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Mastering meiotic recombination

The “Groupement d’Intérêt Scientifique Biotechnologies Vertes” (GIS BV), the “Plant Biology and Breeding” INRA Division and the “Plant Biology and Biotechnologies” working group (GT4) of the AllEnvi Alliance organized in Paris on the 22nd of June 2015 a scientific workshop on «Control of recombination and reproduction for plant breeding». Forty-five scientists from the public and private members of the GIS BV participated in the workshop. The presentations focused on recent discoveries on meiosis control and meiotic recombination and how to use them for plant breeding. They were followed by a global discussion. This paper summarizes the strategies identified for controlling and manipulating plant meiotic recombination and identifies the research opportunities for the French public-private community, in the light of the scientific and socio-economic issues.

Socio economic context

There is an urging demand to increase global crop production for food and feed due to an increasing population that will meet 9 billion people in 2050 as well as to new industrial uses such as biofuels. As cultivated areas and natural resources are limited, this challenge has to be met while facing ecological constraints to reduce the environmental footprints of crop production (i.e. use less water, fertilizers, pesticides, fungicides...). It is thus necessary to speed up the development of new varieties that must not only sustain crop yield, but also improve their quality and nutritional values.

The development of new varieties by conventional breeding methods relies on the creation of new allele combinations, either by mixing alleles existing in elite germplasm or by introgressing genetic variability from genetic resources. The new combinations of alleles can then be propagated through seeds after the generation of homozygous individuals (e.g. wheat) or as F1 hybrids of stable parental lines (e.g. maize, oilseedrape). Only plants amenable to clonal propagation such as fruit trees and grapevine can be reproduced as heterozygous individuals.

The exchange of genes between chromosomes from two different individuals relies on meiotic recombination (or crossovers, COs). However, in each cross between 2 parental lines, the number of COs per meiosis is limited to a few tens per derived individual while there are several 10,000 genes per genome to mix. COs are also the basis of the exploitation of new variability through the introgression of chromosomal regions originating from wild species that often show an interesting level of resistance to biotic and abiotic

stresses. However, in this case, COs are not restricted to chromosomal regions of interest, which can result in linkage drag and the introduction of deleterious genes along with the beneficial ones.

Controlling meiotic recombination in crops would accelerate the release by breeders of improved varieties with new combination of alleles. This relies on the understanding of the factors that control CO rates - that are usually low in crops - as well as those involved in the distribution of the COs along the chromosomes.

Clonal reproduction through seeds - apomixis - represents a holy grail for increasing crop yields, as it would allow propagation of any genotype in particularly of F1 hybrids. It would drastically reduce the cost of F1 favorable genetic combination and allow harnessing hybrid vigor in crops species for which hybrids are not - or only little - commercialized. Engineering clonal reproduction through apomixis for seed-propagated crops would potentially revolutionize plant breeding. It would confer a tremendous competitive advantage to the private breeders who would first use it to create and propagate new varieties.

Meiotic recombination: A universal but limited breeding tool

Meiotic recombination is a fundamental process for all sexual eukaryotes. It is required to produce balanced gametes and therefore is essential to the fertility of species. Meiotic recombination is also the basic tool for any plant or animal breeding program because it allows, through the formation of crossovers (COs), to reshuffle genetic material between individuals and between species and thus reassemble favorable alleles



from different origins in a single genotype. Major international efforts have been made to identify the genes that are involved in meiotic recombination in plants, primarily using *Arabidopsis thaliana* as model system. However, only a few studies have been made in crop species although recombination in these plants suffers from several limitations that hamper their improvement through breeding:

- ⚡ The absolute frequency of crossovers is low, typically one to three per chromosome per generation. Increasing this frequency in crops would facilitate the assembly of multiple favorable alleles that are carried on the same chromosome and will in theory limit the loss of genetic variance in response to breeding pressure. This would also be very useful to improve QTL detection and map-based cloning efficiency in crops;
- ⚡ Meiotic recombination is universally abolished at centromeres and pericentromeric regions. In many plant species – and particularly in cereals – the pericentromeric region devoid of recombination extends to large part of the genomes (up to >80%) and contains numerous genes. This means that breeding is blind to up to 30-50% of the genes, among which some are of major agronomical interest. Unlocking recombination in these regions could improve genetic progress for many traits controlled by these genes. In addition, increasing recombination at specific loci (e.g. clusters of resistance genes) could enhance the repertoire of alleles of interest;
- ⚡ Recombination is commonly abolished in chromosomal regions introgressed from related species, preventing further genetic improvement in these regions. This is for example a major issue in tomato and wheat, two species for which the use of related species as a source of new alleles for genes of agronomic interest – such as biotic and abiotic stresses resistance – is frequent. Unlocking and/or targeting recombination in these regions would be a major progress, especially when the gene of interest is linked to undesired genes conferring deleterious effects (linkage drag).

Emerging technologies that meet breeders' challenges

In the last decade, major progresses have been made in the understanding of the mechanism of meiotic recombination in plants using *Arabidopsis* as model. This offers new opportunities to transfer this knowledge to crops and address the issues listed above. Two complementary technologies directly derived from basic research developed in France emerged.

- ⚡ The HyperRec technology developed by INRA relies on the extinction of a series of recently identified genes that limit meiotic crossover (e.g. *FANCM*, *FIDGL1*, *RECQ4* [1–3]). This allows an increase of recombination as high as eight fold, as shown in *Arabidopsis thaliana* hybrids. This could improve breeding in any species. In addition, manipulation of karyotypes in Brassica crops was shown to increase CO rate several-folds [4,5], in particular in some recombination-cold intervals;
- ⚡ The second technology relies on the targeting of the *SPO11* protein to specific regions of the genome. *SPO11* function is to make double strand breaks to initiate meiotic recombination [6]. This technology was shown to be efficient in budding yeast and is currently tested in plants by the French company Meiogenix. This would allow both improving recombination in regions where it is locked and targeting recombination to break undesirable linkage in homologous or homoeologous regions.

Research targets for future investments

Improving recombination in crops is clearly a need to accelerate the development of new performant varieties in a context of sustainable agriculture. Recent progress achieved on the model species *Arabidopsis* must now be transferred to cultivated crops. INRA is one of the world leaders in the domain both on *Arabidopsis* and crops (oilseed rape, wheat, tomato) and this is clearly an asset to successfully reach this goal and help French breeding companies take a leading position in this area. Different approaches could be applied for specific target crops.

- 1) Increasing crossover frequency at whole-genome scale will necessitate to:
 - a. Implement the HyperRec technology in crops to increase recombination frequency. This could be done by inactivating one or a combination of anti-CO genes by RNAi, CRISPR or TILLING. In theory, the recombination rate limits the efficiency of breeding programs. A Proof Of Concept project on crops will allow assessing: i) the feasibility of knowledge/tools transfer from *Arabidopsis*; ii) the genetic gain resulting from manipulating recombination; and iii) the potential side effects of the manipulation of CO frequency or distribution along the chromosomes on the behavior of derived elite plants.
 - b. Understand the molecular basis underlying the recombination-rate increase in some aneuploid lines – such as the AAC oilseed rape triploids –, and evaluate its transferability to other polyploid crops – such as wheat;

- c.
 - d. Identify additional factors that contribute to the improvement of recombination rate in plants. This will rely on basic research, both in model species (*Arabidopsis*, rice) and crops, to identify the factors that shape the number and distribution of recombination events along the chromosomes.
- 2) Relocating crossovers specifically in naturally cold regions will rely on:
- a. Testing the *SPO11*/Meiogenix technology in crops to target recombination at specific loci. This could be done by defining targets in recombination cold-spots relevant for breeders, for instance introgressed regions from wild species associated with both deleterious and favorable effects. Tomato and wheat will be the most relevant crops for this approach. The specificity of the *SPO11*/Meiogenix technology to induce breakages in homoeologous regions of polyploid plants should also be assessed;
 - b. Evaluating the impact of chromatin structure (condensed or not; heterochromatin/euchromatin) on recombination profiles. Epigenetic landmarks such as DNA or histone methylation are known to impact chromatin structure as well as recombination and analysis of mutants for these traits would be relevant;
 - c. Understanding the differences between male and female meiosis. Heterochiasmy refers to a situation where both male and female gametes recombine, but at different rates, and is widespread throughout the living world. If this phenomenon is well studied in animals, it remains poorly understood especially in plants while it is of major interest especially with regards to double-haploid production;
 - d. Determining the factors that can participate to recombination induction to develop an on/off strategy. This may include environmental factors such as temperature – an increase of temperature modifies recombination profiles but reduces drastically fertility – which will be one of the main problems with global warming. Having chemicals inducing or blocking recombination on demand would be an asset;
- 3) Promoting (or limiting) the formation of crossovers between divergent chromosomes derived from related species:
- a. Identify factors that control homoeologous recombination to improve introgression efficiency in crops. This should include the characterization of the molecular actors involved in the control of homoeologous pairing in polyploid crops such as *Ph2* (wheat) and *PrBn* (oil seed rape);
 - b. Evaluate the effect of the inactivation of target proteins (i.e. *RECQ4*, *FIGL1*, *FANCM*, *MSH2*, *MSH7*) on homoeologous recombination in crops;
 - c. Reduce the size of introgressions (especially in wheat and tomato) which can induce linkage drag with deleterious effects.
- 4) Defining the best (pre-) breeding strategies to take advantage of unbridled recombination. This concerns mostly methodological aspects to apply efficiently the knowledge gained from the above-mentioned studies to breeding. Analyzing the impact of increased CO numbers and reshaped CO distribution on selection response in breeding programs through both computer modelling and proof of concept selection cycles:
- a. Breeding with simulation data and field validation by breeders. This could include also experimental breeding in *Arabidopsis*;
 - b. Pre-breeding with introduction of new genes/alleles of interest in buffer varieties that could be further used in breeding schemes;
 - c. Evaluation of the impact of linkage disequilibrium and gene conversion bias on recombination. Introduce the concept of life and death of recombination hot-spots and further reactivation;
 - d. Interference which prevents the occurrence of additional COs in the vicinity of another type I CO. Understanding how interference act and to what extent it would be helpful to improve recombination rate.
- Ideally, these approaches should be carried out in a continuous dialogue between theory (detailed mechanisms described in model species, modelling...) and practice (transferability to crop species, plant breeding strategies...). Important and complementary crops (vegetable vs. Brassicaceae vs. cereals) should be targeted in order to yield insights with far greater predictive power than if either crop was studied individually. Most notably, a major breakthrough will be the identification of the best possible combination of mutations that increase recombination in regions of interest, which may vary from crop to crop.
- Clonal reproduction through seeds**
- Many plant species –but virtually no crop- reproduce via apomixis but the genetic control of this trait has not been identified, despite large international efforts. A new opportunity to introduce clonal reproduction into crops exists through the recently developed possibility of engineering apomixis *de novo* by tinkering with

sexual processes. This has been demonstrated in the sexual model plant *Arabidopsis*, with the production of ~30% of clonal seeds [7]. This technology requires two steps, (i) turning meiosis into mitosis at high frequency (*MiMe*) [8], which can now be considered as mastered in model species and (ii) the development of embryos without paternal genetic material [9]. This second step represents the current hurdle to the implementation of apomixis, as its efficiency is currently at best 30%, and further research is needed to better master either parthenogenesis or genome elimination.

Research targets include:

- 🌱 Transfer of the *MiMe* technology to crops. Turning meiosis into mitosis, which is a key element of

apomixis has been first engineered in *Arabidopsis* [8], and subsequently transferred to rice (Mieulet et al, in press). This suggests that this technology is amenable in any plant species.

- 🌱 Basic research on genome elimination. The genome elimination step is clearly the limiting step of the current apomixis technology, with an efficiency <30%. Basic research on this phenomenon is required to increase its frequency in both model and crop plants.
- 🌱 Basic research on parthenogenesis. An alternative to genome elimination would be parthenogenesis, the development of an embryo without fertilization. However, the molecular bases of parthenogenesis are unknown.

Overview of strengths, weaknesses, Opportunities and threats of French research public-private community

Strengths	Opportunities
<ul style="list-style-type: none"> 🌱 Worldwide leaders in the meiosis field (IJPB Versailles on <i>Arabidopsis</i>) and near monopoly position over some specific questions (modélisation of CO in <i>Le Moulon</i>, meiosis in Brassica, recombination distribution in wheat...); as a matter of fact, antiCO proteins were identified at IJPB, the discovery that COs get a boost in Brassica AAC hybrids, in particular in otherwise CO-cold regions, was made at INRA (UMR IGEPP and IJPB), many patents have followed these achievements. 🌱 Continuum from fundamental research on meiosis/meiotic recombination based mostly on <i>Arabidopsis</i> (but not only) to species-oriented studies in a wide range of crops (tomato, oilseed rape, pea, wheat, rice, ...) to plant breeding methodology. Proofs of concept that basic knowledge can be translated into innovations (increase CO, targeted CO) is currently being established. 🌱 A recombination network is well-structured and organized (regular annual meetings). It is mostly located in Paris region with well-connected crop-dedicated units (Oilseed Rape in Rennes, Tomato in Avignon/Bordeaux, Wheat in Clermont-Fd, Pea in Dijon, Rice in Montpellier) 🌱 Availability of genetic (collections of mutants for most crops) and genomic resources (genome sequence; CNRGV; genotyping platforms) that are instrumental for translationnal biology 	<ul style="list-style-type: none"> 🌱 The existing network of public labs is ready to mobilize (part of) its forces quickly 🌱 CRISP/CAS9 is democratizing => essential to accelerate translationnal biology (proof-of-concepts; dominant mutations) 🌱 New fields: Genomic selection – it is now possible to create HyperRec reference population; many more QTLs to be cloned (thanks to PIA1) 🌱 <i>Arabidopsis</i> is still the best model to address some basic questions and to provide detailed information on the mechanism(s) at play, which can then be exploited in a broad range of crops: e.g. what cause CO downfall in the pericentromeric regions? Complementary investigations can nonetheless be envisaged in some crops (rice, tomato) 🌱 There are already some small projects in progress that begin to have promising results which deserve to be amplified.

Weaknesses	Threats
<ul style="list-style-type: none">⚡ Connections exist with French private companies but they barely materialize⚡ Some discoveries can still be viewed as upstream from innovations (recessive mutations difficult to use in plant breeding programs)⚡ The transmission belt between basic science and recombination engineering is still wanted in some crop species.⚡ There are not enough (public or private) scientists working on recombination in crops⚡ Difficulty to rise funding from both public (ANR, Europe...) and private (FSOV...) calls because either too much competition or too fundamental.⚡ There is currently a gap between the knowledge, including the Arabidopsis tools, and the applications in breeding. The objective for the private partners is to improve the breeding efficiency and the feasibility of manipulating CO in crops and the benefits of such manipulations are still not known.	<ul style="list-style-type: none">⚡ Monopoly is being challenged for some species (Brassica, wheat...) as many colleagues have been reoriented (part of) their programs abroad to include crops.⚡ As the number of competitors grows worldwide, there is a risk that innovations first arise abroad from scientific insights made in France in the first instance⚡ New models are currently being developed (rice in Asia, barley in the UK); there are still lagging behind Arabidopsis, but for how long?

This paper is endorsed by the strategic committee of the GIS BV, the “Plant Biology and Biotechnologies” working group (GT4) of the AllEnvi Alliance.



Annex 1. References

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Annex 2. List of Participants

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