Review

Managing meiotic recombination in plant breeding

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Crossover recombination is a crucial process in plant breeding because it allows plant breeders to create novel allele combnations on chromosomes that can be used for breeding superior F1 hybrids. Gaining control over this process, in terms of increasing crossover incidence, altering crossover positions on chromosomes or silencing crossover formation, is essential for plant breeders to effectively engineer the allelic composition of chromosomes. We review the various means of crossover control that have been described or proposed. By doing so, we sketch a field of science that uses both knowledge from classic literature and the newest discoveries to manage the occurrence of crossovers for a variety of breeding purposes.

The plant breeders' desire to control crossovers

Plant breeding attempts to combine valuable traits from different parents in new elite varieties. These traits are encoded for by genes on chromosomes. The success of a breeding program depends on the ability of plant breeders to bring the desired alleles together in one hybrid, both by constructing desired combinations of alleles on chromosomes and by designing the right combination of chromosomes. Meiotic recombination has a pivotal role in successful plant breeding because the reshuffling of homologues and chromosome segments takes place only during meiosis. The maximum obtainable amount of meiotic recombination is determined by two factors: the number of chromosomes of a plant (random chromosome assortment) and the number and positions of crossovers on the pairs of homologous chromosomes (crossover recombination). Plant breeders have no direct control over the number of chromosomes, except perhaps by adding artificial chromosomes [1], and therefore look for means of imposing control over that other part of meiotic recombination: crossover formation.

In the past, the lack of practical tools for establishing crossover frequencies hampered systematic studies on crossover management in crops. Determining specific crossover frequencies was costly and laborious and was mostly confined to model species. Nowadays, modern methods for high-throughput genotyping and the development of dense sets of markers provide the tools for efficiently determining crossover frequencies and crossover positions [2]. Research will surely benefit as well from new technologies, such as tetrad analysis in a q*uartet* background (Box 1).

Because they are no longer constrained by technical issues, the interest of plant breeders in control over crossover formation is larger than ever before. We explore the possibilities for controlling crossover formation and describe how several methods have considerable potential. We show how plant breeders can exert influence over crossover frequencies, crossover position and crossover allocation to homoeologous regions. It is even possible to suppress crossover formation completely and reduce the complexity of meiotic recombination to only the random assortment of whole chromosomes. This opens up opportunities to extract and fix whole chromosomes from heterozygous complements (i.e. F1 hybrids). In this paper we will focus on mechanistic aspects of crossover control and point out methods that can be utilized even without a precise understanding of the processes underlying crossover formation (Box 2).

Controlling crossover incidence

The number and distribution of crossovers during meiosis is tightly constrained. There is typically at least one crossover per chromosome pair to ensure proper segregation at metaphase I, known as 'crossover assurance' [3]. The total number and relative position of crossovers on each chromosome is limited to generally one, or perhaps two, per chromosome arm by interference, a phenomenon distributing crossovers in a non-random, semi-uniform pattern [4]. On a smaller scale, crossovers preferentially occur in certain areas called 'recombination hotspots' [5,6], and the areas with almost no crossovers are called 'recombination cold spots'. In the following section we explore variability in crossover incidence and discuss how this can be influenced.

Glossary

Crossover recombination: meiotic recombination resulting from crossovers between chromatid segments in a chromosome pair.
Doubled haploids (DHs): diploid plants grown from (haploid) spores in which genome duplication resulted in diploidy. Chromosomes are identical. DHs are commonly used to directly fix the genotype of meiotic spores.
Homoeologous chromosomes: chromosomes from different species that show a higher degree of sequence divergence than homologous chromosomes do and display less or no pairing at meiotic prophase I. Differences might be of a higher order of magnitude, showing minor structural rearrangements such as inversions, translocations or differences in repetitive sequences. Sequence divergence can be so large that crossover formation during meiosis is impaired.
Homologous chromosomes: chromosomes that are sufficiently similar for regular meiotic pairing but show a limited (allelic) degree of sequence divergence.
Random chromosome assortment: meiotic recombination resulting from an independent assortment of chromosomes.
Univalent: a single chromosome that is not bound to another by a chiasma at anaphase I.

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Box 1. Tetrad analysis in plants

The possibility for tetrad analysis in plants emerged with the discovery in *Arabidopsis thaliana* of the *quartet* mutant, in which the four meiotic spores remain together [56,57]. In combination with fluorescent markers expressed by a pollen-specific promotor, this mutant directly displays the consequences of crossover recombination in pollen grains [58].

Species producing pollen tetrads are widespread among plants [59] and, with the details of the *quartet* mutation known, such phenotypes are possibly inducible in crops [60] or can be identified directly by mutant screens. Insert lines with fluorescent markers are currently being used for *Arabidopsis*, but the construction of such lines for other species would require a considerable investment. However, these insert lines could potentially result in methods for evaluating the effects of treatments for altering recombination frequencies in crops, which would be of enormous benefit, especially since different crops can react differently to certain treatments (as discussed in the main text). A rather similar technique was developed for the direct observation of crossovers in seeds [61] using insertion lines of fluorescent proteins expressed by seed-specific promotors. Although this technique does not require a specific phenotype (e.g. *quartet*), its application in crops might be somewhat more limited because the production of seeds in crops is generally much lower than production of pollen.

Internal factors, such as genetic background and morphological and developmental differences, can have a considerable impact on crossover incidence. Related genotypes can have significantly different crossover frequencies, and up to a 30% difference was reported among barley (*Hordeum vulgare*) cultivars [7]. Similarly, differences of ~17% were found among *Arabidopsis* accessions [8]. More strikingly, recurrent selection for high and low recombining individuals starting from a single heterozygous plant in an F2 population of lima bean (*Phaseolus lunatus)* led to a threefold difference in recombination frequencies in the F6 generation [9].

There is ample evidence for differences between the sexes in crossover frequencies, both in plants and animals [10], and this phenomenon is likely to be caused by differing compaction states of chromatin during meiotic prophase in male and female meiosis [11,12]. In addition, crossovers typically locate more distally at male meiosis in *Arabidopsis* [11]. The physical position of a flower can influence crossover incidence: in *Arabidopsis*, anthers on secondary or tertiary branches have up to 16% more crossovers than those on primary branches [13]. Such effects are species specific: barley and rye (*Secale cereale*), for example, show no variation in crossover incidence in relation to flower position [14].

Box 2. Controlling crossovers: managing the unknown

Crossover formation is a complex process that is regulated at multiple levels, and factors governing crossover formation are still not well understood [62]. It partly depends on the homology search that follows double-strand break (DSB) formation in plants [18]. Mismatch repair proteins, which are involved in homology search, prohibit recombination between non-homologous segments [63,64]. The coordinated remodelling of chromatin affects pairing and recombination affinities between chromosomes [34,65], and the placement of crossovers in a pair of homologues is tightly regulated (as discussed in the main text). Such processes complicate plant breeders' efforts to engineer chromosome structure.

Although high-throughput screening of large populations is sometimes an option for obtaining rare crossovers, recombinants will not always be found. More efficient recombination might be required when, for example, alleles in *trans* of closely linked loci need to be combined. This is especially difficult in regions where recombination is suppressed or absent. In yet other cases, recombination might be required between chromosomes that do not even pair in meiosis.

Some reports have shown how external factors, such as environmental influences and chemical treatments, can alter crossover frequencies. Random environmental variation was shown to result in a twofold difference in recombination frequency between two linked loci in lima bean [9]. In more controlled experiments in which only temperature was varied, recombination rates increased with higher temperature in *Arabidopsis* (up to 18%), as had previously been described for species like *Hordeum vulgare* and *Vicia faba,* whereas high temperatures decreased recombination frequencies in *Allium ursinum* [13]. Crossover frequency in barley, as in rye, is less susceptible to environmental influence [14].

Recombination frequencies can be increased artificially by the use of various chemical agents or physical stress, such as temperature shock or UV exposure [15]. The feasibility of this approach was originally shown by a study in *Hordeum*, where actinomycin D, as well as diepoxubytane, was shown to lead to a threefold increase in recombination frequency between linked markers [16]. A survey study using various chemical agents also showed large (roughly two- to sevenfold) increases in recombination frequencies by the use of various chemical agents, a fourfold increase by heat shock and threefold increase by UV radiation in *Arabidopsis* [15]. These data, however, were based on a relatively small number of plants and might be limited to the specific (pericentromere) genomic regions that were assessed [17].

In recent years, many proteins involved in crossover formation have been identified [18,19]. The possible impact of genetic regulation on crossover formation is illustrated by the uncharacterized *X-ray sensitive4* (*xrs4*) mutant of *Arabidopsis*, in which recombination frequency in certain regions increased over twofold [20]. Such mutants fuelled the idea that either overexpression or silencing of such proteins could modulate recombination frequencies [21]. However, only a few studies on this topic were published, and the extent of their analysis was limited. In tomato (*Solanum lycopersicum*), overexpression of *MutL homolog1* (*MLH1*, which encodes a mismatch repair protein) led to a 10% increase in chiasma frequency [22], whereas a genetic interval in *Arabidopsis* showed a twofold increase of recombination frequency upon overexpression of *RADIATION-SENSITIVE51* (*RAD51*, a gene involved in DNA repair) [23].

Crossovers follow changes in chromosome structure

The location of crossovers along the chromosome field (i.e. proximal versus distal events) is tightly regulated. Whereas in a species like Welsh onion (*Allium fistulosum*) crossovers localize proximally, those in a close relative, *Allium cepa*, localize distally [24], and such distal localization is also found in species such as barley (*Hordeum vulgare*), maize (*Zea mays*) and wheat (*Triticum aestivum*)

[25]. The occurrence of crossovers is in part determined by higher order chromosome structure, and they are less frequent in pericentromere areas [26] (Figure 1a). The presence of tandem repeats in distal heterochromatin blocks in *A. fistulosum* was suggested to move chiasmata away from the ends (L. Khrustaleva, personal communication) (Figure 1b). This strong regulation of crossover placement poses strong constraints on the extent to which the allelic content of a chromosome can be changed by crossover recombination. Because some regions are not subjected to crossover recombination, loci remain tightly or completely linked, which limits breeding potential. In the following section we explore possibilities for altering the positioning of crossover events along the chromosome axis.

It is known that even a short terminal deletion in one of the pairing chromosomes can severely reduce crossover formation in the affected arm [27]. This is likely to be due to a disturbed pairing initiation that generally (but not exclusively) starts at distal chromosome ends [28,29]. In one experiment, radiation induced the deletion of the terminal end of the short arm of chromosome VI in *Petunia hybrida* [30]. Crossover formation in the truncated arm was severely reduced but, interestingly, there was an up to sevenfold increase of crossovers in intervals on the long arm of that same chromosome (Figure 1c). To obtain such deletions, one can use pollen irradiation and then select for the loss of dominant distal markers.

Other types of structural variants that change crossover positions are translocations. Effects of translocations, which also comprise the skewed transmission of alleles due to chromosome deficiencies in gametes, can be well observed in chiasma configurations during meiotic prophase. When a chromosome carries a distal translocation, the translocation introduces strong heterology at the chromosome end in the chromosome pair. This results in a shift of crossovers to interstitial chromosome segments (the area between the centromere and the translocation site) [31]. Although such translocations might be undesired in breeding programs, they illustrate the mechanism by which changes in chromosome structure reallocate crossovers to the homologous sites between the pairing partners.

A related case of chromosome structure alterations has been described for tomato lines carrying introgressed homoeologous segments of related wild species. It was shown that the presence of such segments in otherwise homologous chromosome pairs affected crossover localization: crossover frequencies increased strongly in adjacent homologous sequences [32] (Figure 1d). Comparable observations were made in *Lolium-Festuca* hybrids [27,28,33,34] using allotriploid offspring comprising two homologues of one species in addition to one homologue of the other. Crossovers in the *Lolium* and *Festuca* parents preferentially form in the distal chromosome regions. In the allotriploids, homologous chromosomes behaved similarly, preferentially forming distal crossovers. However, when their homoeologous partner joined in trivalents, it formed crossovers mostly in proximal regions [33,35]

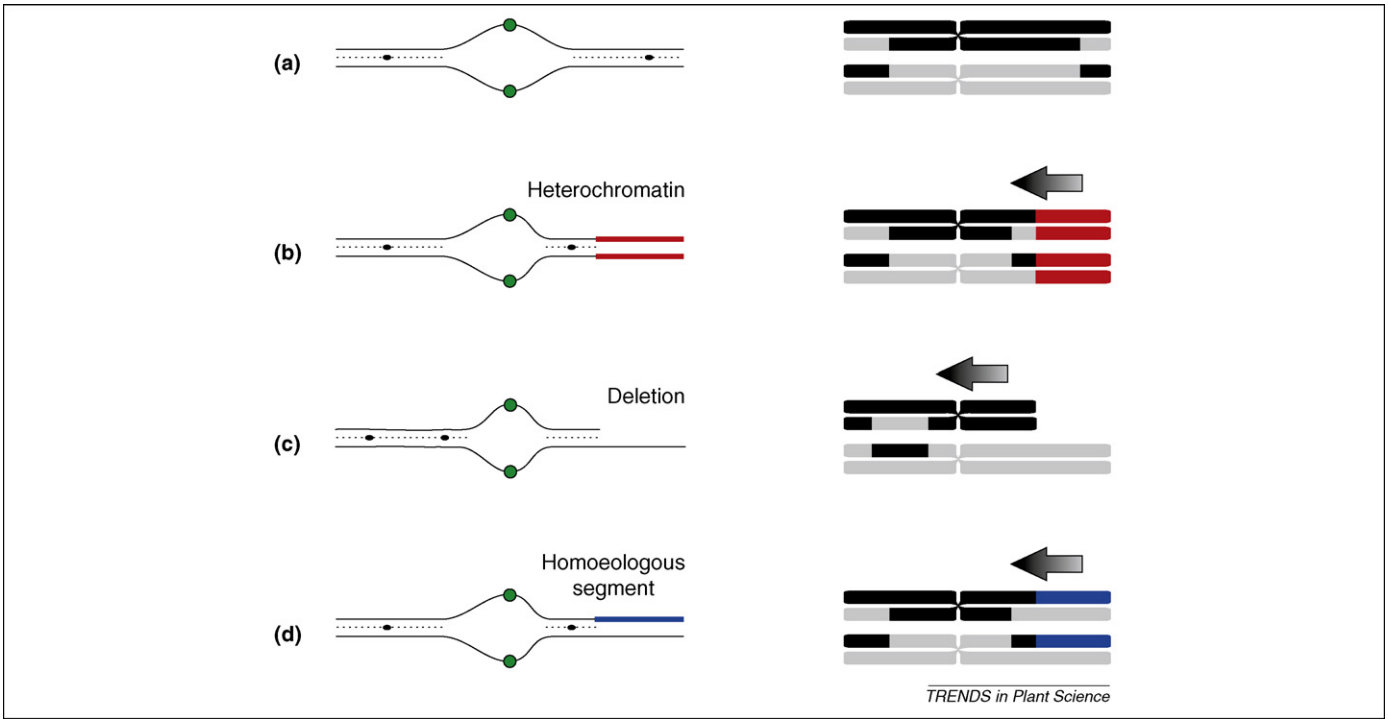

Figure 1. Examples of how structural changes can lead to crossover shifts. Drawings on the left represent a chromosome pair at mid-prophase of meiosis I. Solid lines represent chromosome axes; dotted lines represent the proteinaceous structure keeping paired homologues together during meiotic prophase (the synaptonemal complex); and small black spheres mark the crossover sites that will later form the chiasmata. Green spheres represent the centromere regions. The drawings on the right represent the corresponding recombinant chromosomes at anaphase I. **(a)** Chromosome pairing and crossover recombination of a normal chromosome pair. The region around the centromere is the pericentromere, which is known to synapse later and is poor in crossover events. In these examples we assume that there are two crossovers per chromosome pair and that crossovers occur between only two chromatids (in actuality the number of crossovers and the number of recombining chromatids can vary). **(b)** Pairing and recombination in a chromosome pair containing a recently formed large distal heterochromatic block (represented in red) in one of the chromosome arms. **(c)** Pairing and recombination in a pair in which one partner has the distal end of one arm deleted. **(d)** Pairing and recombination for a chromosome pair in which one partner has a homoeologous chromosome segment (represented in blue).

because homoeology disturbs crossover formation at the distal chromosome ends. In wheat and *Triticeae* species, patterns of homoeologous recombination were shown to vary between different species and homoeologous recombination can, like the *Lolium-Festuca* hybrids, be highly localized (reviewed in [36]).

Approaches for altering crossover localization using transgenic approaches are very scarce. Nicolas *et al.* [36] designed a method for recruitment of SPORULATION-DEFICIENT11 (SPO11) a key protein for crossover initiation, to selected sequences of DNA by designing an artificial fusion protein comprised of SPO11 and a DNA-binding domain. The DNA-binding domain recruits the fusion protein to binding sites on the DNA and induces crossover formation at these sites. This technique has been shown to work well in yeast [37], although the induction of double-strand breaks occurs mostly in binding sites in open chromatin regions and not, or less, in natural cold spots, such as centromere areas [38]. This method, which has been proposed for use in plants, would provide a powerful tool for induction of site-specific crossovers. SPO11 could be fused to a variety of different DNA-binding domains, thus resulting in a suite of fusion proteins that could, in theory, recruit SPO11 to various sites on the DNA.

Crossovers in homoeologous regions

The mechanisms that control crossovers between homologues can be frustrating to plant breeders in their attempts to integrate valuable traits through introgressive hybridization. Examples of such traits are genes for resistance or drought tolerance that might be found in related species. Typically, a cross is made between a recipient crop and a related taxon carrying a trait of interest. This is followed by recurrent backcrosses to the crop in which the introgressed homoeologous region is narrowed down, keeping the gene of interest and removing 'wild' undesired genes (linkage drag). Because crossovers are generally suppressed or absent between the introgressed segment and its homoeologous counterpart, it is imperative to find the mechanisms and genes that control the pairing between homoeologous segments.

To induce crossovers in introgressed segments, one can reallocate crossovers to the homoeologous region by making the other regions in the chromosome pair even more homoeologous [32]. If, for example, crossovers are to be induced in one chromosome arm carrying an introgressed segment, one could provide a pairing partner that carries an introgressed segment of a more distantly related taxon on the opposite chromosome arm. Crossovers then reallocate to the least homoeologous sites. One can predict that any rearrangement could be used to direct crossovers to homoeologous regions of interest. In the same study, it was shown that in tomato hybrids, larger homoeologous segments have a higher incidence of crossovers than shorter segments. This led to the suggestion that in introgression breeding, plant breeders should initially search for those plants with the largest alien insert and then select for single crossovers close to the locus of interest. Crossing two recombinant lines with crossovers on different sides of the locus would then reduce linkage drag to a minimum [39].

Different genes have been identified that influence homoeologous recombination. The best known is *Pairing homoeologous1* (*Ph1*) [40,41], which inhibits homoeologous pairing between wheat chromosomes. In the absence of *Ph1*, pairing and recombination between homoeologous chromosomes is frequent, which greatly facilitates introgressive hybridization [42]. However, the constitutive deletion of *Ph1* can over time lead to rearranged chromosomes in the genome, which can later interfere with further breeding efforts. The use of *Ph1* in plant breeding would greatly benefit from means of temporarily switching off the locus [43]. A gene, *Pairing regulator in Brassica napus* (*PrBn*), with a comparable function was also identified in *Brassica* [44], but it has not been characterized at the molecular level. It was further hypothesized that during meiosis, the knockdown of genes, such as *MutS homolog2* (*MSH2*) or *MSH3,* that encode mismatch repair proteins might promote homoeologous recombination [45,46]. Knockdown of these genes could be achieved, for example, by RNA interference (RNAi) or dominant negative suppression, in which a truncated gene disrupts the functioning of protein complexes [47].

Preserving elite genotypes

Most of the cultivars produced by breeders are heterozygous F1 hybrids, which are bred for their unique combination of alleles and outperform their parents by hybrid vigour. Controlled creation of elite heterozygosity is achieved by simply crossing two carefully selected homozygous parents. Doing the reverse (creating homozygous parents for any heterozygous F1) is, on the contrary, an almost unfeasible task. The allele combinations that give the F1 its unique character are broken apart by recombination when the F1 is used in crosses (Figure 2). The answer to preserving these combinations during meiosis lies in technologies and strategies that reduce the complexity of meiotic recombination; one such strategy is reverse breeding (schematically summarized in Figure 2) [48].

Reverse breeding is based on suppression of crossover formation by RNAi or comparable gene silencing techniques. Studies have shown that RNAi silencing of essential early meiotic genes, such as *DISRUPTED MEIOTIC CDNA1* (*DMC1*), can lead to (almost) complete suppression of crossover formation [49,50]. Consequently, homologues are not joined by chiasmata (the physical manifestation of crossing over) during meiotic prophase I and remain as univalents at anaphase I. These univalents (non-recombinant parental chromosomes) then segregate randomly to daughter cells during the first meiotic division [51]. Most resulting spores will be unbalanced, containing either none, one or two copies of a given chromosome. However, balanced spores, containing one copy of each chromosome, will be formed at a probability of $(1/2)^x$, where x equals the basic chromosome number. Consequently, the chance of obtaining balanced spores decreases exponentially with the chromosome number and seems feasible for species in which the chromosome number equals 12 or less [48].

In reverse breeding, any given elite heterozygote is transformed using an RNAi construct targeting a gene that encodes a protein that mediates the formation of crossovers. The resulting plant is expected to produce

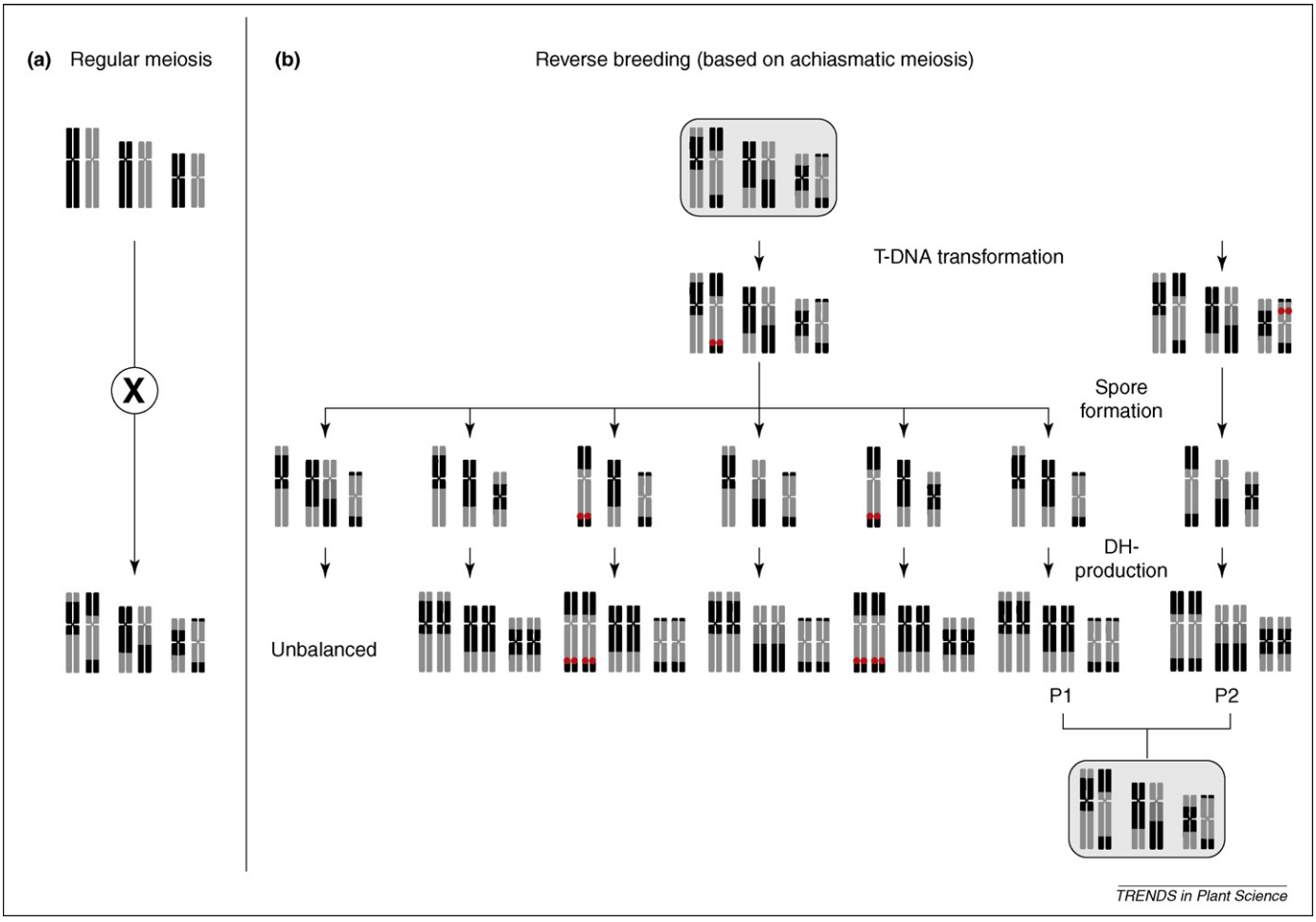

Figure 2. Schematic representation of **(a)** regular meiosis (the selfing of a heterozygote) and **(b)** the reverse breeding technique. We consider a fictive F1 with three chromosome pairs (2n = 2x = 6). During regular meiosis, chromosomes and chromosome segments recombine, giving rise to an infinite number of genotypically different offspring (a). In an alternative approach (b), crossover recombination is prevented by transgenic suppression (RNAi, etc.) of one of the genes essential for crossing over. The red dots represent the transgene. Achiasmatic meiosis gives rise to spores. Note that spores carry non-recombinant chromosomes. Most spores are unbalanced (one possibility drawn), but some spores are balanced (several possibilities drawn). Doubled haploids (DHs) are produced from balanced spores, giving rise to homozygous diploids. Among the DHs produced, reciprocal genotypes (P1, P2) can be recruited that, upon crossing, exactly reconstitute the original F1. These are the homozygous parental lines for the F1 hybrid. Note that P2 is derived from a second transformant carrying the transgene on a different chromosome.

low numbers of viable balanced haploid spores that are then regenerated into doubled haploid, perfectly homozygous plants. Other spores with an unbalanced chromosome number will, if they are still viable, produce aneuploid individuals with poor regeneration rate and vigour. Among the doubled haploids, parents with complementary genotypes can be recruited that, upon crossing, will reconstitute the exact genotype of the elite hybrid again (Figure 2).

In *Arabidopsis*, various mutants that lack crossovers (almost) exclusively produce univalents [18,19], although their chromosome behaviour during meiotic prophase can be different. It was recently shown that in univalent-producing mutants (*desynaptic1* [*dsy1*], *meiotic prophase aminopeptidase1* [*mpa1*]) in which chromsomes pair normally during prophase, univalents segregate preferentially to opposite poles during metaphase I [52]. This suggests that pairing, even without chiasma formation, to some extent orients homologues to opposite poles. Targeting such genes for reverse breeding might be fruitful because the chance of recovering balanced gametes increases substantially. As such, genes such as *PARTING DANCERS* (*PTD*), for which the mutant shows complete pairing and few residual crossovers next to high levels of univalents [53], might also be of interest to reverse breeding. The benefit of its regular segregation might well outweigh the downside of few remaining crossovers.

Reverse breeding provides plant breeders with new possibilities for further breeding. When one transforms a hybrid for which the parents are known, one can directly select chromosome substitution lines from among the produced doubled haploids. These chromosome substitutions have various potential applications, as for example in the generation of near isogenic lines by recurrent backcrosses. Such lines are extremely valuable for mapping quantitative trait loci (QTL) and for advanced forms of marker-assisted breeding [54,55].

Conclusions

The improvement of crop species relies on the possibility to select and carefully produce new allele combinations. Over the last decade, plant breeding practice has been revolutionized by the advent of high-density marker collections that enable high-throughput screening in breeding selection schemes. The ease of genotyping shifted the focus of

breeding to marker-assisted breeding, which greatly increased the predictability of breeding efforts, in which crossovers are and will remain crucial.

In spite of the plethora of genes known to be involved in crossover control, few studies have been published on the practical applications of such genes. This is in contrast to the various patents for crossover control that have been filed, indicating that methods for crossover control have the attention of many researchers and that the economic value of such methods is acknowledged. As we see it, research is progressing along several lines. On the one hand, we expect a revival of classic meiotic research: variability within crops, within the plants or induced by internal and external factors might be evaluated using high-throughput marker technology. On the other hand, we foresee that an increasing knowledge on the molecular control of meiosis might create new applications for plant breeding.

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