



Is Genome Editing Techniques the New Challenge for Plant Breeding?

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Abstract

Plant breeding is the “art and science” of the genetic improvement of crops to produce new varieties that had a large impact on crop yield and food production. Genetic variation is the engine that propels breeding to meet future challenges and this variation is due to intra- and inter-specific recombination. The recombinant DNA technology marked the beginning of a new era, indeed conventional plant breeding was joined to the transgenic approach to produce genetically modified plants (GMP) and new varieties. Transgene technology created great opportunities but many questions about GMP impact and the consideration that many traits of interest are quantitative (QTL) with the problems of pyramiding genes, determined less enthusiasm for this approach. Recently, the knowledge in genome engineering technologies is flashing a new revolution in biological research. Nowadays the researchers can edit the function of DNA sequences in their genetic loci overcoming many problems of GMP, thus considered the enormous potentiality of genome editing the challenge is urgent a regulatory response towards the social acceptance of genome-editing crops, avoiding in the EU the mistakes of the past with GMP. Finally, a global policy for the “new” biotechnology has become necessary debating the integration of genome-editing crops into society.

Key message: “Maximizing crop yield while at the same time minimizing crop failure for sustainable agriculture requires a better understanding of the impacts of plant breeding on crop genetic diversity” is this ancient message prosecutable also by genome editing?

Keywords Conventional plant breeding; Genetically modified plant (GMP); Genome editing (GE); Global policy for new GE varieties

Introduction

Plant breeding since the early 1900s has made a deep impact on food production and it will stand to play a pivotal role in the evolution of modern agriculture [1,2]. Genetics and conventional breeding aims to improve plants and derived products mainly for human food, as well as for many other worldwide needs, being carried out for thousands of years. To achieve more yielded or pathogen and insect-resistant plants, conventional breeding involves crosses between superior plants within the same species (intra-specific recombination). Further, natural variation among wild relatives of cultivated crops is in many species an underexploited resource in plant breeding. Thus, marker-defined genomic regions derived from wild species and introgressed into crop lines (interspecific recombination), provide plant breeders with a key chance to improve the agricultural performance of modern crop varieties.

As considered that genes are the primary elements determining qualitative or quantitative traits desired by breeders, the first step of genetics has been the cross between different parents to obtain thousands of recombinants, as a consequence of natural variation reduction but not only, the intention to mutate these genes specifically became crucial in the second half of last century. As a result, traditional plant breeding by using recombination and selection has been skillful over the last 50 years by mutagenesis using chemical compounds and X-ray application, followed by screening of mutation populations for the desirable traits. Through applications of many effective breeding methods, ranging from introduction, phenotypic selection on natural

variants, selection with controlled mating, to marker-assisted selection for desirable genes, many new cultivars have been developed [3].

Crosses and recombination, mutagenesis, translocation by introgression [4,5] and all others traditional plant breeding techniques is considered indeed largely non-specific, as either a large genome region instead of a single gene is transferred by crossing, the same when random thousands of nucleotides are mutated.

The recombinant DNA technology marked the beginning of a new era. For the first time, molecular biologists gained the ability to manipulate DNA molecules, making it possible to study genes and produce genetic modified organism (GMO). At the end of last century (90' years), the transgenic approach was added to the classical plant breeding techniques in order to produce genetically modified plants (GMP) and new varieties. These aimed to introduce useful genes in plants also from other living organisms (e.g. Bt-toxin from bacteria), and at modifying plants to produce desirable products. When compared to conventional breeding, GMP have gone beyond natural cross barriers, resulting in plants that were unreachable by conventional breeding.

Transgene technology created great opportunities but many questions about GMP impact on health and the environment remained unsolved. A large political discussion took place in 90' years, resulting in formulation of the Cartagena Protocol on Biosafety in 2000. This protocol mainly covers the intentional release of GMO into the environment, and also introduced the precautionary approach. A recent review provided an overview about the last decades of market approval and cultivation of GMPs [6]. The unbalanced opportunities offered by GMP among EU, USA, China, Australia and South America due to the farraginous GMO legislation in EU and also to GMP food

unacceptance by EU consumers have also to be discussed. Finally, the consideration that many traits of interest are quantitative (QTL) with the problems of pyramiding genes, transgenic cannot be considered an alternative to QTL principles especially when genome wide aimed approaches have been demonstrated to be powerful as the theoretical basis for both population improvement and methods of selecting and stabilizing desirable genotypes [7].

Thus, breeding has always been critical for improving crop production especially nowadays with a changing world, a growing population and in the face of extreme environmental change. Molecular assisted selection (MAS) and transgenic breeding are the major procedures of modern plant breeding schemes. What are still the limitations of these approaches? Backcrossing and MAS are laborious, time-consuming and besides unavoidable introgression of closely-linked undesirable traits from donor organisms. On the other hand, transgenic breeding presents the major criticism about the random gene insertions throughout the genome and the disruption of favorable genes function.

More recently, the knowledge in genome engineering technologies are flashing a new revolution in biological research. Rather than studying DNA taken out of the context of the genome, now the researchers can edit the function of DNA sequences in their genetic loci in virtually any organism of choice, enabling them to elucidate the functional organization of the genome at the systems level, as well as identify causal genetic variations [8]. Remarkably, with the advent of gene editing technologies, mainly the CRISPR/Cas9 system, an extremely efficient and simple customized gene modification process has been offered [9].

As well documented, genome editing (GE) can easily target any site of interest as well as cost effective cloning, appearing a promising tool for updating basic research and plant breeding [9]. Gene editing relies on sequence-specific nucleases to trigger DNA at a desired location within the genome. The DNA breaks are then restored by either non-homologous (NH) or homology-directed (HD) repair [10]. The NH repair leads to modifications of the targeted sequence, such as deletions or insertions. Unlikely, native genes can be replaced or corrected by a HD DNA donor repair template to the targeted gene. This latter GE technique is more attractive for plant breeding because of precisely integrates the desired gene with the important agricultural trait from gene pool into a target site within a genome.

In light of the interesting traits for sustainable agriculture (tolerance to abiotic stress and efficient use of nutrients) are typically encoded by multiple and interacting genes, could genome editing overcome the encountered GMP-limit of pyramiding genes modification? The challenges derived by these traits complexity nowadays will appear superable by the enormous knowledge derived by annotated genomes of many crops and GE technology which will create targeted knock-in mutations via site specific DNA integration playing a major role in addressing these challenges [11]. Genome editing appears the way for accelerating the introgression of single gene, even pyramiding some elite genes into the breeding lines, thus provide a high-efficiency and

promising strategy for plant breeding. The evidence of GE impact is demonstrated by the number of publications (e.g. CRISPR publications have increased from 50 in 2010 to 1400 in 2015).

Notwithstanding "Genome editing" had occurred often over time through natural mutations, but also with mutagenesis resulting in thousands genetic changes in plants which have benefited agriculture, the acceptance of these novel plants could become a problem. Presently, the crossroads is how GE technologies are being evaluated and how products from GE should be regulated.

Nowadays a regulatory response is urgently required towards the social acceptance of genome-editing crops, avoiding in the EU the mistakes of the past with GMP. A need of debate on how GE crops have to be released to the consumers is necessary, but a political decision should be implement as soon as possible. Indeed, GE technology is already producing many novel plants [11] that are similar or identical to plants generated by conventional breeding, creating indistinct boundaries with regards to GMP regulations. Thus, a new global policy for the "new" biotechnology has become necessary debating the integration of genome-editing crops into society, in which such movement demands labeling of food which contains genetically engineered ingredients [12].

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