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Review

Interspecific Hybridization for *Brassica* Crop Improvement

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ABSTRACT

Interspecific hybridization is widespread in nature, where it can lead to either the production of new species or to the introgression of useful adaptive traits between species. In agricultural systems, there is also great potential to take advantage of this process for targeted crop improvement. In the *Brassica* genus, several crop species share close relationships: rapeseed (*Brassica napus*) is an ancestral hybrid between turnip (*B. rapa*) and cabbage (*B. oleracea*), and mustard species *B. juncea*, *B. carinata* and *B. nigra* share genomes in common. This close relationship, plus the abundance of wild relatives and minor crop species in the wider Brassiceae tribe which readily hybridize with the *Brassica* crop species, makes this genus an interesting example of the use of interspecific hybridization for crop improvement. In this review we introduce the *Brassica* crop species and their wild relatives, barriers to interspecific and intergeneric hybridization and methods to overcome them, summarize previous successful and unsuccessful attempts at the use of interspecific hybridization for crop improvement in *Brassica*, and provide information about resources available to breeders wishing to take advantage of this method in the *Brassica* genus.

KEYWORDS: *Brassica*; interspecific hybridization; crop improvement; crop wild relatives; genetic diversity

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INTRODUCING THE *BRASSICA* CROP SPECIES AND THEIR WILD RELATIVES

The *Brassica* genus belongs to the tribe Brassiceae (family Brassicaceae). This family comprises 338 genera (assigned to 25 tribes) and 3709 species [1,2]. The members of this family are mostly herbs with annual, biennial or perennial growth habits [3]. Initially this family was known as “Cruciferae” due to its characteristic flower conformation of four petals arranged in a cross-shape [3]. Most of the member species are

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distributed in temperate regions, with the first center of diversification located in the Irano-Turranian region (~150 genera and ~900 species), followed by a second center of diversification in the Mediterranean region (>110 genera and ~630 species)[3].

Brassica is the most prominent genus in the Brassicaceae family and includes 39 species [1]. Many of the species in this genus are cultivated for their edible roots, leaves, stems, buds, flowers, mustard and oilseeds [4]. For 33 of the species the chromosome number has been determined, and ranges from n = 7 up to n = 20 [5]. During the 1930s, the chromosome number and genetic relationships between the cultivated *Brassica* species was established [6,7]. The diploid species *B. rapa* (AA, n = 10), *B. nigra* (BB, n = 8) and *B. oleracea* (CC, n = 9) were determined to be the progenitors of the allopolyploid species *B. juncea* (AABB, n = 18), *B. napus* (AACC, n = 19), and *B. carinata* (BBCC, n = 17), in a relationship known as "U's Triangle" [7]. Based on chloroplast DNA data it was determined that *B. nigra* belongs to a different lineage (Nigra lineage) than *B. rapa* and *B. oleracea* (Rapa/Oleracea lineage)[8], with the two lineages diverging approximately 7.9 Mya [9]. The divergence between *B. rapa* and *B. oleracea* has been estimated to have occurred perhaps 3.75 Mya [10] to about 5 Mya [11]. Later on, approximately 7500 years ago or less, diploid species *B. rapa* and *B. oleracea* hybridized to produce *B. napus* L. [12].

Genetic diversity within *Brassica* species has been broadly studied, with a special focus on the six crop species that form the U's triangle. Of these species, three are highly diverse: *B. oleracea, B. rapa* and *B. juncea* [13,14]. These species are quite morphologically variable, presenting different leaf types, numbers of branches per stem, inflorescence types, and stem thicknesses; these variations also lead to different end-product usage (e.g., oil or vegetable type)[13]. Genetic diversity observed in the *Brassica* allopolyploids can be due to (i) multiple hybridization events with diverse parents (or possibly subsequent backcrossing of the newly formed allotetraploids to the parent species) and (ii) genome changes occurring after polyploidization [15]. Four *Brassica* species are mainly used as oilseed crops: *B. juncea*, *B. rapa, B. carinata* and *B. napus* [16].

THE U'S TRIANGLE SPECIES AS CROPS: USES AND GENETIC DIVERSITY

Brassica napus (rapeseed, oilseed rape, swede) is the most economically important of the *Brassica* crop species, occupying the third position worldwide in the oil vegetable market, after soybean and palm oil. In the year 2016, worldwide production of rapeseed was over 68 million tons (Mt) (www.fao.org/faostat/, November 2018): In Germany, a large proportion of the rapeseed oil produced is used to generate biodiesel (2017: 4 Mt of biodiesel produced, source: European Biodiesel Board). Rapeseed, as well as other members of the Brassicaceae, naturally contain 20–40% erucic acid [17] and high glucosinolates in the seed meal.

However, rapeseed has been extensively bred for low erucic acid and low glucosinolates [18] to produce a type of rapeseed better known as canola. The main producers of rapeseed are Canada, China and India, which together represent almost 60% of the total production worldwide (www.fao.org/faostat/, November 2018). Winter-type rapeseed is mainly grown in Europe, and spring types are mostly grown in Canada, China and Australia [19]. *Brassica napus* (AACC, $2n = 4x = 38$) is thought to have originated in the last 7500 years via at least two different hybridization events between *B. oleracea* and *B. rapa* in agricultural systems [12]. Unfortunately, most of the genetic variation in oilseed rape has been reduced due to intensive selection for low erucic acid and low glucosinolate content traits [20]. Rapeseed is not found in nature as a wild type, and most of the diversity existing nowadays comes from breeding programs or cultivars from different countries [21].

Brassica juncea (AABB, $2n = 4x = 36$) is also used as a vegetable, with leaf mustard or Indian mustard as the common name [19]. A huge diversity of leaf morphotypes is present in this species that is thought to have been influenced by human selection [13], with two representative gene pools: East Europe and Indian [22]. Mustard is mainly grown in India due to climate conditions, where the breeding objectives are mainly focused on improving seed yield [16]. Although genetic resources available for *B. juncea* are not as comprehensive as those available for *B. napus* and its progenitor species, a reference *B. juncea* genome was published in the year 2016 [23].

Brassica rapa (AA, $2n = 2x = 20$), initially named *B. campestris* and commonly known as turnip or Chinese cabbage, has its origins in the Mediterranean and Central Asia [14]. The different subspecies of *B. rapa* can be used as a fodder (e.g., subsp. *rapifera*), vegetables (e.g., subsp. *chinensis* or *pekinensis*), or as an oilseed crop (e.g., subsp. *oleifera*)[14]. *Brassica rapa*, Chinese cabbage accession Chiifu-401-42, was the first *Brassica* species to get its genome sequenced [24]. Of the estimated genome size of 485 Mb, 283.8 Mb was initially assembled [24]. Later on, an improved assembly was released (v2.0) that increased the size of the genome assembly to 389.2 Mb [25]. The *B. rapa* genome is rich in transposable elements, accounting for 32.3% (~54 Mb) of the assembled sequence [25], much more than the 10.0% observed in the related genome of *Arabidopsis thaliana* [26].

Brassica oleracea (CC, $2n = 2x = 18$) is mainly used as an edible vegetable. This species is composed of several varieties and morphotypes are usually referred to as coles. These vegetables are rich in vitamin C, folate and calcium [27]. Different varieties include Brussels sprouts (var. *gemmifera*), cabbage (var. *capitata*), cauliflower (var. *botrytis*), and Chinese kale (var. *alboglabra*)[27]. In the year 2016, the worldwide production of cauliflower and broccoli surpassed 25 million tons (www.fao.org/faostat/, November 2018). Some new vegetables have also been produced by crossing different varieties within this genus, such as broccolini [27]. Two draft genome references for *B. oleracea* were published in 2014 [28,29].

Brassica carinata (BBCC, $2n = 4x = 34$), also called Ethiopian mustard, possesses wide genetic variability and is also used as an oilseed crop [30]. This crop has also been considered for use in biodiesel production [31] and for other purposes including as a condiment, medicine and vegetable [19].

Brassica nigra (BB, $2n = 2x = 16$) was previously used as a condiment mustard but has now been mostly replaced by *B. juncea* [19]. Compared to the major *Brassica* crops, *B. nigra* contains little variety in physical appearance [13], but it nevertheless possesses different agronomical traits of great value such as resistance to *Phoma lingam* [32]. Although *B. nigra* is the least agriculturally significant of the six *Brassica* crop species, a scaffolded genome assembly (not yet assembled into pseudomolecules) was made available in 2016 alongside the *B. juncea* genome [23], and a new chromosome-level assembly was released in 2019 [33].

THE *BRASSICA* WILD RELATIVES: COENOSPECIES AND CYTODEMES

In the 1970s, Harberd defined the term “coenospecies” for those species or genera that have sufficient relatedness to the six *Brassica* crops to be able to exchange genetic material with them [34,35]. The coenospecies are composed of almost 100 wild species and genera that can potentially be used to increase diversity, and to introgress useful traits such as disease resistance or abiotic stress [36]. Harberd also classified the *Brassica* coenospecies into biological units called “cytodemes” [34,35,37]. Each cytodeme can contain more than one genus or species, but all species within a cytodeme should have the same chromosome number, and readily cross with other species in the same cytodeme to produce fertile, vigorous hybrids. Based on these criteria, the *Brassica* coenospecies were initially classified into 38 cytodemes [35], covering nine genera from the subtribe Brassiceae (*Brassica, Coincya, Diplotaxis, Eruca, Erucastrum, Hirschfeldia, Sinapis, Sinapidendron,* and *Trachystoma*) and two genera from subtribe Raphaninae (*Enarthrocarpus and Raphanus*). This was later updated to 63 [38], after the addition of three genera (*Moricandia*, *Pseuderucaria*, and *Rytidocarpus*) from the related subtribe Moricandiinae [39]. The crossability between cytodemes is low, but certain tools can be used to increase success rates (as discussed in later sections of this review). Crossability can also be influenced by the direction of the cross, *i.e.*, which species is used as the maternal parent, which is referred to as “unilateral incompatibility” [40].

An extended list of potentially useful agronomic traits for crop improvement present in wild allies of the *Brassica* species can be found in [41]. Examples include resistance to white rust (*Albugo candida*) in *Brassica maurorum* [42] and *Eruca versicaria* ssp. *sativa* [43], resistance to *Alternaria* blight in *Brassica fruticulosa* [44] and *Trachystoma ballii*

[45], resistance to beet cyst nematode in *Raphanus sativus* [46] and *Sinapis alba* [47], and resistance to blackleg/Phoma disease (*Leptosphaeria maculans*) in *Sinapis arvensis* [48], *Sinapis alba* [49], *Thlaspi arvense* [50], and *B. tournefortii* [51]. The *Brassica* crop species also contain unique, useful traits: examples include resistance to powdery mildew (*Hyaloperonospora parasitica*) in *Brassica oleracea* [52], resistance to clubroot disease (*Plasmodiophora brassicae*) in *B. rapa*, *B. oleracea* and *B. napus* [53], and pod shatter resistance and tolerance to heavy metals in *B. juncea* [54]. More exotic traits of interest include a C_3–C_4 intermediate photosynthetic system in *Moricandia* [55] and *Diplotaxis* species [56,57], and high erucic acid levels in *Crambe abyssinica* [58]. Cytoplasmic male sterility in *Brassica* could also be conferred by hybridization with *Sinapis incana* [59] and *Diplotaxis siifolia* [60], among other examples.

HYBRIDIZATION BETWEEN *BRASSICA* SPECIES AND WILD RELATIVES

Direct wide hybridization has been attempted many times between *Brassica* and various wild relative species, with different levels of success (reviewed in [61]). Originally such hybrids were produced to resolve chromosome homoeology (phylogenetic relationships) or simply out of curiosity [62]. However, crossing with distant relatives is today attracting increasing recognition as a method with which to improve agronomic traits in high-end varieties. There are many examples of the successful introgression of new traits into *Brassica* crops. Initial attempts to create hybrids between *Brassica* species started in the early 1800s. At this time, some crosses were made between *B. napus* × *B. rapa* and *B. oleracea* × *B. rapa*. Different success rates were reported and the results were published in 1925 by [63]. Later on, a compilation of crossability between species in the *Brassica*, *Raphanus* and *Sinapis* genera was published, showing that interspecific hybrids can be made between the *Brassica* crops and many closely-related wild species [61].

The occurrence of natural hybridization between distant relatives in natural conditions is low. For instance, [64] found that hybridization between *Brassica napus*, *B. rapa* and *B. juncea* and their two weedy relatives *B. nigra* and *Sinapis arvensis* does not occur under open pollination conditions in the field, although *B. rapa*, *B. juncea* and *B. napus* all readily produce hybrid progeny with each other under the same conditions. The cross between *B. napus* ($2n = 38$) and *Raphanus raphanistrum* ($2n = 18$)[65] has also been assessed under field conditions. In this case, just two allopolyploid hybrids ($2n = 56$) were obtained from more than 52 million *B. napus* seedlings when this species was used as a female, showing a hybridization frequency of 4×10^{-8} in field conditions. These results indicate that the likelihood of this cross in the wild is low, which shows the importance of conducting such hybridizations under controlled conditions.

TRANSFER OF USEFUL TRAITS INTO *BRASSICA* CROP SPECIES THROUGH INTROGRESSION BREEDING

Disease Resistance

The introgression of genes for disease resistance between species has been widely studied in *Brassica*. One example is the utilization of the B genome as a source of resistance against *Leptosphaeria maculans* (blackleg) from diploid and tetraploid species. For instance, chromosome B4 from *B. nigra* was introgressed into rapeseed variety “Darmor”, which showed high resistance with the addition of this chromosome [66]. Similarly, high resistance from *B. juncea* was obtained in selected recombinant lines of *B. napus* carrying a resistance gene located on chromosome B8 [67]. A similar study [68] successfully introgressed a B-genome chromosome from *B. carinata* to *B. napus*, with plants carrying this chromosome showing variation in traits such as blackleg resistance, days to flowering, days of maturity, and fatty acid composition. Another example is the improvement of resistance against *Erysiphe polygoni* (which can cause powdery mildew disease). Resistance in 100% of BC_1 progeny was successfully demonstrated in hybrids obtained by hand crossing and embryo rescue between *B. carinata* (donor) × *B. oleracea* [69]. Other cases of resistance transfer include transfer of blackrot resistance from *B. carinata* to *B. oleracea* [70], resistance to *Brassica* leaf blight caused by *Alternaria brassicae* from *B. hirta* to *B. juncea* [71] and transfer of powdery mildew resistance from *B. carinata* to *B. oleracea* through embryo rescue followed by backcrossing to *B. oleracea* [69].

Yellow Seededness

Yellow seededness is a desirable trait in *Brassica*, as yellow seeds have less fiber, higher protein, and higher oil content than black seeds. Although *B. juncea* and *B. rapa* contain yellow-seeded traits, this trait is not found in rapeseed (*B. napus*). Using monosomic alien addition lines from the cross *B. rapa* × *B. oleracea*, Heneen *et al.* [72] found that seven of the nine C chromosomes carry genes that affect seed color, showing the complexity of this phenotype. Interspecific crosses between *B. alboglabra*, *B. rapa* var. “yellow sarson”, yellow seeded *B. carinata* and black seeded *B. napus* have been carried out previously to attempt to produce yellow-seeded *B. napus*, with interspecific hybrid progeny showing different degrees of seed colour [73]. However, this study demonstrated that the combination of the C genome of yellow-seeded *B. carinata* with the A genome of “yellow sarson” does not result in a yellow-seeded *B. napus.* The expression of this trait also appears to be heavily affected by the environment. Rashid *et al.* [74] crossed [(*B. napus* × *B. juncea*) × *B. napus*] × [(*B. napus* × *B. carinata*) × *B. napus*] and successfully obtained yellow seeds. However, when these plants were tested in the field the color was found to be highly affected by temperature [75].

Male Sterility

A common use of wild relatives for *Brassica* crop improvement is in the production of male sterile lines to facilitate hybrid production. Male sterility is often conferred when cytoplasm from an alien species is present in the genetic background of another species: this is referred to as cytoplasmic male sterility, or CMS. The most successful example of this approach in *Brassica* is the Ogura CMS system, where alien cytoplasm was obtained from crossing *Brassica napus* to Japanese radish (*Raphanus sativus*)[76]. This system was subsequently widely used in *B. napus*, *B. juncea* and *B. oleracea* [77]. Several other CMS systems have also been successfully developed from interspecific hybridization events, including a novel CMS system in *B. juncea* incorporating the cytoplasm of *B. fruticulosa* [78], and the Nsa CMS system in *B. napus* utilizing *Sinapis arvensis* cytoplasm [79]. On the other hand, several attempts to produce additional CMS lines through interspecific hybridization have also been unsuccessful. Seventeen crosses between *Diplotaxis* species and *B. napus* were done in order to introgress CMS, but out of hundreds of crossings using conventional techniques only crosses with *D. muralis* and *D. erucoides* were successful, and no CMS system was consequently established [80]. Protoplast fusion has been used to transfer *Ogu* cytoplasmic male sterility factor from *Brassica napus* to *Brassica juncea* and for the improvement of male sterile lines in hybrid breeding systems [81]. Somatic hybridization between *B. juncea* and *B. oleracea* has also been used to transfer cytoplasmic male sterility and resistance to Turnip mosaic virus from *B. oleracea* to *B. juncea* [82,83]. Prakash *et al*. [84] successfully obtained both stable CMS *B. juncea* and an introgression line carrying the restorer gene via somatic hybridization between *M. arvensis* and *B. juncea* followed by backcrossing with *B. juncea*.

Oil Quality Traits

Interestingly, oil quality traits have also been successfully transferred between species for crop improvement in *Brassica*. In the case of rapeseed, low erucic acid and low glucosinolate content originate from two *B. napus* cultivars: "Liho" with low erucic acid and "Bronowski" with low glucosinolate content [20]. Another possible source of these oil quality traits is *Capsella bursa-pastoris*, which can show less than 1% erucic acid and less than 16 μmol/g of glucosinolates in the seeds, as well as high resistance to *Sclerotinia sclerotiorum* [85]. Previously, several chromosomes and chromosomal fragments from *C. bursa-pastoris* were successfully introgressed into *B. napus* and *B. rapa* [85]. Another wild relative with favorable fatty acid content is *Orychophragmus violaceus*, which has been successfully crossed with *B. napus* [86,87]. From this cross, advanced progenies with $2n = 38$ chromosomes, ≥70% oleic acid, 28% linoleic acid and low glucosinolate content in the seeds (<30 μmol/g oil free meal) were produced [87].

Other Traits of Agronomic Interest

Moricandia arvensis is a plant that expresses an intermediate C_3–C_4 photosynthetic mechanism [88]. This trait was introgressed into *B. napus* by somatic hybridization by [89], who obtained three hybrid plants that expressed C_3–C_4 intermediate photosynthesis characteristics. Dwarfism is a useful agronomic characteristic which helps avoid lodging, and which was introgressed by [90] from a mutant *B. rapa* into natural *B. napus* via production of a resynthesized *B. napus* from the mutant *B. rapa* with a normal *B. oleracea*, followed by four generations of backcrossing with natural *B. napus.* Pod shatter resistance has also been introgressed into *B. napus* from *B. juncea* via direct hybridization [91]. Finally, drought tolerance has been introgressed from *Sinapis alba* into *B. napus* by somatic hybridization, and was identified at the vegetative stage in the BC_3F_1 vegetation, although the original target was yellow-seededness [92].

RESYNTHESIS OF *BRASSICA* ALLOTETRAPLOID CROP SPECIES

Interspecific hybridization has two major outcomes: introgression and speciation. While introgression transfers just a limited number of alleles, hybrid speciation produces a new hybrid species. Resynthesis is the process of reproducing an already existing species from its progenitor species. This is most often done to increase the genetic diversity of the existing allotetraploid species by incorporating some of the greater genetic diversity of the progenitor species. Resynthesis as a tool of crop improvement has many benefits. Polyploidy induced during the process of resynthesis can overcome crossing barriers due to endosperm failure in interploidy crosses [93]. The genetic diversity of some *Brassica* allotetraploid crops is limited due to the few hybridization events that gave rise to these species [12]. In the case of *B. napus*, geographic isolation, extensive breeding and selection for low erucic acid and glucosinolate content has further eroded the genetic diversity of this species [37,94]. Resynthesizing the *Brassica* allotetraploids from their diploid parents is a means of increasing the genetic diversity of these species. Studies of this method abound: Seyis *et al.* [95] resynthesized 165 *Brassica napus* lines by crossing *B. rapa* and *B. oleracea* progenitor species; analysis of these resynthesized lines using RFLP markers showed they were highly genetically divergent from established oilseed rape cultivars, and also showed a high degree of morphological diversity. Abel *et al.* [96] also developed resynthesized *Brassica napus* to study fixed heterosis by crossing 21 *B. rapa* and 16 *B. oleracea* species, and showed that the direction of the cross affects hybridization outcome, although the diversity of this population and its effect on fixed heterosis was not reported in this study. Several other studies have also reported on resynthesis of *B. napus* in order to expand the available gene pool [97–100], and to test for new traits such as resistance to cabbage stem weevil *Ceutorhnchus pallidactylus* [101]. *Brassica juncea* has also been resynthesized by crossing its progenitor species *B. rapa* and *B. nigra* to broaden the genetic base of this species [102–104]. Bansal *et al.* [105] resynthesized new *B. juncea* genotypes, and found the resynthesized *B. juncea* to be morphologically diverse compared to natural *B. juncea*. *Brassica carinata* has also been resynthesized from its progenitor species, with hybrids showing morphological variation potentially useful for crop improvement [106,107].

These new synthetic polyploids are not usually being bred to become a new crop nor in competition with the elite varieties, but rather as a source of diverse new agronomic traits, where they are used to cross with and introgress these traits into high-performance cultivars [62]. One successful example is the synthetic clubroot-resistant allotetraploid *B. napus* RS 15/04, which was created by crossing a resistant kale (*B. oleracea* ECD-15) and turnip rape (*B. rapa* ECD-04). This synthetic *B. napus* was subsequently crossed with WOSR cv. “Falcon”, and a DH line created from the F_1. This line was then backcrossed with cv. “Falcon” until the BC_2F_1 where three dominant genes specific to a particular race of the clubroot pathogen were present. Further breeding was done, and in 2001 the clubroot-resistant winter oilseed rape cv. “Mendel” was released [20]. Newly synthesized *Brassica* polyploids can also present extensive genome change at very early stages and also throughout further generations (F_1–F_5)[108]. This variation can also be phenotypically observed in traits like flowering time [109] and hybrid vigor in synthetic *B. juncea* [102], and may comprise a means of generating entirely new traits.

NOVEL GENOME COMBINATIONS AND CROP TYPES

Efforts on *Brassica* improvement through polyploid synthesis have not only been limited to the naturally occurring allotetraploids. Several attempts have been made to synthesize a new, fertile and meiotically stable allohexaploid *Brassica* ($2n$ = AABBCC), with varying success rates that appear dependent on both genotype and method used (reviewed by [110]). Synthetic allohexaploids produced from crosses between *B. carinata* and *B. rapa* followed by chromosome doubling showed bigger flowers, high silique setting and high fertility, the latter increasing from the F_2 to F_4: this trend is expected to continue across generations, leading to a potentially stable species which could be of benefit to agriculture [111]. Other studies on allohexaploid *Brassica* have focused on using these hybrids as a bridge between species (reviewed by [94]), such as in the creation of novel *Brassica napus* genotypes exhibiting useful traits like yellow seededness via hybridization between *B. rapa* and *B. carinata* to produce $2n$ = AABBCC types followed by backcrossing to *B. napus* and elimination of the B genome [112–114].

The *Raphanus* genome has also been used to develop synthetic allotetraploids, as radicole (CCRR, $2n$ = 36)[115] or Raparadish (AARR, $2n$ = 38)[116]. Both of these hybrids feature a fodder-like crop with the advantage of resistance to the beet cyst nematode. Although *B. napus* has some resistance to this nematode, transfer of this high-resistance trait from Raparadish to *B. napus* was attempted in 1993 [117]. Surprisingly, there was no significant difference in the number of hybrids produced based on the *B. napus* cultivar or accession used in the crosses. In the F_1 population (AACR, $2n = 38$), nematode resistance was found to be intermediate between the two parental species. The meiosis observed in the F_1 plants was also very variable, producing a high frequency of unbalanced and unreduced gametes.

BARRIERS TO INTERSPECIFIC HYBRIDIZATION

Near and far relatives of major crop species provide us with an enormous untapped reservoir of agriculturally important traits. Transferring this genetic variation to crops through introgression breeding has helped produce improved, high yielding crops resilient to prevailing climatic conditions [118]. The *Brassica* A, B and C genome species and other wild relatives contain valuable genetic variation for crop improvement, including genes or alleles for defense against pests and diseases [67,69] and drought tolerance [119,120]. Extensive interspecific and intergeneric hybridization has been performed between cultivated species, and between cultivated species and wild relatives, to develop more potentially useful cultivars with improved biotic and abiotic stress tolerances [61].

However, despite the potential of using hybridization to transfer useful traits from related crop species or wild relatives, there are barriers that limit the usefulness of this process. Interspecific and intergeneric hybridization barriers can be divided into two categories: pre-fertilization and post-fertilization barriers. Pre-fertilization barriers can arise due to failure of pollen germination, pollen tube growth or pollen tube penetration of the embryo [121,122]. Degradation or death of the hybrid embryo and male and female sterility in hybrid plants are some of the causes of post-hybridization barriers and hybrid sterility [123]. Fertilization in interspecific crosses can still occur, but later on can produce embryo abortion related to problems with endosperm development [124]. This often happens in one direction (*i.e.*, when one species is used as the maternal parent, but not when it is used as the paternal parent) and it can be overcome when the reciprocal cross direction is tested [124]. This has been recorded, and some examples show more success when *B. napus* is used as a female in interspecific hybridization events [16]. Similarly, in some attempted crosses between *B. carinata* and *B. rapa*, F_1 hybrids were only obtained when *B. carinata* was used as the female [125]. The challenge of creating interspecific hybrids increases as the phylogenetic distance between the combining species increases [126]. Opportunities for and success of interspecific crosses are also dependent on a number of other factors: physical distance between the species/parent plants, synchrony of flowering, the specific parental genotypes used, the method of pollen dissemination, the direction of the cross (which parent is female), environmental factors, and whether one parent is male-sterile [127,128].

In *Brassica* it is difficult to make a simple statement about reproductive compatibility and incompatibility, as reproductive compatibility relationships are complicated, with partial reproductive barriers between many species [61,127]. Despite years of research on hybridization in *Brassica*, the degree of reproductive compatibility between many species combinations remains untested. Detailed summaries of the extent of interspecific hybridization in *Brassica* have been reported by various sources [13,41,61]. Given that several factors need to be considered in creating successful interspecific hybrids, different methods have been developed to transfer useful traits between different *Brassica* species and to increase the genetic diversity of *Brassica* crops.

METHODS TO FACILITATE INTERSPECIFIC HYBRIDIZATION AND THE TRANSFER OF TRAITS BETWEEN SPECIES

Early and *in Vitro* Fertilization and Embryo Rescue

Failure of foreign pollen to germinate on the stigma, to grow pollen tubes or to subsequently fertilize ovules, and for fertilized ovules to develop into seeds, are all commonly observed in interspecific hybridization attempts. However, a number of strategies exist to overcome these pre- and post-fertilization barriers (reviewed by [129]). Early pollination of stigmas (before buds open and before full maturity) or stump pollination can help in overcoming reproductive incompatibilities between some genotypes of *Brassica* species [130], while in other cases *in vitro* pollination of the stigma or pistils and/or opened ovules and ovaries may facilitate the interspecific fertilization event [131]. Seed abortion post-fertilization is also often observed in crosses between plants of different species or ploidy levels [132]. In cases where seeds cannot be obtained from crossing, a technique where the embryo is “rescued” from the putatively hostile maternal environment, usually into tissue culture or a sterile medium, can sometimes allow the production of hybrid plants. The technique of *in vitro* culture to rescue interspecific hybrid embryos was first used in crosses between *Lolium perenne* and *L. austriacum* [133]. Wide crosses between many crop plants and their wild relatives have now become possible through the use of embryo rescue techniques, as embryo rescue and subsequent culture *in vitro* helps to overcome post-fertilization barriers [70]. In the production of *Brassica* interspecific hybrids, embryo rescue is commonly used to overcome natural reproductive barriers [94,134]. Embryo rescue was first used in *Brassica* by [135]. Following this study, extensive investigations have been carried out to improve this method [136,137].

The successful application of this technique depends on the developmental stage of the embryo being rescued [70].

Several studies have demonstrated the importance and success of this technique in transferring useful traits between *Brassica* species. Using embryo rescue, triazine resistance has been transferred from *B. napus* to *B. oleracea* [133]. Yao *et al.* [138] produced allohexaploids by crossing *B. maurorum* with all three *Brassica* allotetraploids. Herbicide resistance was transferred from *Sinapis arvensis* to *B. juncea* and *B. rapa* using embryo rescue [139]. Cytoplasmic male sterility has been transferred from *B. juncea* and *B. napus* to *B. oleracea* [140]. Zhang *et al.* [98] resynthesized *B. napus* from interspecific hybridization between *B. rapa* and *B. oleracea*, and new type *B. napus* types showing resistance to *Verticillium longisporum* were synthesized from a diverse set of *B. rapa* and *B. oleracea* through embryo rescue [141].

Somatic Fusion

Somatic fusion is an important means of transferring useful traits from one species to another. Somatic fusion has the advantage that it can bypass these incompatibility barriers and transfer genes between sexually incompatible species [142]. Besides the transfer of agronomically important traits, protoplast fusion can be used to modify organellar traits, as chloroplasts and mitochondria from both parental species are combined with somatic fusion, rather than only the maternal cytoplasm being inherited by the interspecific hybrid as is the case for sexual crosses. *Brassica* species were among the first crops used for protoplast isolation, as most parts of the plant are suitable for releasing totipotent protoplasts [142,143]. Regeneration of plants from isolated protoplasts has been reported in all *Brassica* species following the first report of successful plant regeneration from *B. napus* mesophyll tissue [144]. Somatic hybridization has successfully been used to transfer traits such as disease resistance, oil quality, cold and drought tolerance and herbicide resistance between species [142,143]. In one example, somatic hybrids between *B. rapa* and *B. oleracea* were used to create improved *B. rapa* cultivars resistant to soft rot by backcrossing somatic hybrids to *B. rapa* [145]. Asymmetric somatic hybridization has also been used to transfer resistance to blackleg disease from *B. juncea*, *B. rapa* and *B. carinata* into *B. napus* [113].

Genetic Transformation

Genetic transformation can play an important role in variety improvement and functional analysis of *Brassica* crops. It has paved the way for the development of new *Brassica* varieties producing biodegradable plastics, pharmaceuticals and nutritive compounds by introducing new genes from unrelated sources [146]. Conventional breeding of *Brassica* is time consuming, labor and resource intensive. On the other hand, genetic transformation provides a direct means of introducing specific genes or traits without negatively affecting the desirable genetic background [147]. In addition, certain important traits may not be available in the existing germplasm [148]. Under such circumstances, genetic transformation has shown to be a powerful means of effectively transferring genes across reproductive barriers [149].

Genetic transformation systems have been developed in almost all the economically important *Brassica* species, including *B. napus* [150], *B. oleracea* [151], *B. juncea* [152], *B. nigra* [153], *B. carinata*, and *B. rapa* [154]. Different plant transformation methods exist. The direct method, where naked DNA is introduced into the protoplasts of intact cells, can be mediated by methods such as polyethylene glycol (PEG) treatment, microinjection and electroporation. Alternatively, indirect methods requiring an intermediate biological vector can be used; usually *Agrobacterium tumefaciens* transformation is suitable for this purpose in *Brassica* [149].

Genetic transformation has led to the introduction of new traits in to *Brassica* crops far beyond the species boundary: genes not present in the *Brassica* species. Traits improved through genetic transformation include resistance to herbicides such as glyphosate, glufosinate, sulfonylurea, bromoxynil, and bromoxynil resistance [155–157]. Oil quality improvement has also been a target of transformation. *Brassica juncea* and *B. napus* with high oleic acid have been produced by silencing the endogenous oleate desaturase [158]. Also, transformation of the d12-desaturase genes from the fungus *Mortierella alpina* has led to the production of canola with high gamma-linolenic acid [159].

Insect and disease resistance have also been important target traits for improvement of *Brassica* crops. *Brassica napus* producing an endogenous endotoxin of *Bacillus thuringiensis* poisonous to the diamondback moth have been produced through transformation with the *Bt cry1* gene [160,161]. Novel insect resistance in *B. napus* has also been developed by transformation of chitinase and scorpion genes [161]. Transformation has been used to convert *Brassica* crops to biofactories producing pharmaceutical and industrial products such as biodegradable polymers [162]; the anticoagulant protein hirudin has been produced in *B. carinata* [163].

The development of male sterile lines and restoration system has also been a significant advancement in *Brassica* transformation. Male sterile plants were obtained in *B. juncea* by introducing the *barnase* gene with tapetum-specific promoters, following which the fertility of the male sterile line was restored by crossing it with a barstar containing transgenic line [164].

Genome Editing

Recently, the clustered regularly interspaced short palindromic repeat (CRISPR)-CRISPR associated protein 9 (CRISPR/Cas9) system has emerged as a versatile molecular tool for genome editing in different organisms [165]. It has been shown that the CRISPR/Cas9 system is able to achieve efficient gene editing in plants through either transient experiments or in the production of transgenic plants [166]. In this system, the endonuclease Cas9 is directed to a specific DNA target by a synthetic guide RNA [167]. It is an innovative genetic tool that can modify the genome of any species with high precision and accuracy [168]. Although this technology is still in its early stages, its application has been demonstrated not only in model species such as *Arabidopsis thaliana* [169], but also in crops such as tomato (*Solanum lycopersicum*)[170] and wheat (*Triticum aestivum*)[171]. In *Brassica napus*, proof of concept was recently demonstrated by [172], who targeted the two homologues and four alleles of the *BnALC* gene, which is responsible for fruit dehiscence in *Brassica*. Similar reports have since followed, such as [173] who determined the mutation efficiency of CRISPR-Cas9 in 12 gene families. CRISPR/Cas 9 has also been used to modify the fatty acid desaturase 2 (*FAD2*) gene which catalyzes the desaturation of oleic acid in *B. napus* leading to the production of *B. napus* with high oleic acid [168]. The application of this system has been demonstrated also in *B. oleracea* [174] and *B. carinata* [175]. CRISPR/Cas9 therefore promises to be an important tool in *Brassica* improvement. In future, linking genetic and genomic information to germplasm bank resources could extend the reach of this genome editing technique to many genetic variants of agricultural significance present within the wild relatives of the *Brassica* crop species, allowing direct editing of crops to mimic wild relative variants.

AVAILABLE GERMPLASM RESOURCES AND INFORMATION ON *BRASSICA* CROPS AND WILD RELATIVES

Wild Brassiceae species can be found around the world in temperate climates [176], and hence may constitute a valuable source of locally-adapted germplasm for use in crop improvement. Although all of the cultivated *Brassica* species are thought to originate from roughly around the Mediterranean region, with wider distributions from Europe to North Africa to the Middle East and West Asia [176], Brassiceae germplasm has also been identified in North America in archeological and ethnobotanical studies [177], with wild mustard relative *Sinapis arvensis* widespread 2000 years ago in North Eastern American states ([178] as cited in [176]). Other Brassiceae weeds and crop species have been identified in weedy habitats in Canada [179], the United States and Mexico [180,181], as well as in Australia [9], and of course Europe and Asia [176]. Germplasm resources and collections of *Brassica* crops and related species, which are either cultivated (domesticated lines) or growing in the natural environment, are mostly (90%) conserved as seeds in cold storage in gene banks [182]. These collections generally comprise elite and domesticated breeding lines, plus a few wild relatives which are being conserved for breeding as well as for research purposes. Overall, conservation methods can basically be categorized into either *in situ* or *ex situ* conservation.

In Situ Conservation of Germplasm Resources

In situ conservation is the primary form of conservation for crop wild relatives, and either takes place in farmers' fields or in natural environments. *In situ* conservation is promoted because landraces can be an essential component of indigenous cultures and show highly specialized local adaptations [183,184]. Growth of plants in the natural environment also allows selection and adaptation to changing environmental conditions and is highly cost effective [182]. Growing interest in the use of wild species in breeding [185,186] has underlined the need to also create national *in situ* inventories to encourage conservation. *In situ* conservation also includes conservation in natural or wilderness areas, national parks and special management areas. Understanding the genetic potential of *Brassica* crops and wild relatives is critical for the establishment of long term breeding programs. Useful agronomic traits which can potentially be introgressed from wild relatives into elite crops include resistance traits [176], salt tolerance [187,188] and cold tolerance [189]. However, to date *ex situ* conservation remains the most common form of germplasm conservation.

Ex Situ Conservation of Germplasm Resources: Genebanks

Ex situ conservation of plant genetic resources started in the mid-twentieth century, as an initiative to prevent the rapid loss of plant biodiversity resulting from the introduction of improved varieties to replace landraces [182,190,191]. Therefore, germplasm (or "gene") banks were established with the intention to preserve genetic material which might be useful in future for cultivation or as material in breeding programs [192]. The major world germplasm collections of *Brassica* today include the Centre for Genetic Resources (CGN, The Netherlands), the Institute for Horticultural Plant Breeding (IVT, The Netherlands), the Horticultural Research Institute (HRI, UK) and the Gene Bank of the Crop Research Institute (UK)[193]. Other genebanks include the United States Department of Agriculture (USDA) (https://www.ars-grin.gov/npgs/) in the United States, the Australian Grains Genebank (https://grdc.com.au/resources-and-publications/groundcover/gc110/australian-genebank), and the Nordic Genetic Resource Centre (NordGen) (https://www.nordgen.org/en/) in Norway. In Spain, the *Brassica* genebank MBG-CSIC (http://www.mbg.csic.es/es/) started its activities in 1985. This gene bank holds a collection of Galician *Brassica* crops belonging to the species *B. oleracea* L., *B. rapa* L. and *B. napus* L., and houses a total of 644 accessions. *B. oleracea* varieties include kales (*B. oleracea* var. *acephala*), cabbages (*B. oleracea* var. *capitata*), and Tronchuda cabbage (*B. oleracea* var. *costata*). *Brassica rapa* includes the turnips, turnip greens, and turnip tops; and *B. napus* appears only in the form known as “nabicol” or leaf rape [194]. The United Kingdom Vegetable Genebank (UKVGB) managed by the University of Warwick conserves approximately 14,000 accessions of crops including *Brassica* types [195]. *Brassica* genetic resources hosted at the UKVGB have been incorporated into several germplasm panels, including (amongst others) the European clubroot differential series (ECD) to help identify races of the clubroot-causing pathogen *Plasmodiophora brassicae* [196], *Brassica S* allele (self-incompatibility) collections [195] which comprise *Brassica* lines with characterized *S*-allele haplotypes, and other collections of *B. oleracea* and *B. napus* fixed diversity sets (homozygous doubled-haploid (DH) or inbred lines)[197]. In total, about 74,000 *Brassica* accessions from various sources have been identified: mostly conserved in Europe (41%) and Asia (41%) as well as a few in the Americas (12%)[38]. *Brassica oleracea* and *B. rapa* species, which comprise the most important *Brassica* vegetables, are represented worldwide by about 20,000 (27%) and 18,000 (25%) accessions, respectively [198]. The European *Brassica* database (Bras-EDB; www.cgn.wageningen-ur.nl/pgr/collections/brasedb/) contains detailed accession data on 32 collections from 22 European countries.

A total of 412 accessions of wild relatives have also been identified in gene banks (mostly European) including 179 species at the University of Madrid in Spain, and 97 species at the Leibniz-Institut für Pflanzengenetik und Kultur Pflanzenforschung (IPK) in Gatersleben, Germany [199]. However, wild species are still under-represented in most *ex situ* collections [198].

Information Databases

Brassica databases are another important resource for crop improvement. These comprise freely available online databases which provide genomic and genetic data for important *Brassica* crops, including genome sequence information, predicted genes and associated annotations, and genetic marker information. In addition, several databases provide cytogenetic and taxonomy data, such as Brassibase (https://brassibase.cos.uni-heidelberg.de/), or species distribution and observation data (usually for specific countries or regions) for *Brassica* crops and wild relatives growing in the natural environment. In Canada for example, an electronic database provides taxonomy and synonymy information for 338 Brassicaceae genera and 3709 species (14,000 taxonomic names) found distributed across Canada: http://www.cbif.gc.ca/eng/species-bank/?id=1370403266204 [1]. The *Brassica* database (BRAD (https://brassicadb.org)) has a specific focus on genome annotations and deep mining of the assembled *Brassica* crop genomes to provide information for breeding and research [200]. Another database, brassica.info, contains links to browsers and downloads for annotated reference genomes of *B. napus*, *B. rapa* and *B. oleracea* as well as *Brassica* linkage maps and molecular marker collections (www.brassica.info/

genome/linkage_maps.html). The *Brassica* genome databases (http://www.plantgdb.org/BrGDB) mainly focus on genome data dissemination via CropStore and the *Brassica* Genome Database (*Brassica*DB). The *Brassica* CropStore was initially developed to collate and disseminate information from crop research communities [201,202] as well as provide data information for *Brassica* phenotypic and genetic maps from different projects [203,204]. CropStore is an integral part of InterStoreDb which provides a platform for the utilization of a set of interlinked databases to assist linking phenotype to QTL regions for a particular trait. Data contained within CropStore can be accessed via a web interface [201,204].

In the era of fast growing technologies such as genome editing, sequencing and biotechnology tools, there is scope to improve the efficient utilization of information and resources provided by gene banks. Future gene banks should also aim to conserve DNA as well as products of genome editing and transgenic approaches, alongside genomic sequence information for plant accessions [194]. If possible, current gene banks should aim to provide genotypic as well as phenotypic information on *Brassica* species and wild relative collections in the form of an online portal or databases. A number of online *Brassica* species databases have been in existence since the era of reduced cost genome sequencing: the incorporation of these online databases with traditional germplasm banks would provide breeders and scientists with considerable resources for efficient crop improvement.

FUTURE OUTLOOK

In this review we describe the progress that has been made to date in the use of interspecific hybridization for *Brassica* crop improvement. But what may be possible in future? Recent technological advances in genome sequencing and editing have the potential to revolutionize the use of genetic diversity present in the wild relatives for *Brassica* crop improvement. Putatively, *Brassica* wild relatives with useful phenotypic diversity can be identified through screening of diverse populations under different environmental conditions, phenotype data then coupled with genome and resequencing data to link phenotypes to genotypes, followed by gene editing to directly install these genetic variants into the major *Brassica* crop species. Although this process may still be more speculative than realistic, the technological basis for this approach already exists today. High-throughput phenotyping platforms are available and under constant improvement for glasshouse and field environments [205–207]. In natural environments, traits have also been successfully linked to genetic loci through sequencing of contrasting species populations in different habitats [208]. Whole genome sequencing and resequencing is becoming increasingly cheap and available, with major strides being made in both improving genomic resources available for the *Brassica* crop genomes [209–212] and in the availability of additional genomic resources for *Brassica* wild relatives [213,214]. As previously mentioned, genetic transformation and genome editing protocols have already been established for many of the *Brassica* crop species [172,174,175]. In future, we expect the true value of interspecific hybridization and the use of wild relatives for crop improvement in the agriculturally significant *Brassica* genus to be realized, with implementation of new technologies supported by gene banks and information resources for breeding and research outcomes.

AUTHOR CONTRIBUTIONS

All authors contributed to conceptualization and writing of the review.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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REFERENCES

1. Warwick SI, Francis A, Al-Shehbaz IA. Brassicaceae: Species checklist and database on CD-Rom. Plant Syst Evol. 2006 Jul;259(2-4):249-58.
2. Al-Shehbaz IA, Beilstein MA, Kellogg EA. Systematics and phylogeny of the Brassicaceae (Cruciferae): An overview. Plant Syst Evol. 2006;259(2-4):89-120.
3. Al-Shehbaz IA. The tribes of Cruciferae (Brassicaceae) in the southeastern United States. J Arnold Arbor. 1984;65(3):343-73.
4. Rakow G. Species origin and economic importance of *Brassica*. In: Pua EC, Douglas CJ, editors. *Brassica* Biotechnology in Agriculture and Forestry. Berlin (Germany): Springer; 2004. p. 3-11.
5. Warwick SI, Al-Shehbaz IA. Brassicaceae: Chromosome number index and database on CD-Rom. Plant Syst Evol. 2006;259:237-48.
6. Nagai K, Tsunetaro S. The number of chromosomes in the cultivated *Brassica*. Jpn J Genet. 1930;5(3-4):151-8.
7. UN. Genome-analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn J Bot. 1935;7:389-452.
8. Warwick SI, Black LD. Molecular systematics of *Brassica* and allied genera (Subtribe Brassicinae, Brassiceae)—chloroplast genome and cytodeme congruence. Theor Appl Genet. 1991;82(1):81-92.

9. Lysak MA, Koch MA, Pecinka A, Schubert I. Chromosome triplication found across the tribe Brassiceae. Genome Res. 2005;15(4):516-25.
10. Inaba R, Nishio T. Phylogenetic analysis of Brassiceae based on the nucleotide sequences of the *S*-locus related gene, *SLR*$_1$. Theor Appl Genet. 2002;105(8):1159-65.
11. Cheng F, Sun R, Hou X, Zheng H, Zhang F, Zhang Y, et al. Subgenome parallel selection is associated with morphotype diversification and convergent crop domestication in *Brassica rapa* and *Brassica oleracea*. Nat Genet. 2016;48:1218-24.
12. Chalhoub B, Denoeud F, Liu S, Parkin IAP, Tang H, Wang X, et al. Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. Science. 2014;345(6199):950-3.
13. Prakash S, Hinata K. Taxonomy, cytogenetics and origin of crop *Brassicas*, a review. Opera Bot. 1980;55:1-57.
14. Sun R. Economic/academic importance of *Brassica rapa*. In: Wang X, Cole C, editors. The *Brassica rapa* genome Compendium of Plant Genomes. Berlin (Germany): Springer; 2015. p. 1-15.
15. Song K, Tang K, Osborn TC, Lu P. Genome variation and evolution of *Brassica* amphidiploids. Acta Hortic. 1996 Apr;(407):35-44.
16. Gupta SK, editor. Biology and Breeding of Crucifers. Boca Raton (US): CRC Press; 2010. p. 394.
17. Mag TK. Canola oil processing in Canada. J Am Oil Chem Soc. 1983 Feb;60:380-4.
18. Eskin NAM, Przybylski R. Rapeseed oil/canola. In: Caballero B, editor. Encyclopedia of Food Sciences and Nutrition. 2nd ed. Oxford (UK): Academic Press; 2003. p. 4911-6.
19. Kumar A, Banga SS, Meena PD, Kumar PR, editors. *Brassica* oilseeds: breeding and management. Oxfordshire (UK): CABI Publishing; 2015.
20. Friedt W, Tu J, Fu T. Academic and economic importance of *Brassica napus* rapeseed. In: Liu S, Snowdon R, Chalhoub B, editors. The *Brassica napus* genome. 1st ed. Cham (Switzerland): Springer; 2018. p. 1-20. (Compendium of Plant Genomes).
21. Rahman H. Review: Breeding spring canola (*Brassica napus* L.) by the use of exotic germplasm. Can J Plant Sci. 2013 May;93(3):363-73.
22. Banga SS, Banga S. Genetic diversity and germplasm patterns in *Brassica juncea*. In: Rajpal V, Rao S, Raina S, editors. Gene Pool Diversity and Crop Improvement Sustainable Development and Biodiversity. Cham (Switzerland): Springer; 2016. p. 163-86.
23. Yang J, Liu D, Wang X, Ji C, Cheng F, Liu B, et al. The genome sequence of allopolyploid *Brassica juncea* and analysis of differential homoeolog gene expression influencing selection. Nat Genet. 2016;48:1225-32.
24. Wang X, Wang H, Wang J, Sun R, Wu J, Liu S, et al. The genome of the mesopolyploid crop species *Brassica rapa*. Nat Genet. 2011;43(10):1035-9.
25. Cai C, Wang X, Liu B, Wu J, Liang J, Cui Y, et al. *Brassica rapa* genome 2.0: a reference upgrade through sequence re-assembly and gene re-annotation. Mol Plant. 2017;10(4):649-51.

26. Kaul S, Koo HL, Jenkins J, Rizzo M, Rooney T, Tallon LJ, et al. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature. 2000;408(6814):796-815.
27. Fahey JW. Brassicas. In: Caballero B, editor. Encyclopedia of Food Sciences and Nutrition. 2nd ed. Oxford (UK): Academic Press; 2003. p. 606-15.
28. Liu SY, Liu YM, Yang XH, Tong CB, Edwards D, Parkin IAP, et al. The *Brassica oleracea* genome reveals the asymmetrical evolution of polyploid genomes. Nat Commun. 2014;5:3930.
29. Parkin IAP, Koh C, Tang H, Robinson SJ, Kagale S, Clarke WE, et al. Transcriptome and methylome profiling reveals relics of genome dominance in the mesopolyploid *Brassica oleracea*. Genome Biol. 2014 Jun 10;15(6):R77.
30. Alemayehu N, Becker H. Genotypic diversity and patterns of variation in a germplasm material of Ethiopian mustard (*Brassica carinata* A. Braun). Genet Resour Crop Evol. 2002;49(6):573-82.
31. Cardone M, Prati MV, Rocco V, Seggiani M, Senatore A, Vitolo S. *Brassica carinata* as an alternative oil crop for the production of biodiesel in Italy: engine performance and regulated and unregulated exhaust emissions. Environ Sci Technol. 2002;36(21):4656-62.
32. Zhu JS, Struss D, Röbbelen G. Studies on resistance to *Phoma lingam* in *Brassica napus—Brassica nigra* addition lines. Plant Breed. 1993;111(3):192-7.
33. Wang W, Guan R, Liu X, Zhang H, Song B, Xu Q, et al. Chromosome level comparative analysis of *Brassica* genomes. Plant Mol Biol. 2019;99(3):237-49.
34. Harberd DJ. A contribution to the cyto-taxonomy of *Brassica* (Cruciferae) and its allies. Bot J Linn Soc. 1972;65(1):1-23.
35. Harberd DJ. Cytotaxonomic studies of *Brassica* and related genera. In: Vaughan JG, MacLeod AJ, Jones BMG, editors. The Biology and Chemistry of the Cruciferae. London (UK): Academic Press; 1976. p. 47-68.
36. Kirti PB, Prakash S, Baht SR, Chopra VL. Protoplast fusion and *Brassica* improvement. Indian J Biotechnol. 2003;2:76-84.
37. Prakash SR, Bhat SR, Quiros CF, Kirti PB, Chopra VL. *Brassica* and its close allies: cytogenetics and evolution. Plant Breed Rev. 2009 Nov;31:21-187.
38. Gómez-Campo C, editor. Biology of *Brassica* coenospecies. Amsterdam (The Netherlands): Elsevier; 1999.
39. Warwick SI, Black LD. Phylogenetic implications of chloroplast DNA restriction site variation in subtribes Raphaninae and Cakilinae (Brassicaceae, tribe Brassiceae). Can J Bot. 1997 Jun;75(6):960-73.
40. Harrison BJ, Darby LA. Unilateral hybridization. Nature. 1955;176(4490):982.
41. Warwick SI, Francis A, Gugel RK. Guide to wild germplasm of *Brassica* and allied crops (tribe Brassiceae, Brassicaceae). 3rd ed. Available from: http://www.brassica.info/info/publications/guidewild/Guide_ed.3_Introd_16July2009.pdf. Accessed 2019 Jul 18.
42. Chrungu B, Verma N, Mohanty A, Pradhan A, Shivanna KR. Production and characterization of interspecific hybrids between *Brassica maurorum* and crop Brassicas. Theor Appl Genet. 1999;98(3-4):608-13.

43. Kirti PB, Narasimhulu SB, Prakash S, Chopra VL. Somatic hybridization between *Brassica juncea* and *Moricandia arvensis* by protoplast fusion. Plant Cell Rep. 1992 Jun;11(5-6):318-21.
44. Siemens J. Interspecific hybridisation between wild relatives and *Brassica napus* to introduce new resistance traits into the oilseed rape gene pool. Czech J Genet Plant Breed. 2002 Aug;38:155-7.
45. Kirti PB, Narasimhulu SB, Prakash S, Chopra VL. Production and characterization of intergeneric somatic hybrids of *Trachystoma ballii* and *Brassica juncea*. Plant Cell Rep. 1992 Mar;11(2):90-2.
46. Lelivelt CLC, Krens FA. Transfer of resistance to the beet cyst nematode (*Heterodera schachtii* Schm.) into the *Brassica napus* L. gene pool through intergeneric somatic hybridization with *Raphanus sativus* L. Theor Appl Genet. 1992 Apr;83(6-7):887-94.
47. Lelivelt CLCC, Leunissen EHMM, Frederiks HJ, Helsper JPFGFG, Krens FA. Transfer of resistance to the beet cyst nematode (*Heterodera schachtii* Schm.) from *Sinapis alba* L. (white mustard) to the *Brassica napus* L. gene pool by means of sexual and somatic hybridization. Theor Appl Genet. 1993 Feb;85(6-7):688-96.
48. Snowdon RJ, Winter H, Diestel A, Sacristán MD. Development and characterisation of *Brassica napus-Sinapis arvensis* addition lines exhibiting resistance to *Leptosphaeria maculans*. Theor Appl Genet. 2000;101(7):1008-14.
49. Ryschka U, Schumann G, Klocke E, Scholze P, Neumann M. Somatic hybridization in Brassicaceae. Acta Hortic. 1996 Apr;(407):201-8.
50. Pedras MSC, Chumala PB, Suchy M. Phytoalexins from *Thlaspi arvense*, a wild crucifer resistant to virulent *Leptosphaeria maculans*: structures, syntheses and antifungal activity. Phytochemistry. 2003 Nov;64(5):949-56.
51. Liu J-H, Dixelius C, Eriksson I, Glimelius K. *Brassica napus* × *B. tournefortii*, a somatic hybrid containing traits of agronomic importance for rapeseed breeding. Plant Sci. 1995;109(1):75-86.
52. Greenhalgh JR, Mitchell ND. The involvement of flavour volatiles in the resistance to downy mildew of wild and cultivated forms of *Brassica oleracea*. New Phytol. 1976;77:391-8.
53. Piao Z, Ramchiary N, Lim YP. Genetics of clubroot resistance in *Brassica* species. J Plant Growth Regul. 2009 Sep;28(3):252-64.
54. Belimov AA, Safronova VI, Demchinskaya SV, Dzyuba OO. Intraspecific variability of cadmium tolerance in hydroponically grown Indian mustard (*Brassica juncea* (L.) Czern.) seedlings. Acta Physiol Plant. 2007 Sep;29(5):473-8.
55. Rawsthorne S, Hylton CM, Smith AM, Woolhouse HW. Photorespiratory metabolism and immunogold localization of photorespiratory enzymes in leaves of C3 and C3–C4 intermediate species of *Moricandia*. Planta. 1988 Mar;173(3):298-308.

56. Hinata K, Konno N. Studies on a male sterile strain having the *Brassica campestris* nucleus and the *Diplotaxis muralis* cytoplasm. I On the breeding procedure and some characteristics of the male sterile strain. Jpn J Breed. 1979 Dec;29(4):305-11.
57. Pellan-Delourme R, Renard M. Identification of maintainer genes in *Brassica napus* L. for the male-sterility-inducing cytoplasm of *Diplotaxis muralis* L. Plant Breed. 1987 Oct;99(2):89-97.
58. Wang YP, Sonntag K, Rudloff E. Development of rapeseed with high erucic acid content by asymmetric somatic hybridization between *Brassica napus* and *Crambe abyssinica*. Theor Appl Genet. 2003;106(7):1147-55.
59. Horovitz A, Galil J. Gynodioecism in East Mediterranean *Hirschfeldia incana*. Cruciferae. Botanical Gazette. 1972;133:127-31.
60. Rao GU, Batra-Sarup V, Prakash S, Shivanna KR. Development of a new cytoplasmic male-sterility system in *Brassica juncea* through wide hybridization. Plant Breed. 1994 Mar;112(2):171-4.
61. FitzJohn RG, Armstrong TT, Newstrom-Lloyd LE, Wilton AD, Cochrane M. Hybridisation within *Brassica* and allied genera: evaluation of potential for transgene escape. Euphytica. 2007;158(1-2):209-30.
62. Prakash S, Bhat SR, Quiros CF, Kirti PB, Chopra VL. *Brassica* and its close allies: cytogenetics and evolution. In: Janick J, editor. Plant Breed Rev. Hoboken (US): John Wiley & Sons, Inc.; 2009. p. 21-187.
63. Kakizaki Y. A preliminary report of crossing experiments with cruciferous plants, with special reference to sexual compatibility and matroclinous hybrids. Jpn J Genet. 1925;3:49-82.
64. Bing DJ, Downey RK, Rakow GFW. Hybridizations among *Brassica napus*, *B. rapa* and *B. juncea* and their two weedy relatives *B. nigra* and *Sinapis arvensis* under open pollination conditions in the field. Plant Breed. 1996;115(6):470-3.
65. Rieger MA, Potter TD, Preston C, Powles SB. Hybridisation between *Brassica napus* L. and *Raphanus raphanistrum* L. under agronomic field conditions. Theor Appl Genet. 2001 Sep;103(4):555-60.
66. Chevre AM, Eber F, This P, Barret P, Tanguy X, Brun H, et al. Characterization of *Brassica nigra* chromosomes and of blackleg resistance in *B. napus—B. nigra* addition lines. Plant Breed. 1996;115(2):113-8.
67. Chèvre AM, Barret P, Eber F, Dupuy P, Brun H, Tanguy X, et al. Selection of stable *Brassica napus-B. juncea* recombinant lines resistant to blackleg (*Leptosphaeria maculans*). 1. Identification of molecular markers, chromosomal and genomic origin of the introgression. Theor Appl Genet. 1997;95(7):1104-11.
68. Navabi ZK, Parkin IAP, Pires JC, Xiong Z, Thiagarajah MR, Good AG, et al. Introgression of B-genome chromosomes in a doubled haploid population of *Brassica napus* × *B. carinata*. Genome. 2010;53(8):619-29.
69. Tonguç M, Griffiths PD. Transfer of powdery mildew resistance from *Brassica carinata* to *Brassica oleracea* through embryo rescue. Plant Breed. 2004 Dec;123(6):587-9.

70. Sharma BB, Kalia P, Singh D, Sharma TR. Introgression of black rot resistance from *Brassica carinata* to cauliflower (*Brassica oleracea botrytis* group) through embryo rescue. Front Plant Sci. 2017 Jul;8:1255.
71. Mohapatra D, Bajaj YPS. Interspecific hybridization in *Brassica juncea—Brassica hirta* using embryo rescue. Euphytica. 1987;36(1):321-6.
72. Heneen WK, Geleta M, Brismar K, Xiong ZY, Pires JC, Hasterok R, et al. Seed colour loci, homoeology and linkage groups of the C genome chromosomes revealed in *Brassica rapa-B. oleracea* monosomic alien addition lines. Ann Bot. 2012;109(7):1227-42.
73. Rahman MH. Production of yellow-seeded *Brassica napus* through interspecific crosses. Plant Breed. 2001;120:463-72.
74. Rashid A, Rakow G, Downey RK. Development of yellow seeded *Brassica napus* through interspecific crosses. Plant Breed. 1994;112(2):127-34.
75. Rakow G, Raney JP, Relf-Eckstein J. Agronomic performance and seed quality of a new source of yellow seeded *Brassica napus*. Proceedings of the 12th International Rapeseed Congress; 1999 Sep 26-29; Canberra, Australia.
76. Ogura H. Studies on the new male-sterility in Japanese radish, with special reference to the utilization of this sterility towards the practical raising of hybrid seeds. Mem Fac Agric Kagoshima Univ. 1968;6(2):39-78.
77. Yamagishi H, Bhat SR. Cytoplasmic male sterility in Brassicaceae crops. Breed Sci. 2014 May;64(1):38-47.
78. Atri C, Kaur B, Sharma S, Gandhi N, Verma H, Goyal A, et al. Substituting nuclear genome of *Brassica juncea* (L.) Czern & Coss. in cytoplasmic background of *Brassica fruticulosa* results in cytoplasmic male sterility. Euphytica. 2016 May;209(1):31-40.
79. Liu J, Xiang RY, Wang WM, Mei DS, Li YC, Mason AS, et al. Cytological and molecular analysis of *Nsa* CMS in *Brassica napus* L. Euphytica. 2015;206(2):279-86.
80. Ringdahl EA, McVetty PBE, Sernyk JL. Intergeneric hybridization of *Diplotaxis spp.* with *Brassica napus*: a source of new CMS systems? Can J Plant Sci. 2010;67(1):239-43.
81. Kirti P, Banga S, Prakash S, Chopra V. Transfer of *Ogu* cytoplasmic male sterility to *Brassica juncea* and improvement of the male sterile line through somatic cell fusion. Theor Appl Genet. 1995;91(3):517-21.
82. Lian Y-J, Lin G-Z, Zhao X-M, Lim H-T. Production and genetic characterization of somatic hybrids between leaf mustard (*Brassica juncea*) and broccoli (*Brassica oleracea*). Vitr Cell Dev Biol Plant. 2011 May;47(2):289-96.
83. Chen L-P, Zhang M-F, Li C-S, Hirata Y. Production of interspecific somatic hybrids between tuber mustard (*Brassica juncea*) and red cabbage (*Brassica oleracea*). Plant Cell Tissue Organ Cult. 2005 Mar;80(3):305-11.
84. Prakash S, Kirti PB, Bhat SR, Gaikwad K, Kumar VD, Chopra VL. A *Moricandia arvensis*-based cytoplasmic male sterility and fertility restoration system in *Brassica juncea*. Theor Appl Genet. 1998 Aug;97(3):488-92.

85. Chen H-F, Wang H, Li Z-Y. Production and genetic analysis of partial hybrids in intertribal crosses between *Brassica* species (*B. rapa*, *B. napus*) and *Capsella bursa-pastoris*. Plant Cell Rep. 2007 Sep;26(10):1791-800.
86. Hu Q, Hansen LN, Laursen J, Dixelius C, Andersen SB. Intergeneric hybrids between *Brassica napus* and *Orychophragmus violaceus* containing traits of agronomic importance for oilseed rape breeding. Theor Appl Genet. 2002 Nov;105(6-7):834-40.
87. Ma N, Li Z-Y, Cartegena JA, Fukui K. GISH and AFLP analyses of novel *Brassica napus* lines derived from one hybrid between *B. napus* and *Orychophragmus violaceus*. Plant Cell Rep. 2006;25:1089-93.
88. Holaday AS, Chollet R. Photosynthetic/photorespiratory characteristics of C3–C4 intermediate species. Photosynth Res. 1984;5(4):307-23.
89. O'Neill CM, Murata T, Morgan CL, Mathias RJ. Expression of the C3–C4 intermediate character in somatic hybrids between *Brassica napus* and the C3–C4 species *Moricandia arvensis*. Theor Appl Genet. 1996;93(8):1234-41.
90. Muangprom A, Mauriera I, Osborn TC. Transfer of a dwarf gene from *Brassica rapa* to oilseed *B. napus*, effects on agronomic traits, and development of a "perfect" marker for selection. Mol Breed. 2006;17(2):101-10.
91. Prakash S, Chopra VL. Introgression of resistance to shattering in *Brassica napus* from *Brassica juncea* through non-homologous recombination. Plant Breed. 1988 Sep;101(2):167-8.
92. Li A, Jiang J, Zhang Y, Snowdon RJ, Liang G, Wang Y. Molecular and cytological characterization of introgression lines in yellow seed derived from somatic hybrids between *Brassica napus* and *Sinapis alba*. Mol Breed. 2012 Jan;29(1):209-19.
93. Tonosaki K, Sekine D, Ohnishi T, Ono A, Furuumi H, Kurata N, et al. Overcoming the species hybridization barrier by ploidy manipulation in the genus *Oryza*. Plant J. 2018 Feb;93(3):534-44.
94. Chen S, Nelson MN, Chèvre AM, Jenczewski E, Li Z, Mason AS, et al. Trigenomic bridges for *Brassica* improvement. CRC Crit Rev Plant Sci. 2011;30(6):524-47.
95. Seyis F, Snowdon RJ, Luhs W, Friedt W. Molecular characterization of novel resynthesized rapeseed (*Brassica napus*) lines and analysis of their genetic diversity in comparison with spring rapeseed cultivars. Plant Breed. 2003;122(6):473-8.
96. Abel S, Möllers C, Becker H. Development of synthetic *Brassica napus* lines for the analysis of "fixed heterosis" in allopolyploid plants. Euphytica. 2005;146(1-2):157-63.
97. Zhang B, Lu CM, Kakihara F, Kato M. Effect of genome composition and cytoplasm on petal colour in resynthesized amphidiploids and sesquidiploids derived from crosses between *Brassica rapa* and *Brassica oleracea*. Plant Breed. 2002 Aug;121(4):297-300.
98. Zhang GQ, Tang GX, Song WJ, Zhou WJ. Resynthesizing *Brassica napus* from interspecific hybridization between *Brassica rapa* and *B. oleracea* through ovary culture. Euphytica. 2004 Jan;140(3):181-7.

99. Zou J, Fu D, Gong H, Qian W, Xia W, Pires JC, et al. De novo genetic variation associated with retrotransposon activation, genomic rearrangements and trait variation in a recombinant inbred line population of *Brassica napus* derived from interspecific hybridization with *Brassica rapa*. Plant J. 2011 Oct;68(2):212-24.
100. Girke A, Schierholt A, Becker HC. Extending the rapeseed gene pool with resynthesized *Brassica napus* II: Heterosis. Theor Appl Genet. 2012;124(6):1017-26.
101. Eickermann M, Ulber B, Vidal S. Resynthesized lines and cultivars of *Brassica napus* L. provide sources of resistance to the cabbage stem weevil (*Ceutorhynchus pallidactylus* (Mrsh.)). Bull Entomol Res. 2011;101(3):287-94.
102. Prakash S. Artificial synthesis of *Brassica juncea* Coss. Genetica. 1973;44:249-63.
103. Srivastava A, Mukhopadhyay A, Arumugam N, Gupta V, Verma JK, Pental D, et al. Resynthesis of *Brassica juncea* through interspecific crosses between *B. rapa* and *B. nigra*. Plant Breed. 2004;123(2):204-6.
104. Yadav P, Bhat S, Prakash S, Mishra L, Chopra V. Resynthesized *Brassica juncea* lines with novel organellar genome constitution obtained through protoplast fusion. J Genet. 2009;88(1):109-12.
105. Bansal P, Kaur P, Banga S, Banga S. Augmenting genetic diversity in *Brassica juncea* through its resynthesis using purposely selected diploid progenitors. Int J Plant Sci. 2009;3(1):41-5.
106. Jourdan P, Salazar E. *Brassica carinata* resynthesized by protoplast fusion. Theor Appl Genet. 1993;86(5):567-72.
107. Narasimhulu SB, Kirti PB, Prakash S, Chopra VL. Resynthesis of *Brassica carinata* by protoplast fusion and recovery of a novel cytoplasmic hybrid. Plant Cell Rep. 1992;11(8):428-32.
108. Song KM, Lu P, Tang KL, Osborn TC. Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. Proc Natl Acad Sci U S A. 1995;92(17):7719-23.
109. Schranz ME, Osborn TC. Novel flowering time variation in the resynthesized polyploid *Brassica napus*. J Hered. 2000;91(3):242-6.
110. Gaebelein R, Mason AS. Allohexaploids in the Genus *Brassica*. CRC Crit Rev Plant Sci. 2018 Sep 3;37(5):422-37.
111. Malek MA, Rahman L, Das ML, Hassan L, Rafii MY, Ismail MR. Development of hexaploid *Brassica* (AABBCC) from hybrids (ABC) of *Brassica carinata* (BBCC) × *B. rapa* (AA). Aust J Crop Sci. 2013;7(9):1375-82.
112. Meng J, Shi S, Gan L, Li Z, Qu X. The production of yellow-seeded *Brassica napus* (AACC) through crossing interspecific hybrids of *B. campestris* (AA) and *B. carinata* (BBCC) with *B. napus*. Euphytica. 1998;103(3):329-33.
113. Sjödin C, Glimelius K. Transfer of resistance against *Phoma lingam* to *Brassica napus* by asymmetric somatic hybridization combined with toxin selection. Theor Appl Genet. 1989;78(4):513-20.
114. Li M, Qian W, Meng J, Li Z. Construction of novel *Brassica napus* genotypes through chromosomal substitution and elimination using interploid species hybridization. Chromosom Res. 2004;12(5):417-26.

115. Karpechenko GD. Polyploid hybrids of *Raphanus sativus* L. X *Brassica oleracea* L—on the problem of experimental species formation. Z Indukt Abstamm Vererbungsl. 1928;48(1):1-85.
116. Lange W, Toxopeus H, Lubberts JH, Dolstra O, Harrewijn JL. The development of Raparadish (× *Brassicoraphanus*, $2n = 38$), a new crop in agriculture. Euphytica. 1989;40(1-2):1-14.
117. Lelivelt CLC, Lange W, Dolstra O. Intergeneric crosses for the transfer of resistance to the beet cyst nematode from *Raphanus sativus* to *Brassica napus*. Euphytica. 1993;68(1-2):111-20.
118. King J, Armstead I, Harper J, Ramsey L, Snape J, Waugh R, et al. Exploitation of interspecific diversity for monocot crop improvement. Heredity. 2013 May;110(5):475-83.
119. Ahuja I, Rohloff J, Bones AM. Defence Mechanisms of Brassicaceae: Implications for Plant-Insect Interactions and Potential for Integrated Pest Management. In: Sustainable Agriculture. Volume 2. Dordrecht (The Netherlands): Springer; 2011. p. 623-70.
120. Gunasekera CP, French RJ, Martin LD, Siddique KHM. Comparison of the responses of two Indian mustard (*Brassica juncea* L.) genotypes to post-flowering soil water deficit with the response of canola (*B. napus* L.) cv. Monty. Crop Pasture Sci. 2009 Apr;60(3):251.
121. van Tuyl JM. Interspecific hybridization of flower bulbs: a review. Acta Hortic. 1997 Dec;(430):465-76.
122. DeVerna JW, Myers JR, Collins GB. Bypassing prefertilization barriers to hybridization in *Nicotiana* using *in vitro* pollination and fertilization. Theor Appl Genet. 1987 Sep;73(5):665-71.
123. De Jeu MJ, Jacobsen E. Early postfertilization ovule culture in *Alstroemeria* L. and barriers to interspecific hybridization. Euphytica. 1995 Jan;86(1):15-23.
124. Haig D, Westoby M. Genomic imprinting in endosperm: its effect on seed development in crosses between species, and between different ploidies of the same species, and its implications for the evolution of apomixis. Philos Trans Biol Sci. 1991;333(1266):1-13.
125. Choudhary BR, Joshi P, Ramarao S. Interspecific hybridization between *Brassica carinata* and *Brassica rapa*. Plant Breed. 2000;119:417-20.
126. Kaneko Y, Bang SSW. Interspecific and intergeneric hybridization and chromosomal engineering of Brassicaceae crops. Breed Sci. 2014;64(1):14-22.
127. Scheffler J, Dale P. Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species. Transgenic Res. 1994;3:263-78.
128. Choudhary BR, Joshi P. Genetic diversity in advanced derivatives of *Brassica* interspecific hybrids. Euphytica. 2001;121(1):1-7.
129. van Tuyl JM, de Jeu MJ. Methods for overcoming interspecific crossing barriers. In: Sawhney VK, Shivanna KR, editors. Pollen Biotechnology for Crop Production and Improvement. Cambridge (UK): Cambridge University Press; 1997. p. 273-92.
130. Bhat S, Sarla N. Identification and overcoming barriers between *Brassica rapa* L. em. Metzg. and *B. nigra* (L.) Koch crosses for the resynthesis of *B. juncea* (L.) Czern. Genet Resour Crop Evol. 2004;51(5):455-69.

131. Sosnowska K, Cegielska-Taras T. Application of *in vitro* pollination of opened ovaries to obtain *Brassica oleracea* L. × *B. rapa* L. hybrids. Vitr Cell Dev Biol Plant. 2014;50(2):257-62.
132. Sekine D, Ohnishi T, Furuumi H, Ono A, Yamada T, Kurata N, et al. Dissection of two major components of the post-zygotic hybridization barrier in rice endosperm. Plant J. 2013 Dec;76(5):792-9.
133. Ayotte R, Harney PM, Machado VS. The transfer of triazine resistance from *Brassica napus* L. to *Brassica oleracea* L . 1. Production of F1 hybrids through embryo rescue. Euphytica. 1987;36(2):615-24.
134. Momotaz A, Kato M, Kakihara F. Production of intergeneric hybrids between *Brassica* and *Sinapis* species by means of embryo rescue techniques. Euphytica. 1998;103(1):123-30.
135. Nishi S, Kawata J, Toda M. On the breeding of interspecific hybrids between two genomes, “c” and “a” of *Brassica* through the application of embryo culture techniques. Jpn J Breed. 1959;8(4):215-22.
136. Bajaj YPSS, Mahajan SK, Labana KS. Interspecific hybridization of *Brassica napus* and *B. juncea* through ovary, ovule and embryo culture. Euphytica. 1986;35(1):103-9.
137. Wen J, Tu JX, Li ZY, Fu TD, Ma CZ, Shen JX. Improving ovary and embryo culture techniques for efficient resynthesis of *Brassica napus* from reciprocal crosses between yellow-seeded diploids *B. rapa* and *B. oleracea*. Euphytica. 2008;162(1):81-9.
138. Yao X, Ge X, Li Z. Different fertility and meiotic regularity in allohexaploids derived from trigenomic hybrids between three cultivated *Brassica* allotetraploids and *B. maurorum*. Plant Cell Rep. 2012;31(4):781-8.
139. Mithila J, Hall JC. Transfer of auxinic herbicide resistance from *Brassica kaber* to *Brassica juncea* and *Brassica rapa* through embryo rescue. Vitr Cell Dev Biol Plant. 2013 Aug;49(4):461-7.
140. Chamola R, Balyan H, Bhat S. Transfer of cytoplasmic male sterility from alloplasmic *Brassica juncea* and *B. napus* to cauliflower (*B. oleracea* var. *botrytis*) through interspecific hybridization. Indian J Genet. 2013;73(2):203-10.
141. Rygulla W, Snowdon RJ, Eynck C, Koopmann B, von Tiedemann A, Lühs W, et al. Broadening the genetic basis of *Verticillium longisporum* resistance in *Brassica napus* by interspecific hybridization. Phytopathology. 2007 Nov;97(11):1391-6.
142. Christey MC. *Brassica* Protoplast Culture and Somatic Hybridization. In: Biotechnology in Agriculture and Forestry. Berlin (Germany): Springer; 2004. p. 119-48.
143. El-Esawi MA. Somatic hybridization and microspore culture in *Brassica* improvement. In: Anis N, Ahmad N, editors. Plant Tissue Culture: Propagation, Conservation and Crop Improvement. Singapore (Singapore): Springer; 2016. p. 599-609.
144. Glimelius K, Fahlesson J, Landgren M, Sjödin C, Sundberg E. Gene transfer via somatic hybridization in plants. Trends Biotechnol. 1991;9(1):24-30.

145. Ren JP, Dickson MH, Earle ED. Improved resistance to bacterial soft rot by protoplast fusion between *Brassica rapa* and *B. oleracea*. Theor Appl Genet. 2000 Mar;100(5):810-9.
146. Bhalla PL, Singh MB. *Agrobacterium*-mediated transformation of *Brassica napus* and *Brassica oleracea*. Nat Protoc. 2008 Feb;3(2):181-9.
147. Cardoza V, Stewart CN. Canola (*Brassica napus* L.). In: Agrobacterium Protocols. New Jersey (US): Humana Press; 2006. p. 257-66.
148. Taylor DC, Falk KC, Palmer CD, Hammerlindl J, Babic V, Mietkiewska E, et al. *Brassica carinata*—a new molecular farming platform for delivering bio-industrial oil feedstocks: case studies of genetic modifications to improve very long-chain fatty acid and oil content in seeds. Biofuels Bioprod Biorefin. 2010 Sep;4(5):538-61.
149. Cardi T, D'Agostino N, Tripodi P. Genetic transformation and genomic resources for next-generation precise genome engineering in vegetable crops. Front Plant Sci. 2017 Feb;8:241.
150. Moloney MM, Walker JM, Sharma KK. High efficiency transformation of *Brassica napus* using *Agrobacterium* vectors. Plant Cell Rep. 1989;8(4):238-42.
151. De Block M, De Brouwer D, Tenning P. Transformation of *Brassica napus* and *Brassica oleracea* using *Agrobacterium tumefaciens* and the expression of the *bar* and *neo* genes in the transgenic plants. Plant Physiol. 1989 Oct;91(2):694-701.
152. Barfield D, Pua E. Gene transfer in plants of *Brassica juncea* using *Agrobacterium tumefaciens*-mediated transformation. Plant Cell Rep. 1991;10(6-7):308-14.
153. Gupta V, Lakshmi Sita G, Shaila MS, Jagannathan V. Genetic transformation of *Brassica nigra* by *Agrobacterium* based vector and direct plasmid uptake. Plant Cell Rep. 1993 May;12(7-8):418-21.
154. Radke SE, Turner JC, Faccioti D. Transformation and regeneration of *Brassica rapa* using *Agrobacterium tumefaciens*. Plant Cell Rep. 1992;11:499-505.
155. Zhong R, Zhu F, Liu Y, Li S, Kang L, Luo P. Oilseed rape transformation and the establishment of a bromoxynil-resistant transgenic oilseed rape. Acta Bot Sin. 1997;39(1):22-7.
156. Blackshaw RE, Kanashiro D, Moloney MM, Crosby WL. Growth, yield and quality of canola expressing resistance to acetolactate synthase inhibiting herbicides. Can J Plant Sci. 1994 Oct;74(4):745-51.
157. Qing CM, Fan L, Lei Y, Bouchez D, Tourneur C, Yan L, et al. Transformation of Pakchoi (*Brassica rapa* L. ssp. *chinensis*) by *Agrobacterium* infiltration. Mol Breed. 2000;6(1):67-72.
158. Stoutjesdijk PA, Hurlestone C, Singh SP, Green AG. High-oleic acid Australian *Brassica napus* and *B. juncea* varieties produced by co-suppression of endogenous Delta12-desaturases. Biochem Soc Trans. 2000 Dec;28(6):938-40.
159. Liu JW, DeMichele S, Bergana M, Bobik EJ, Hastilow C, Chuang LT, et al. Characterization of oil exhibiting high γ-linolenic acid from a genetically transformed canola strain. J Am Oil Chem Soc. 2001;78(5):489-93.

160. Liu H, Guo X, Naeem MS, Liu D, Xu L, Zhang W, et al. Transgenic *Brassica napus* L. lines carrying a two gene construct demonstrate enhanced resistance against *Plutella xylostella* and *Sclerotinia sclerotiorum*. Plant Cell Tissue Organ Cult. 2011 Jul;106(1):143-51.
161. Wang J, Chen Z, Du J, Sun Y, Liang A. Novel insect resistance in *Brassica napus* developed by transformation of chitinase and scorpion toxin genes. Plant Cell Rep. 2005 Nov;24(9):549-55.
162. Houmiel KL, Slater S, Broyles D, Casagrande L, Colburn S, Gonzalez K, et al. Poly(β-hydroxybutyrate) production in oilseed leukoplasts of *Brassica napus*. Planta. 1999 Oct;209(4):547-50.
163. Chaudhary S, Parmenter DL, Moloney MM. Transgenic *Brassica carinata* as a vehicle for the production of recombinant proteins in seeds. Plant Cell Rep. 1998 Jan;17(3):195-200.
164. Jagannath A, Arumugam N, Gupta V, Pradhan A, Burma PK, Pental D. Development of transgenic barstar lines and identification of a male sterile (barnase)/restorer (barstar) combination for heterosis breeding in Indian oilseed. Curr Sci. 2002;82(1):46-52.
165. Haurwitz RE, Jinek M, Wiedenheft B, Zhou K, Doudna JA, Snijders AP, et al. Sequence- and structure-Specific RNA processing by a CRISPR endonuclease. Science. 2010 Sep;329(5997):1355-8.
166. Tsai SQ, Joung JK. Defining and improving the genome-wide specificities of CRISPR–Cas9 nucleases. Nat Rev Genet. 2016 May;17(5):300-12.
167. Liu H, Ding Y, Zhou Y, Jin W, Xie K, Chen L-L. CRISPR-P 2.0: An Improved CRISPR-Cas9 Tool for Genome Editing in Plants. Mol Plant. 2017;10(3):530-2.
168. Okuzaki A, Ogawa T, Koizuka C, Kaneko K, Inaba M, Imamura J, et al. CRISPR/Cas9-mediated genome editing of the fatty acid desaturase 2 gene in *Brassica napus*. Plant Physiol Biochem. 2018;131(April):63-9.
169. Feng Z, Mao Y, Xu N, Zhang B, Wei P, Yang D-L, et al. Multigeneration analysis reveals the inheritance, specificity, and patterns of CRISPR/Cas-induced gene modifications in *Arabidopsis*. Proc Natl Acad Sci U S A. 2014;111(12):4632-7.
170. Brooks C, Nekrasov V, Lippman Z, Van Eck J. Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. Plant Physiol. 2014;166:1292-7.
171. Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, et al. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol. 2014 Sep;32(9):947-51.
172. Braatz J, Harloff H-J, Mascher M, Stein N, Himmelbach A, Jung C. CRISPR-Cas9 targeted mutagenesis leads to simultaneous modification of different homoeologous gene copies in polyploid oilseed rape (*Brassica napus*). Plant Physiol. 2017 Jun;174(2):935-42.
173. Yang H, Wu J-J, Tang T, Liu K-D, Dai C. CRISPR/Cas9-mediated genome editing efficiently creates specific mutations at multiple loci using one sgRNA in *Brassica napus*. Sci Rep. 2017 Dec;7(1):7489.

174. Lawrenson T, Shorinola O, Stacey N, Li C, Østergaard L, Patron N, et al. Induction of targeted, heritable mutations in barley and *Brassica oleracea* using RNA-guided Cas9 nuclease. Genome Biol. 2015 Dec;16(1):258.
175. Kirchner M, Schneider S. CRISPR-Cas: from the bacterial adaptive immune system to a versatile tool for genome engineering. Angew Chem Int Ed Engl. 2015 Nov;54(46):13508-14.
176. Warwick SI, Beckie HJ, Thomas AG, McDonald T. The biology of Canadian weeds. 8. *Sinapis arvensis*. L. (updated). Can J Plant Sci. 2000;80(4):939-61.
177. Arnason T, Hebda RJ, Johns T. Use of plants for food and medicine by Native Peoples of eastern Canada. Can J Bot. 1981;59(11):2189-325.
178. Jacobson HA, Petersen JB, Putnam DE. Evidence of pre-Columbian *Brassica* in the northeastern United States. Rhodora. 1988;90:355-62.
179. Gulden RH, Warwick SI, Thomas AG. The biology of Canadian weeds. Can J Plant Sci. 2008;71(3):885-6.
180. Al-Shehbaz IA. The genera of Brassiceae (Cruciferae; Brassicaceae) in the southeastern United States. J Arnold Arbor. 1985;66(3):279-351.
181. Lemke DE, Worthington RD. *Brassica* and *Rapistrum* (Brassicaceae) in Texas. Southwest Nat. 1991;36(2):194-7.
182. Gepts P. Plant genetic resources conservation and utilization: The accomplishments and future of a societal insurance policy. Crop Sci. 2006;46(5):2278-92.
183. Ceccarelli S. Landraces: importance and use in breeding and environmentally friendly agronomic systems. In: Maxted N, editor. Agrobiodiversity Conserv Secur Divers Crop Wild Relatives and Landraces. Oxfordshire (UK): CABI Publishing; 2012. p. 103-17.
184. Villa TCC, Maxted N, Scholten M, Ford-Lloyd B. Defining and identifying crop landraces. Plant Genet Resour. 2005;3(3):373-84.
185. Hajjar R, Hodgkin T. The use of wild relatives in crop improvement: A survey of developments over the last 20 years. Euphytica. 2007;156(1-2):1-13.
186. McCouch S, Genomics CWR, Baute GJ, Bradeen J, Bramel P, Bretting PK, et al. Agriculture: Feeding the future. Nature. 2013;499(7456):23-4.
187. Boyd RS, Barbour MG. Relative salt tolerance of *Cakile edentula* (Brassicaceae) from lacustrine and marine beaches. Am J Bot. 1986;73(2):236-41.
188. Popova OV, Yang O, Dietz K, Golldack D. Differential transcript regulation in *Arabidopsis thaliana* and the halotolerant *Lobularia maritima* indicates genes with potential function in plant salt adaptation. Gene. 2008;423:142-8.
189. Laroche A, Geng X-M, Singh J. Differentiation of freezing tolerance and vernalization responses in Cruciferae exposed to a low temperature. Plant Cell Environ. 1992;15(4):439-45.
190. Van De Wouw M, Kik C, Van Hintum T, Van Treuren R, Visser B. Genetic erosion in crops: Concept, research results and challenges. Plant Genet Resour Charact Util. 2010;8(1):1-15.
191. Khoury CK, Bjorkman AD, Dempewolf H, Ramirez-Villegas J, Guarino L, Jarvis A, et al. Increasing homogeneity in global food supplies and the implications for food security. Proc Natl Acad Sci U S A. 2014;111(11):4001-6.

192. Tanksley SD, McCouch SR. Seed banks and molecular maps: Unlocking genetic potential from the wild. Science. 1997;277(5329):1063-6.
193. van der Meer QP, Toxopeus H, Crisp P. The collection of land-races of cruciferous crops in EC countries. Wageningen (The Netherlands): Institute for Horticultural Plant Breeding, Wageningen University & Research; 1984. IVT (Rapport/Instituut voor de Veredeling van Tuinbouwgewassen no. 198)-210.
194. Díez MJ, De la Rosa L, Martín I, Guasch L, Cartea ME, Mallor C, et al. Plant genebanks: present situation and proposals for their improvement. The case of the Spanish network. Front Plant Sci. 2018;9(December):1-13.
195. Davies LR, Allender CJ. Who is sowing our seeds? A systematic review of the use of plant genetic resources in research. Genet Resour Crop Evol. 2017;64(8):1999-2008.
196. Buczacki ST, Toxopeus H, Mattusch P, Johnston TD, Dixon GR, Hobolth LA. Study of physiologic specialization in *Plasmodiophora brassicae*: Proposals for attempted rationalization through an international approach. Trans Br Mycol Soc. 2009;65(2):295-303.
197. Walley PG, Teakle GR, Moore JD, Allender CJ, Pink DAC, Buchanan-Wollaston V, et al. Developing genetic resources for pre-breedingin *Brassica oleracea* L.: An overview of the UK perspective. J Plant Biotechnol. 2012;39(1):62-8.
198. Maggioni L. Conservation and use of vegetable genetic resources: a European perspective. Acta Hortic. 2004;637:13-30.
199. Boukema IW, Cristea N, van Hintum TJL, Menting F. The European *Brassica* database: version 2001. In: Maggioni L, Thomas G, Lipman E, editors. Report of a Working Group on *Brassica*. Vila Real (Portugal): IPGRI; 2002. p. 14-8.
200. Cheng F, Wu J, Liu B, Wang X, Liu S, Sun S, et al. BRAD, the genetics and genomics database for *Brassica* plants. BMC Plant Biol. 2011;11(1):136.
201. Love CG, Andongabo AE, Wang J, Carion PWC, Rawlings CJ, King GJ. InterStoreDB: a generic integration resource for genetic and genomic data. J Integr Plant Biol. 2012;54(5):345-55.
202. Leibovici DG, Anand S, Santos R, Mayes S, Ray R, Al-Azri M, et al. Geospatial binding for transdisciplinary research in crop science: the GRASPgfs initiative. Open Geospat Data Softw Stand. 2017;2(1):20.
203. Qiu D, Morgan C, Shi J, Long Y, Liu J, Li R, et al. A comparative linkage map of oilseed rape and its use for QTL analysis of seed oil and erucic acid content. Theor Appl Genet. 2006;114(1):67-80.
204. Eckes AMH, Gubała T, Nowakowski P, Szymczyszyn T, Wells R, Irwin JA, et al. Introducing the *Brassica* Information Portal: Towards integrating genotypic and phenotypic *Brassica* crop data. F1000Research. 2017;6(0):465.
205. Yang W, Duan L, Chen G, Xiong L, Liu Q. Plant phenomics and high-throughput phenotyping: accelerating rice functional genomics using multidisciplinary technologies. Curr Opin Plant Biol. 2013;16:180-7.
206. Araus JL, Cairns JE. Field high-throughput phenotyping: the new crop breeding frontier. Trends Plant Sci. 2014;19(1):52-61.

207. Andrade-Sanchez P, Gore MA, Heun JT, Thorp KR, Carmo-Silva AE, French AN, et al. Development and evaluation of a field-based high-throughput phenotyping platform. Funct Plant Biol. 2014;41(1):68-79.
208. Arnold BJ, Lahner B, DaCosta JM, Weisman CM, Hollister JD, Salt DE, et al. Borrowed alleles and convergence in serpentine adaptation. Proc Natl Acad Sci U S A. 2016;113(29):8320-5.
209. Edwards D, Batley J. Plant genome sequencing: applications for crop improvement. Plant Biotechnol J. 2010;8:2-9.
210. Scheben A, Batley J, Edwards D. Genotyping-by-sequencing approaches to characterize crop genomes: choosing the right tool for the right application. Plant Biotechnol J. 2017;15:149-61.
211. Golicz AA, Bayer PE, Barker GC, Edger PP, Kim HR, Martinez PA, et al. The pangenome of an agronomically important crop plant *Brassica oleracea*. Nat Commun. 2016;7:13390.
212. Scheben A, Edwards D. Bottlenecks for genome-edited crops on the road from lab to farm. Genome Biol. 2018;19(1):178.
213. Gan X, Hay A, Kwantes M, Haberer G, Hallab A, Ioio RD, et al. The *Cardamine hirsuta* genome offers insight into the evolution of morphological diversity. Nat Plants. 2016;2:16167.
214. Nikolov LA, Shushkov P, Nevado B, Gan X, Al-Shehbaz IA, Filatov D, et al. Resolving the backbone of the Brassicaceae phylogeny for investigating trait diversity. New Phytol. 2019;222(3):1638-51.

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