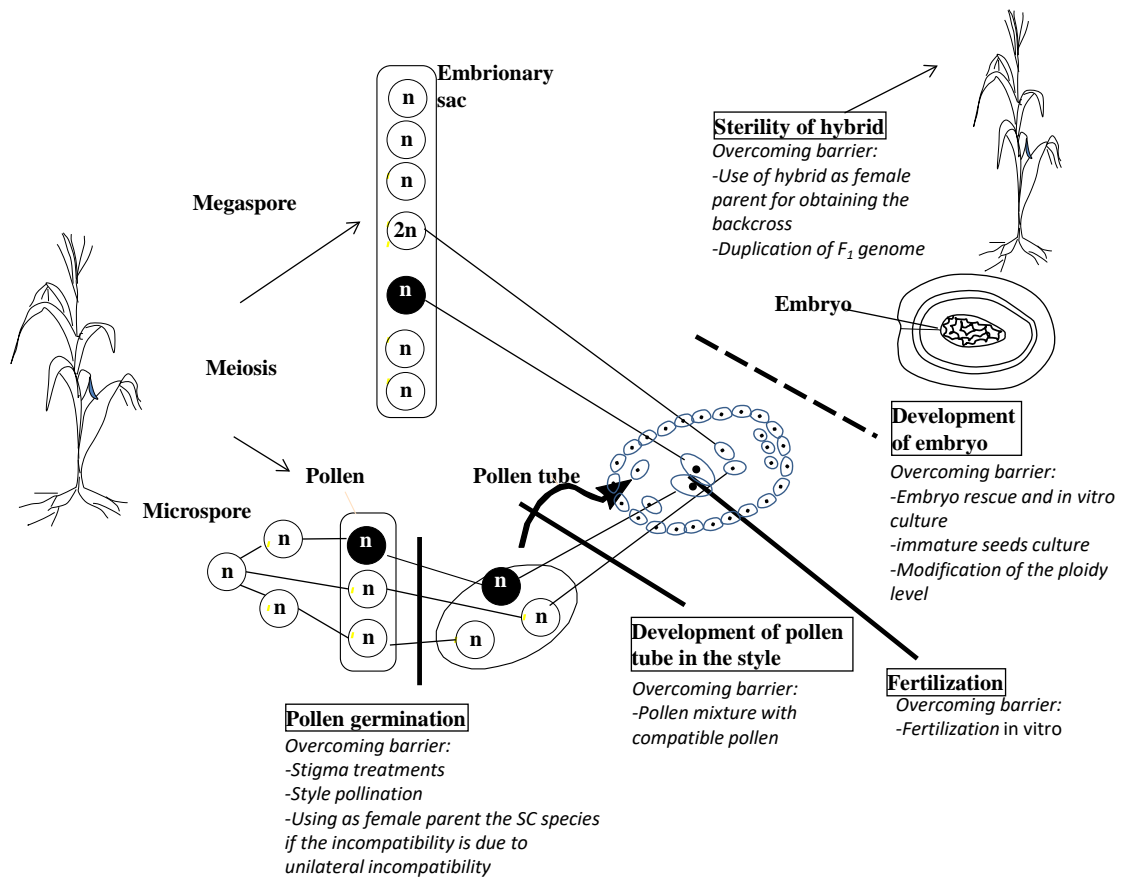
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1056 Figure 1. Outline of the introgressiomics pre-breeding approach workflow process, from the  
 1057 use of genetic resources to the development of elite materials with introgressions from crop  
 1058 wild relatives (CWRs).

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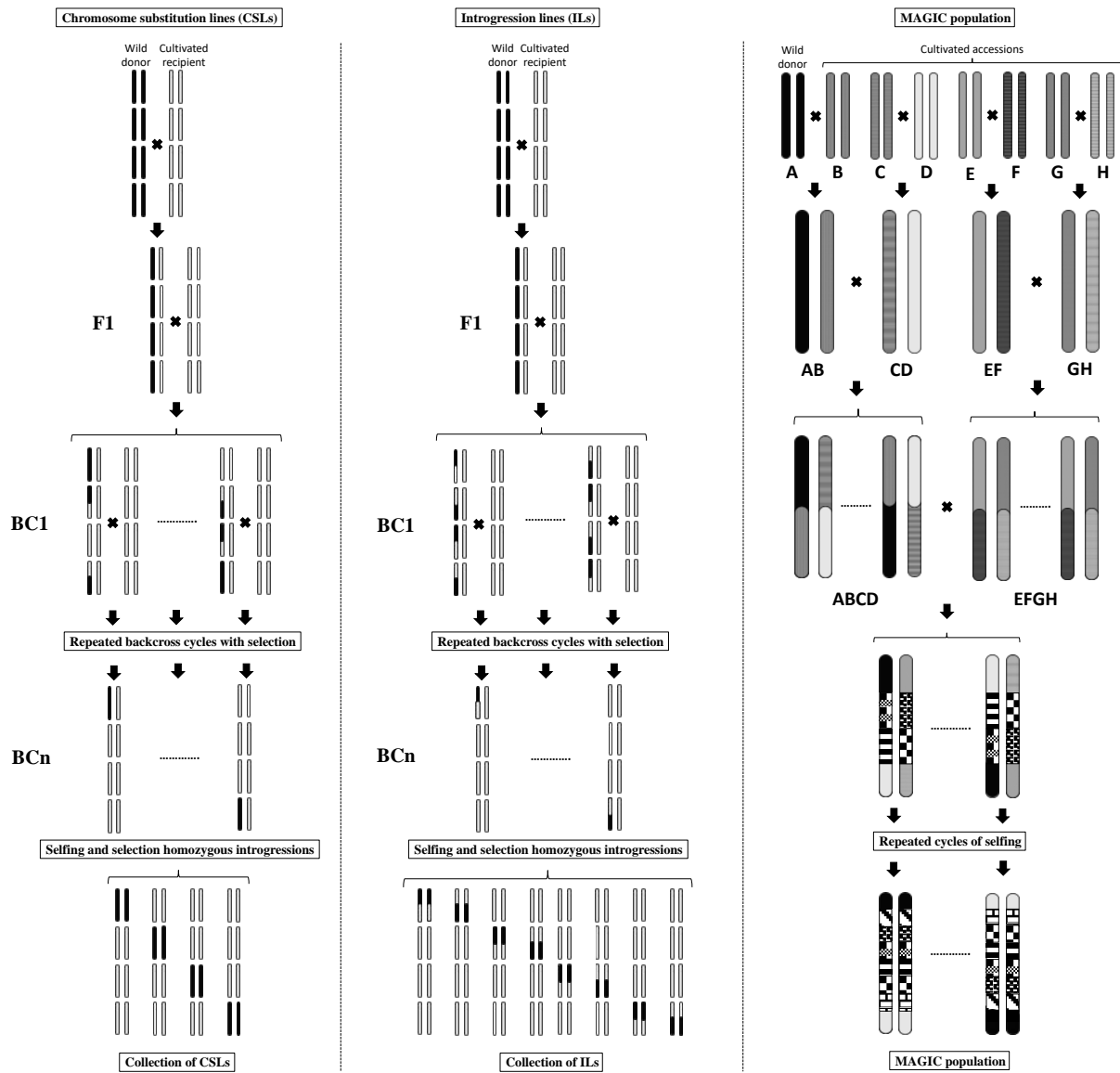
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1063 Figure 2. Pre- and post-zygotic barriers difficulting interspecific hybridization and introgression

1064 breeding with crop wild relatives (CWRs) and strategies to overcome them.



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1066 Figure 3. Scheme for the development of several types of introgressions populations:  
 1067 chromosome substitution lines (CSLs, left); introgression lines (ILs, center); and, multi-parent  
 1068 advanced inter-cross (MAGIC) lines (right).