CellPress

Review

Opportunities for Products of New Plant Breeding Techniques

Jan G. Schaart,[1,*] Clemens C.M. van de Wiel,[1]
Lambertus A.P. Lotz,[2] and Marinus J.M. Smulders[1]

Various new plant breeding techniques (NPBT) have a similar aim, namely to produce improved crop varieties that are difficult to obtain through traditional breeding methods. Here, we review the opportunities for products created using NPBTs. We categorize products of these NPBTs into three product classes with a different degree of genetic modification. For each product class, recent examples are described to illustrate the potential for breeding new crops with improved traits. Finally, we touch upon the future applications of these methods, such as cisgenic potato genotypes in which specific combinations of *Phytophthora infestans* resistance genes have been stacked for use in durable cultivation, or the creation of new disease resistances by knocking out or removing S-genes using genome-editing techniques.

Trends

Several NPBTs are currently being implemented and represent a significant step forward for crop improvement compared with traditional breeding.

NPBTs make use of a genetic modification step, but the resulting endproducts do not contain any foreign genes. Consequently, NPBT products are genetically similar to, or may be even indistinguishable from, traditionally bred plants.

Recent studies show the remarkable potential of NPBTs for the production of innovative crop varieties.

New Plant Breeding Techniques Facilitate Breeding of Improved Crop Varieties

Crop improvement is an important endeavor if we are to meet the demands of a growing population (a worldwide population of 9 billion people is projected for 2050), for which food production needs to be increased, while at the same time the environmental impact of food production needs to be reduced. To respond adequately, we should optimally apply all existing tools to breed improved crops and maximize any potential future applications for increasingly sustainable food production.

Plant breeding has resulted in numerous improved food, feed, ornamental, and industrial crop varieties and traditional breeding based on crossing and selection remains an important activity for crop improvement. Although the efficiency of crossing and selection has been improved by using marker-assisted selection, it faces limitations in crops with complex genetics (e.g., due to polyploidy, heterozygosity, or self-incompatibility) or a long generation time (e.g., fruit trees). In addition, the search for useful genetic variation is often laborious, and introgressing such variation from wild relatives into the cultivated germplasm through crossing can be tedious.

Mutation breeding using chemical mutagens or ionizing radiation is used to create new genetic variation. The selection of mutants was originally based on phenotypic variation, but the availability of new advanced genomics technologies has facilitated the selection of plants with desired mutations. Nevertheless, screening for mutants remains time consuming and expensive, and requires large populations. Once mutant plants have been selection, subsequent breeding steps are required to achieve homozygous mutations and to remove undesired mutations. Although mutation breeding is regarded as genetic modification (GM), it is exempted from GM legislation by annex IB of Directive 2001/18/EC.

[1]Wageningen UR Plant Breeding, Droevendaalsesteeg 1, NL-6708 PB Wageningen, The Netherlands
[2]Wageningen UR Agrosystems Research, Droevendaalsesteeg 1, NL-6708 PB Wageningen, The Netherlands

*Correspondence: jan.schaart@wur.nl (J.G. Schaart).

 http://dx.doi.org/10.1016/j.tplants.2015.11.006

Over the past 15 years, several new techniques have been developed and are being implemented to facilitate breeding of improved crop varieties. Compared with traditional breeding, these techniques increase the precision of making changes in the genomes and thereby reduce the time and effort that is needed to produce varieties that meet new requirements. A common denominator of these techniques is that they make use of a GM step, but result in products in which no foreign genes (i.e., genes other than from the species itself or from cross-compatible species) are present. The exception is the use of genome editing to insert transgenes using **sequence-specific nuclease technology** (SSN-3; see Glossary), where the innovation is that genes can be inserted at a precise, predefined location, without the need for T-DNA border sequences, occurrence of small deletions, and so on.

In 2007, a New Techniques Working Group was established by the European Commission with the task of evaluating these new techniques of GM with respect to the current European Union (EU) genetically modified organism (GMO) legislation. This working group identified several NPBTs [1], and a short definition of each is given in the Glossary. In addition to these NPBTs, we also consider a new development, which we call '**induced early flowering**', as a NPBT.

The regulatory issues with regard to biosafety of NPBT products are complex, because the techniques vary greatly with regards to the technologies used and the impact of the applied modification on the plant genome. Some of the end products are indistinguishable from conventional plant breeding products. In the EU, the current GM legislation (European Directive 2001/18/EC) is process based, triggered by the use of a GM step. A product-based approach would be more flexible when new technological developments are applied. Such an approach is also more consistent with current scientific understandings of the risks involved with GM and would provide a more flexible approach to regulation. The regulatory issues were recently reviewed by Araki *et al.* [2], who addressed the fact that some of these techniques blur the current boundaries of product- and process-based regulations, but how they do that also differs between jurisdictions. The lack of clarity regarding the regulatory issues undermines confidence in the new technologies and, therefore, stifles investment and innovation [3]. Other recent reviews have also focused on regulatory uncertainty [3], social acceptance [4], and the technical side of NPBTs, in particular on current developments in genome-editing technology (e.g., [5–7]).

Here, we focus on the potential for innovative crop varieties made by using these new techniques, as has already been demonstrated in recent studies. We survey the type of products that can be made with various NPBTs and provide three product classes with a different degree of modification. For each product class, relevant examples of plants recently produced by NPBTs are described to illustrate the potential for new crops with improved traits. Finally, we touch upon possible future applications for these methods.

NPBTs Produce Three Types of Improved Plant

Products from NPBTs may be grouped into three classes: (i) improved plants that contain a new DNA fragment (usually a new gene); (ii) improved plants that do not contain a new DNA fragment, but have a mutation or modification in their own DNA; and (iii) improved plants that do not contain a new DNA fragment or any modification of their DNA (Figure 1, Key Figure). Below, we describe these different product classes.

NPBT Products that Contain New DNA Fragments

Products made with **cisgenesis**, **intragenesis** and specific cases of genome editing using SSN3 technology contain new DNA fragments (Table 1, Improved plant 1). Both cisgenesis and intragenesis are concepts relevant to genetic transformation technology and concern the origin of the inserted DNA. For cisgenesis, a copy of a complete natural gene, including the promoter

Glossary[†]

Agroinfiltration: a technique using *Agrobacterium* as a tool to achieve temporary and local expression of genes in plant tissue. Agroinfiltration is applied for testing the reaction of target plants to transgenic proteins, or for functional gene analysis in plants.

Cisgenesis: the production of plants by genetic modification using only genes from the species itself or from a species that can be crossed with this species using traditional methods (for overview of these traditional methods, see [iv]). The genes used are added as an extra copy and are natural variants.

Grafting on GM rootstock: the top of a nonGM plant is grafted on a GM rootstock. In such a graft, the scion may benefit from traits conferred by transgenes in the rootstock, but its products do not contain the transgene itself.

Induced early flowering: a recombinant gene that induces flowering is introduced into a plant. The gene confers flowering of the seedlings in the first year and, thus, speeds up breeding in species with a long generation time. The recombinant gene is removed in the final cross(es) leading to the end product that will be marketed. In an alternative approach, the gene is delivered in the form of a virus [44].

Intragenesis: similar to cisgenesis, because it uses only gene sequences from the species itself or from a crossable species, but the genes introduced are novel combinations of functional elements originating from different genes.

Oligo-directed mutagenesis (ODM): introduces specific mutations at defined locations in the plant genome. Synthetic oligonucleotides homologous to the target DNA, but containing mismatches, are introduced into plant cells. The mismatches in base pairing between the oligonucleotide and target DNA are corrected by the native repair mechanism of the plant, resulting in point mutations in the targeted DNA.

Reverse breeding: an approach for the generation of homozygous parental lines from any heterozygous line. By crossing these homozygous parental lines, the heterozygous line is reproduced as a form of hybrid seed production. Reverse breeding may make use of genetic