International Seed Federation
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Future Proofing Policies for Products of Plant Breeding Innovation:

Application of the International Seed Federation (ISF) Criteria for the Scope of Regulatory Oversight

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Addendum/Explanatory Note to the ISF Position Paper: Plant breeding innovation: Consistent criteria for the scope of regulatory oversight

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BACKGROUND

The scientific basis and understanding of the genetics of crop plants is continuously advancing as is the range of innovative plant breeding tools and their applications in breeding programs. The ISF policy principles for determining the scope of regulatory oversight for products of plant breeding innovation (first published in 2016) provide a framework for ensuring that policy and regulatory approaches are "future proof" in the context of the expanding use of innovative technology applications in plant breeding. These technologies and their applications are discussed in the appendix to this paper.

The continuous development of breeding tools is a result of an increase in scientific understanding of the genetics and biology of plants. The most recent of these new breeding tools falls into the category of genome editing. Therefore, this paper and appendix primarily focus on the different applications of genome editing.

The underlying basis for the ISF criteria[1] for determining the scope of regulatory oversight for products of plant breeding innovation is that the application of the latest plant breeding methods can result in genetic changes that are indistinguishable from, or similar to, the changes in plants obtained through earlier breeding methods or can result in changes that can happen in nature. Therefore, under the ISF criteria, plant varieties should not be covered under the scope of existing biotechnology/GMO regulations if one of the criteria below is met:

a) There is no novel combination of genetic material (i.e. there is no stable insertion in the plant genome of one or more genes that are part of a designed genetic construct), or;

b) The final plant product solely contains the stable insertion of genetic material from sexually compatible plant species, or;

c) The genetic variation is the result of spontaneous or induced mutagenesis.

Regulatory policies in many countries have been established based on criteria similar to those developed by ISF. These policies provide a useful benchmark for governments that are still in the process of reviewing their policy approaches for the regulation of products of innovations in plant breeding, particularly applications of genome editing. The ISF criteria ensure a proportionate and science-based approach toward plant breeding innovations. The criteria can also guide discussions on future proofing policies in the context of the growing diversification and expanded application of innovative breeding tools driven by advancing scientific and technological developments.

USE OF THE ISF CRITERIA FOR THE EXPANDING RANGE OF GENOME EDITING APPLICATIONS

The ISF criteria remain appropriate for determining the scope of regulatory oversight for plant products produced through a broad range of applications of genome editing. Examples of applications that can be achieved through genome editing include:

- mutations, such as deletions, substitutions, as well as chromosomal rearrangements
- gene duplications

Such application can also be obtained with earlier breeding methods or can occur in nature. Therefore, these products should not fall within the scope of GMO/biotechnology regulatory

oversight in line with the ISF criteria. A more detailed description of these applications can be found in the appendix.

POLICY IMPLICATIONS

Policy approaches for products of genome editing have been implemented in several countries[2] [3]. Other countries are in the process of reviewing current GMO/biotechnology regulations within the context of genome editing applications and some are in active discussions around *de novo* policies for applications of these innovative tools. It is critical that any policies/regulations that have been, or will be, put in place be proportionate, science-based and predictable, as well as flexible enough to take into account the evolution in scientific knowledge and the application of that knowledge. This is often called "future proofing" government policy and is essential for the continued enabling of innovation.

Those countries that have policies in place have implemented an overarching principle that applications of new methods like genome editing resulting in genetic changes comparable to the outcomes that could occur spontaneously or via traditional breeding should be excluded from current GMO/biotechnology regulatory oversight. On a more technical level, in alignment with the ISF criteria, countries have defined specific criteria to use in determining whether a genome edited product would not fall under the scope of GMO/biotechnology regulations. Criteria such as the presence/absence of a new or novel combination of genetic material[4], absence of foreign DNA/genes in the final product[5], or similarity to conventional/traditional breeding have been used as such a determinant of regulatory scope.

CONCLUSION

The range of methods and applications of innovative breeding methods like genome editing continues to grow, supported by the advancements in science and technology. While such applications are often described as new when they first become available, they are used by breeders to achieve the same breeding end goals as with earlier breeding methods. The question then becomes how countries—both those that have already implemented policies and those that are in the process of developing policies—will approach the expanding range of innovative genome editing applications. This underlines the importance of flexible and "future proof" policies that can keep pace with these advancements so that plant breeders can successfully incorporate these innovations to improve the efficiency of their work. The ISF criteria for the scope of regulatory oversight for plant breeding innovations provide the required flexibility to define when the resulting products should be excluded from the scope of existing GMO/ biotechnology regulations.

APPENDIX

PLANT BREEDING AND THE IMPORTANCE OF GENETIC VARIATION

The goals of plant breeders have always been to create new variations of plant characteristics, to provide solutions for diseases and pests, to increase tolerance to environmental stress, to improve quality and yields, and to meet consumer expectations. Plant breeding depends upon genetic variability within and across related species as a basis for developing new plant varieties with improved traits.

The science of plant breeding began to develop at the beginning of the 20th century with the growing understanding of the rules of inheritance discovered by Gregor Mendel and better knowledge of crop plants' reproductive biology. Plant Breeding is the professional application of different science disciplines such as cell biology, genome and proteome research, gene mapping and molecular markers for the genetic improvement of plants. It is often said to be a process not of selection, but one of elimination. Any off-types, unstable breeding lines, or lines showing undesirable characteristics such as significant differences in nutrient content, detrimental responses to environmental stresses, susceptibility to diseases, or the presence of other undesirable traits are discarded during the plant breeding process as soon as they are identified. This process of selection and elimination takes place over the course of several years in numerous geographical locations and in diverse environments so that the lines identified for prospective commercial release will perform as expected.[6]

With the discovery of DNA in the 1950s, breeders gradually began to understand and characterize the enormous and continuously evolving genetic variation in plants and to reliably associate genotypes with major phenotypic characteristics. Today, breeders have access to a multitude of tools including genomics, sophisticated imaging and other analytical techniques to improve the effectiveness and efficiency of the breeding process and to deliver plant varieties that reliably produce safe, nutritious, and good tasting food.

Using the increased understanding of plant genetics, plant breeders continue to develop a variety of breeding methods to safely increase the precision of the breeding process so that farmers can continue to produce more food more efficiently than today to meet the challenges of rapid environmental changes, as well as to reduce the environmental footprint of farming. Breeders and farmers are overcoming obstacles, such as drought and plant diseases, with improved seeds, healthier soils, precision equipment and useful data as well as other basic tools of modern agriculture. Precision breeding methods, including genome editing, will contribute to these sustainable farming systems. Virtually all crops today have gone through some degree of domestication and improvement through human intervention. Over the last several thousand years, humans have directed the evolution of plants by selectively saving and planting seeds from the wild, gathering plants value which were the results of mutations in key genes resulting in attributes of human value; for example, healthier plants, better flavors, larger fruits, fewer thorns, more nutrients and seed/grain that is readily harvested. This practice began with the domestication of some wild crops. As an example, wheat and barley were domesticated some 13,000 years ago in the fertile crescent of Mesopotamia and the Levant. The process of constant genetic improvement continues today in a more systematic way using the science of plant

breeding. As is true for evolution by natural selection, genetic diversity is the essential resource upon which plant breeding programs are built.

Genome editing and genomics offer the potential to accelerate the adaptation of unimproved plant species opening the door to new, heartier crops that are both nutritious and convenient[7]. It also holds the promise to shorten the breeding cycle in response to a rapidly changing environment. For example, genome editing has been used to improve the agronomic characteristics of several, unadapted, plant species, such as groundcherries, wild tomatoes, and perennial intermediate wheatgrass.

GENETIC VARIATION THROUGH SPONTANEOUS, INDUCED AND TARGETED MUTATIONS

Plant breeders have long utilized inherent and spontaneous genetic variation--such as mutations, horizontal gene transfer, homologous recombination-- to produce plants with improved characteristics. Spontaneous mutations occur continuously in plants at low frequency and bring about genetic changes that are the basis of evolution[8] [9]. These spontaneous genetic changes can result in different changes at DNA level, such as deletions, rearrangements, or insertions of nucleotides as well as larger rearrangements of DNA sequences or genomic regions. Genomic rearrangements also occur in every generation due to chromosomal recombination during meiosis (the process of formation of pollen and egg cells in plants). All types of changes and rearrangements are fundamental to a plant breeder's ability to make improvements to plant performance.[10] [11] [12] [13] Moreover, many important agronomic traits have been introduced through the introgression (transfer) of large fragments of DNA from related species carrying desired features such as disease resistance. Examples of such introgressions are the transfer of rye disease resistant genes into wheat[14].

The frequency of beneficial, spontaneous mutations is so low that their identification, when at all feasible, is challenging and is only possible through extensive and careful screening of a large number of plants. Once identified, desired changes must be incorporated into breeding lines to ultimately obtain new varieties with desired characteristics.[15] Semi-dwarf cereal crops are an example of a spontaneous mutation creating a characteristic that has helped to improve yield and has been broadly incorporated into modern breeding lines. Because spontaneous, beneficial mutants occur at such a low frequency, breeders have employed methods, such as chemical treatments and irradiation, to increase the rate of DNA mutations and thus increase the chance of identifying desirable mutations. Since the 1950s, well over 3200 crop varieties have been directly developed by selection of induced mutations.[16] [17] [18]

Today, with a deepened understanding of gene functions, certain applications of genome editing have enabled refinements of earlier mutation breeding methods and enable targeted introduction of desired genetic changes in the plant genome. Besides the improved specificity and precision of the methods, the resulting DNA changes are analogous to and indistinguishable from the deletions, insertions and rearrangements that can be obtained using earlier mutagenesis techniques or spontaneous changes by nature.[19] [20] [21] The improved precision of genome editing – the introduction of desired changes at predetermined locations in the genome - is due to the ability to introduce a targeted DNA "modification" at or near the location of the desired change.

Diverse and adaptable plant systems are essential to meet changes in weather patterns, land uses, production systems, and ecology that create ever-changing and new stresses on the crops or

plants that we choose to grow. Changes in markets, prices, technology, and farmers' as well as consumer needs create challenges as well as opportunities. To address these challenges and opportunities, plant breeders have always focused on developing varieties with new characteristics and utilizing all the breeding tools available in order to meet their plant breeding goals.

THE APPLICATION RANGE OF THE LATEST BREEDING METHODS

Increasing understanding of the genetics and biology of plants is being translated into the development of new breeding methods as well as into new applications of those methods. The most recent of these breeding tools falls into the category of genome editing. Therefore, the following paragraphs primarily address different applications of innovative tools like genome editing.

There are a number of genome editing tools that are used by plant breeders: Meganucleases (MN), Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs) and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated proteins. These tools are commonly grouped together under the name of Site Directed Nucleases (SDNs). SDNs are protein complexes that consist of a DNA recognition and 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. Second and third generation genome editing tools have evolved that don't rely on nucleases (see below), introducing genetic and epigenetic changes without DNA breaks. Because the field is moving rapidly, it is important to note that nomenclature, such as "SDN" is becoming less relevant. Oligonucleotide-Directed Mutagenesis (ODM) is another category of gene editing tool for targeted mutagenesis, employing a specific oligonucleotide (a short stretch of DNA or RNA sequence) to mediate the introduction of small, often single DNA base changes in the plant genome.

For agricultural applications, the ability to introduce targeted and specific changes to plant genomes has expanded the possibility to successfully tackle diverse breeding objectives -- from improving the efficiency and reducing the duration of the breeding process to the improvement of specific traits. In addition to the applications in breeding, genome editing has become a standard research tool to study gene function.

The science around genome editing continues to evolve and with that the range of genome editing methods that breeders can select from continues to expand. Examples of recent additions to the family of genome editing tools include but are not limited to:

- **Base Editing**: a tool for targeted nucleotide substitutions by catalytically impaired ("dead") Cas enzyme fused to deaminase enzyme. This approach does not require the generation of a DNA double strand break (DSB) or the use of DNA repair template[22].
- **Prime Editing**: a tool for targeted nucleotide substitutions, small insertions or deletions by a Cas9 nickase domain and an engineered reverse transcriptase domain. This approach also does not require the DNA DSB and the desired DNA sequences changes are encoded in a reverse transcription template

- **Epigenetic Editing**: a tool for making targeted changes in gene function that do not entail a change in DNA sequence. Targeted changes in DNA methylation through genome editing can result in changes in gene expression (epigenetic changes) that in some cases can be inherited over several generations.

With the ever-growing interest in the further development and improvement of genome editing methods, it is expected that new tools and refinements of existing tools will continue to emerge.

Alongside the improvement and diversification of genome editing tools, we are also witnessing a diversification and expansion in the ways these tools are being applied in breeding to achieve specific outcomes in a more rapid and directed fashion. Thus, as the tools and methods evolve so do the applications of those methods. Below, are examples of naturally occurring genetic changes that can also be induced by traditional breeding methods but can also now be achieved with improved efficiency using tools like genome editing:

- epigenetic changes of the genome without changing the DNA sequence[23] [24] [25] [26]
- disruption of genetic linkage drag and increase in genetic variation [27] [28] [29] [30]
- gene duplication, in the same genomic location, (i.e., cisgenic "insertion" without using a repair template DNA) [31] [32] [33] [34] [35]
- different chromosomal rearrangements as they occur in nature and used in breeding, e.g., inversions[36], deletions or translocation[37] of a chromosomal segment
- chromosomal replacement/duplication/substitution/additions[38]

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