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Towards social acceptance of plant breeding by genome editing

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Although genome-editing technologies facilitate efficient plant breeding without introducing a transgene, it is creating indistinct boundaries in the regulation of genetically modified organisms (GMOs). Rapid advances in plant breeding by genome-editing require the establishment of a new global policy for the new biotechnology, while filling the gap between process-based and product-based GMO regulations. In this Opinion article we review recent developments in producing major crops using genome-editing, and we propose a regulatory model that takes into account the various methodologies to achieve genetic modifications as well as the resulting types of mutation. Moreover, we discuss the future integration of genome-editing crops into society, specifically a possible response to the 'Right to Know' movement which demands labeling of food that contains genetically engineered ingredients.

The need for regulatory models

Genome-editing via technologies such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas systems (e.g., Cas9) offers the ability to perform robust genetic engineering in many species [1–3]. For example, by utilizing plant genomic information, genome-editing is expected to generate many new crop varieties with traits that can satisfy the various demands for commercialization. However, one of the new plant breeding techniques (http://ipts.jrc.ec.europa.eu/publications/pub.cfm?id=4100), genome-editing, allows plant breeding without introducing a transgene, and this has led to new challenges for the regulation and social acceptance of GMOs [4–8]. This modern genome-editing technology can produce novel plants that are similar or identical to plants generated by conventional breeding techniques, thus creating indistinct boundaries with regards to GMO regulations [4–8]. Therefore an appropriate regulatory response is urgently required towards the social acceptance of genome-editing crops. Here, we review the recent development of genome-editing of major crops and propose a concept of appropriate regulatory models by unraveling the indistinct boundaries. In addition, we discuss how breeders should respond to the Right to Know movement which demands labeling of genome-editing crops that are released to consumers.

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Genome-editing-mediated plant breeding

Conventional genetic engineering begins with extracellular DNA manipulation to construct a plasmid vector harboring the gene or specific DNA sequence to be transferred into the chosen organism. The entire plasmid or only the DNA fragment is then shot into plant cells by using particle bombardment or delivered into the cells by polyethylene glycol or *Agrobacterium*-mediated transformation. The modified plant cells are then used to generate a GM plant. When the gene is derived from an unrelated, cross-incompatible species, the process is referred to as transgenesis. When an identical copy of a gene from a cross-compatible species (cisgene) is transferred to a related species, the process is termed cisgenesis [9]. In intragenesis, transferring a DNA sequence creates a new combination of gene elements (promoter, coding region, and terminator) that are derived from different genes within the cross-compatible species [9]. However, because homologous recombination rarely occurs in plants, exogenously delivered DNA molecules, even if they are designed to induce homologous recombination in a target gene, frequently integrate into nonspecific sites in the plant cell genome [10,11] via non-homologous end-joining (NHEJ) [12]. Thus, conventional genetic engineering is labor-intensive and requires time-consuming screens to identify the desired plant mutants. By contrast, genome-editing is an advanced genetic engineering tool that can more directly modify a gene within a plant genome [1–3]. The desired genetic modification is initiated by inducing double-stranded breaks (DSBs) into a target sequence by using nucleases, and is subsequently attained by DNA repair through NHEJ or homology-directed repair (HDR) [13]. The NHEJ pathway efficiently yields a small insertion or deletion (referred to as indel) in a specific locus without the use of exogenous DNA. By contrast, the HDR pathway can introduce a desired DNA sequence or gene into a targeted site, depending on the length of the exogenous DNA that is delivered to the plant cells together with the nucleases. Recent reports regarding genome-editing of major crops, including barley (*Hordeum vulgare*), maize (*Zea mays*), rice (*Oryza sativa*), soybean (*Glycine max*), sweet orange (*Citrus sinensis*), tomato (*Solanum lycopersicum*), and wheat (*Triticum*), have demonstrated a high efficiency of indels [14–25] in addition to the introduction of exogenous DNA in a targeted locus [17,26] (Table 1). Some reports have demonstrated that NHEJ-mediated indels can lead to disease resistance without the need to use a transgene [16–18,25]. Most notably, three homeoalleles of

Table 1. Examples of reported genome-editing-mediated gene modifications in major crops

Species	Target locus	Genome-editing technique	Modification type	Efficiency of modification	Off-target mutation	Genotyped subject	Refs
Barley	*HvPAPhy_a*	TALEN	Indel	16–31%	N.D.	Plantlets	[14]
Maize	*ZmIPK1*	ZFN	Inserting PAT	3.4–22.1% (autonomous)[a] 16.7–100% (non-autonomous)[a]	No	Calli	[26]
	ZmIPK	TALEN	Indel	39.1%	N.D.	Plants	[15]
	ZmIPK	Cas9	Indel	13.1%	N.D.	Protoplasts	[15]
Rice	*OsSWEET14*	TALEN	Biallelic indel	6.7–27%	N.D.	Plants	[16]
	OsPDS-SP1, OsBADH2, OsMPK2	Cas9	Biallelic indel	3.1% (*OsPDS-SP1*), 0% (*OsBADH2*), 0% (*OsMPK2*)	No (*OsPDS-SP1)* Yes (*OsMPK2*) N.D. (*OsBADH2*)	Plants	[17]
	OsSWEET11, OsSWEET14	Cas9	Indel	91% (*OsSWEET11*)[b] 90% (*OsSWEET14*)[b]	N.D.	Protoplasts	[18]
	OsBADH2, OsCKX2	TALEN	Biallelic indel	12.5% (*OsBADH2*), 3.4% (*OsCKX2*)	N.D.	Calli	[19]
	OsBEL	Cas9	Biallelic indel	2.2%	No	Plants	[20]
	OsPDS	Cas9	Introducing *Kpn*I + *Eco*RI sites	6.9%	No	Protoplasts	[17]
Soybean	*DCL4b*	ZFN	Biallelic indel	25%	N.D.	Plants	[21]
	FAD2	TALEN	Biallelic indel	33.3%	No.	Plants	[22]
Sweet orange	*CsPDS*	Cas9	Indel	3.2–3.9%	No	Leaf	[23]
Tomato	*PROCERA*	TALEN	Biallelic indel	2.5%	N.D.	Plants	[24]
Wheat	*TaMLO*	Cas9	Indel	28.5%	N.D.	Protoplasts	[17]
	TaMLO	TALEN	Heterozygous indel for all three homoeoalleles	3.7%	N.D.	Plants	[25]

Abbreviation: N.D., not determined.

[a]Two different donor constructs containing short homology arms were used: one with an autonomous herbicide tolerance gene expression cassette (PAT), the other with a non-autonomous donor that relied on precise trapping of the endogenous *ZmIPK1* promoter for expression of the marker.

[b]Indicates the results of sequencing after the enrichment of mutated alleles.

TaMLO were simultaneously edited in hexaploid bread wheat, resulting in heritable resistance to powdery mildew [25]. Moreover, maize which has indels in *ZmIPK1* is expected to have improved nutritional value as a result of decreased phosphorus content [15,26]. Furthermore, rice with indels in *OsBADH2* [17,19] may appeal to consumers in view of its improved fragrance [27,28]. Such results show that genome-editing dramatically simplifies plant breeding even in major crops, with potential impact on the future of agriculture and human nutrition. However, modification efficiency appears to vary based on the locus selected [17,19], although the selection of genome-editing systems [15] and crop species [17,23] has no significant effect on the efficiency (Table 1). Moreover, although Cas9-treated rice showed off-target mutations in *OsMPK2* [17] (Table 1), in most cases no off-target mutations were observed [17,20,22,23,26] (Table 1). However, most of these reports did not address potential off-target mutations. The occurrence of off-target mutations is one of the crucial issues in the agricultural use of genome-editing. Some off-target mutations are likely to result in silent or loss-of-function mutations, others might lead to immunogenicity or toxicity in the food products by changing amino acids within a protein, although there has been no documented instance of any adverse effect resulting from foods produced from GM plants [29]. It has also been speculated that the cultivation of crops with off-target mutations might affect an ecosystem as a result of crossbreeding. Notably, a plant with an entirely new trait, the resistance to two different herbicides, was recently found in North Dakota, USA [30]. It was reported that the herbicide resistance developed in the field owing to crossbreeding of wild type canola with herbicide-resistant genetically modified canola.

Although it is difficult to identify off-target mutations in the plant genome, breeders should demonstrate that no significant off-target mutations are associated with potential health or environmental risks. Otherwise, the imprudent use of genome-editing may lead to its rejection in agricultural and environmental applications.

Regulatory controversies

According to the Cartagena Protocol on Biosafety, a 'living modified organism' (the technical legal term that is close to GMO) is stipulated as 'any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology' (http://bch.cbd.int/protocol/text/). This definition suggests that some plants modified by genome-editing may be outside the scope of current GMO regulations because genome-editing can produce a null segregant (lines that lack the transgenic insert), thus blurring the boundaries in the GMO regulations [4–8].

The regulatory response to genome-editing of plants has been considered (or a decision has already been made) in Argentina, Australia, the EU, New Zealand, and the USA [4,8]. We have analyzed such regulatory responses and have summarized them in two categories regarding the presumed