Guide: Evaluation of Genome Edited Plants

Background

Over thousands of years, humans have directed the evolution of plants by selectively saving and planting seed from wild, gathered plants with attributes of human value; for example, better flavors, larger fruits, fewer thorns, more nutrients and seeds that do not shatter. As is true of evolution by natural selection, genetic variation is the essential resource upon which plant breeding is based.

Plant breeders use and introduce genetic variation through crosses with related plants and wild relatives with desirable characteristics, such as yield, size, shape, color, taste or disease resistance. They also utilize genetic variation created through random mutations - spontaneous and induced - to develop plant varieties with improved characteristics. Using these traditional breeding methods, it normally takes many generations of repeated cycles of selection of plants, or recurrent backcrossing to an elite parent, to generate plants with the best combination of characteristics to produce a commercial variety. During the breeding and selection cycles, any off-types, unstable lines, or lines showing undesirable characteristics due to their genetic make-up, such as poor response to environmental stress and disease susceptibility, are discarded. Despite being time and labor intensive, traditional breeding methods are effective and are responsible for the abundance of plant varieties we have today.

Today, plant breeders can also use genome editing tools to introduce genetic variation. There are different types of genome editing tools. Those that use site-directed nucleases (SDNs) rely on custom-designed DNA nucleases to introduce a double-stranded break at a predetermined, specific genomic location. Repair of this double-stranded break by the cell's natural repair process leads to the introduction of mutations or DNA insertions at the break site. SDNs currently utilized in plant research include Meganucleases (MN), Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs) and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated proteins. In each case, SDNs consist of a DNA binding region (protein, or in the case of CRISPRs, RNA) and an endonuclease. The DNA binding regions are custom-designed to bind to a unique, predetermined DNA sequence in the plant genome. Oligonucleotide-Directed Mutagenesis (ODM) is another

gene editing tool for targeted mutagenesis, employing a specific oligonucleotide to produce predetermined DNA base changes in the plant genome.

Genome editing tools can introduce a range of targeted changes in endogenous plant genes, from small mutations in the plant genome, such as nucleotide deletions or additions, to allele swaps. An allele swap recreates an allele (gene variant) directly in an elite variety by using the SDN to make a DNA break, and a template to direct the repair of that break. Thus, an allele for a desirable trait, such as a disease-resistance gene from a different variety or a wild relative, can be reproduced directly in the elite genetic material. This avoids the laborious process of backcrossing, used to keep the desired traits while discarding plants that express undesirable traits. All new plant varieties, regardless of how the specific combination of characteristics was achieved, will typically undergo multiple years of standard product selection and testing under geographically diverse commercial field/production conditions to confirm that the lines identified for commercial release will perform as expected.

There is the possibility that genome editing tools can result in off-target genetic changes; the potential for off-target genetic changes can be mitigated but it is also important that off-target genetic changes be considered specifically within the context of plant breeding:

- Gene editing tools increase the specificity of mutation breeding strategies by predominantly binding to sequences that are extremely similar to those for which they are designed. Therefore, appropriate protocols and design criteria for editing reagents, such as the choice of the target and guide RNA design, are the most important factors to minimize the potential for off-target activity (see guidance below).
- If an off-target genetic change should occur through the gene editing process, these changes are genetically comparable, yet much less likely to occur, than multiple changes from spontaneous, chemically- or radiation-induced mutations historically used as sources of genetic diversity by breeders.
- Well-established plant breeding and selection practices for new plant variety development effectively identify and remove off-type plants while retaining plants with improved characteristics. Off-types due to introduced mutations, regardless of their source, spontaneous, induced, or through gene editing, will similarly be removed and not be selected for commercialization.

Objective of Guidance

The focus of this Guide is limited to those applications of genome editing that use the plant's gene pool to create genetic variability, leading to a new plant variety with improved plant characteristics or performance. These applications result in a plant variety that does not contain any DNA sequences from a non-sexually compatible species. The goal of the Guide is to assist developers in their characterization and evaluation of these types of genome edited products. By applying the guidance outlined in this document, a developer can identify and assess the most

appropriate way to confirm these genome edited products contain the targeted genotypic and phenotypic changes and are subjected to the processes that plant breeders have historically used to develop safe and efficacious new plant varieties.[1]

Guidance on Product Design and Characterization

This Guide is focused on the genome edited plant and not on the genome editing process itself. There may be different considerations depending on the genome edit and delivery method used, such as RNA-protein based methods or modular protein based methods. An overarching commitment is compliance with relevant laws, regulations and standards in the country in which the developer operates.

1. If DNA vector sequences are used, verification/determination of the absence of those vector derived sequences should be confirmed by one of the standard, acceptable methods to show that the plant does not contain vector-derived DNA.
    - A developer should identify an appropriate molecular analysis to confirm with confidence the absence of unintended vector-derived, transgenic sequences utilized in the genome editing process. For example, analysis utilizing southern blots, genome sequencing, and/or Polymerase Chain Reaction (PCR) amplification should be considered.

2. A developer should identify an appropriate molecular method to confirm and describe the targeted DNA change(s) that were made.
    - A developer should identify an appropriate molecular analysis using commonly accepted techniques to determine the DNA sequence of the targeted region to characterize the DNA change.

3. A developer should identify and implement appropriate protocols and design criteria for the editing reagents, such as guide RNA, to minimize the potential for off-target edits (considering potential off-targets to homologous gene sequences).

4. A developer should apply appropriate breeding and selection process(es) to confirm the intended phenotype, and identify and eliminate off-type plants.
    - Refer to the ASTA document, Common Practices of Plant Breeders, which can be found here.

[1] This document is intended as general guidance to assist companies and does not prescribe the only practices that can be used to achieve the objectives of the guidance.

References and Resources

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