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OBM Genetics

Opinion

Breeding "CRISPR" Crops[1]

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Academic Editor: Masahiro Sato

Special Issue: Genome Editing

OBM Genetics
2020, volume 4, issue 3
doi: 10.21926/obm.genet.2003115

Received: April 21, 2020
Accepted: August 04, 2020
Published: August 13, 2020

Abstract

The challenges which face the world today can be summed up in a few words: An increasingly congested world with dwindling areas of viable cultivated land and accelerating climate instability. The combined effect of these realities, together with the trend of striving to extend the average human life, puts the world on the path toward future catastrophe. This situation makes it imperative to seek realistic and practical solutions, which must be able to address food shortages and climate problems in a timely manner. In this article, an elucidative argument is presented with the intention of revealing the need for humanity to step back and consider more objectively the long-term benefits of crop-genome-editing for food security, looking beyond the unfounded negative notions about safety issues. If the faulty interpretations and arguments, which claim the CRISPR/Cas technology as being just another undesirable form of crop genetic modification stay unchallenged, they will continue to becloud the minds of decision makers and the public at large with inaccurate information. Eventually, the technology will be dismissed as a mere academic exercise with little or no benefit to future generations.

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[1] In loving memory of my beloved wife, Jean Georges.

Keywords

Agriculture; CRISPR/Cas; COVID-19; crop breeding; food safety; food security; GMO; genome editing; mutagenesis; plant productivity; regulatory issues

1. Introduction

In the plant kingdom, genetic variability enforced by necessity has been nature's most effective tool contributing to the vast diversity of plant types and species. These natural variations are brought about as a result of genetic refinements spanning millions of years in evolutionary alterations. In order to produce more efficient food crops and to meet the current world demands for food security, mankind must mimic nature's tools but not its longanimity, since we cannot afford millions, thousands or even hundreds of years to cope with modern food demands. Therefore, we need to be able to proactively anticipate future problems and consider available solutions without limiting our choices to inadequate alternatives.

Despite the impressive advances made in the area of plant genetic engineering (GE) over the past four decades and the production of genetically modified (GM) commercial crops with enhanced traits, the technology neither gained the popularity it deserved nor was it allowed to offer long term solutions to the ever growing demands for securing adequate world food supplies [1]. Nevertheless, in addition to its many documented benefits [2, 3], the technology also proved instrumental in building a powerful foundational understanding of gene function and regulation at the molecular level.

Further research provided the necessary infrastructure for what has now become one of the most significant accomplishments in the history of biology: Genome Editing (GenEd). The combination of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and the Cas9 endonuclease enzyme-system (and derivatives thereof), generically known as the CRISPR/Cas gene editing technology, is the most prominent concept now being used in crop GenEd projects [4]. Much of what has been promised or accomplished by the GE technology, and more, is now achievable with the GenEd counterpart.

The art of GenEd typically simulates film editing, where precise cuts are introduced in the film sequence to remove unwanted frames/clips or insert new ones, followed by end joining to create the desired edit. Likewise, GenEd is performed by accurately cutting DNA sequences using engineered site-specific nucleases (SSNs) to generate double-stranded breaks (DSB) in the target sequence in a sequence-dependant highly specific manner. Depending on the type of system used, the generated ends are then repaired and joined in a non-homologous fashion to create out-of-frame mutations, or by inserting or replacing a DNA clip through homologous recombination, thereby adding or deleting certain genetic information [5], resulting in the expression of a new trait.

A variant of this scenario also exists, where certain deaminases are used to effect targeted base-changes without introducing DNA breaks, whereby facilitating protein engineering through the introduction of amino acid changes, and (or) leading to expression termination or activation of genes [6].

Thus, the GenEd concept and its associated technologies constitute a form of an 'artificially induced' evolution process, which makes it possible to provide, both expeditiously and cost-effectively, what took millions of years for nature to achieve through its much slower version, breeding by natural evolution.

Within the limited scope of this article I will briefly reflect on the concept's positive long-term impacts on agriculture and food production by selectively looking at a few of the many innovative examples reported to date (section 2). I will also explore some of the potential barriers that need to be overcome, political or otherwise (section 3), and project a general outlook on opportunities that would be lost or gained with some additional food for thought (sections 4 and 5).

2. Relevant New GenEd Achievements

The importance of crop traits is perceived on the basis of qualitative and (or) quantitative measures. The former may entail nutritional issues or environmental pattern changes, while the latter is determined by the need for higher productivity. Since crop improvement for higher productivity implies higher yields (e.g., grain size and (or) number), resilience against losses to pests, diseases and other environmental factors, much of the focus of GenEd research has been to address these direct and indirect ways of yield improvement.

Higher yields may also be measured in terms of increased capacity for productivity per plant as opposed to greater number of plants per area (i.e., enhanced vertical productivity, EVP). An increase in the number of branches, for example, can achieve EVP.

2.1 Direct Yield Improvement

Direct yield improvement of this type was demonstrated in a GenEd experiment using CRISPR/Cas9, where disruption of the enzyme CCD7, involved in the biosynthesis of the shoot branching inhibitors, strigolactones, afforded increased tillering in rice [7]. A similar effect was also observed through the silencing of the rice *LAZY1* gene in an earlier CRISPR GenEd study [8].

Alternatively, EVP may also be achieved using the CRISPR/Cas9 system to influence grain number (GN) or size (GS). This was demonstrated by knocking out the yield-related *Gn1a*, *DEP1* and *GS3* genes in rice, producing mutants with enhanced grain number and larger grain size, respectively [9].

Moreover, as many genes exist in variant or multiple forms (e.g., alleles, homeologs, etc.), the high versatility of the GenEd methodologies is demonstrated by the ability to target common specific CRISPR sequences in the various forms of the gene and achieve multiple-gene knockouts simultaneously (multiplex GenEd) [10]. For example, in wheat, CRISPR/Cas9-based GenEd disruption of expression of homeologs of the grain weight determinant gene, *GW2*, was shown to enhance grain weight and protein content, which is another form of EVP [11].

2.2 Indirect Yield Improvement

Indirect yield improvement through GenEd includes minimizing losses to pests and diseases (biotic) or to environmental challenges such as temperature extremes, water availability, soil conditions, etc. (abiotic). Although these can be viewed from varied perspectives, they all converge into one ultimate goal, productivity and performance enhancement for better yields.

Two of the most visited stress-causing agents by scientists around the world are diseases and competition by weeds. Biotic stresses imposed by pathogenic microorganisms and insect infestations cause substantial yield losses, which can severely reduce global food production [12, 13]. Effective control of these types of stresses can be challenging, time consuming and expensive in view of the unpredictability of outbreaks and the efficacy of existing solutions and regimes [1].

Although disease and (or) herbicide resistant crop varieties have been developed through GE techniques or by using conventional breeding protocols [3], in retrospect, both approaches required long periods of time to obtain a good overall phenotype. The speed advantage of CRISPR GenEd breeding was demonstrated in tomato when the conserved *SlMlo1* gene was targeted for a 48bp-long deletion, leading to a new powdery mildew-resistant variety much more rapidly than would be expected of conventional mutagenesis [14]. Similarly, tomato protection against bacterial speck was achieved through editing of the *SlJAZ2* gene [15].

In wheat, simultaneous mutagenesis of multiple gene homeologs of *TaEDR1* resulted in protection against powdery mildew [16], while in citrus blocking of the disease susceptibility *CsLOB1* gene afforded protection against citrus canker [17]. Similar results were also obtained by the GenEd deletion of a specific region in the *CsLOB1* gene promoter common to other citrus gene alleles [18]. Thus, gene deactivation through GenEd can also be achieved by disrupting untranslated regulatory regions in the genome.

External abiotic factors that can indirectly hinder crop productivity and reduce yields are usually contributed by the crop’s environment. These range from drought, temperature extremes, high wind and poor soil properties. To date, drought and temperature extremes are two of the most worrisome abiotic stresses in agriculture, perhaps in recognition of changing global weather conditions in addition to soil and climate factors that affect crop survival.

Among the recent examples in which GenEd has proven its superiority in breeding is the achievement of drought resistance in maize [19]. In this example the CRISPR approach did not only afford drought stress resistance in maize plants but also led to EVP by providing a significant improvement of grain yield.

2.3 Enhancement of the Nutritional Value

Enhancement of the nutritional value of crops through GenEd has also received a significant amount of interest in line with its importance to future resources. For example, ratio modulation of the types of fatty acid constituents in an oil-producing crop would substantially increase its health benefits or industrial value. Although this area has been successfully explored by GE methodologies, it is now transcended by the more precise CRISPR GenEd techniques and appears to hold greater promise for the oil industry. A candidate crop in point is soybean, where recent CRISPR GenEd experiments demonstrated highly increased levels of oleic acid of up to >65% of the fatty acid composition as a result of mutating the corresponding desaturase *FAD2-2* gene [20].

3. Potential Challenges to Overcome

Despite the overwhelming technological advances and scientific innovations associated with it, for the GenEd technology to be adopted as a tool with high potentials for future food security, it must first overcome formidable obstacles embodied in the negative publicity, the fluctuating political views (particularly in Europe) and the subsequent societal distrust [1].

Despite the doubt instilled in the public minds by antagonists toward plant biotechnology in its various forms, in the medical field patients do not seem concerned about using drugs produced by genetic engineering including insulin, first marketed in 1986. Many new drugs are proteins produced by genetically engineered bacteria [21]. Vaccines and other immunity boosters used to fend against viral diseases can also be developed through biotechnological approaches, including GenEd.

Other complications that are being used against the technology are the intrinsic, and often encountered, off-target mutations, addressed below.

3.1 Technological Barriers

An inherent anomaly associated with the GenEd technology has been the possible existence of non-specific recognition sites that are very similar to the target sequence along the genome, where the Cas-type proteins could generate indiscriminate off-target cuts and produce unplanned sequence changes. For example, in some mammalian studies, CRISPR-Cas9 systems were shown to cause unintended genome modifications, potentially hampering gene therapy applications [22]. Human gene therapy requires that the intended modifications be exact and completely void of side-effects. On the other hand, with the off-target mutations being more accommodable in plant systems, they are still considered undesirable and have been observed and studied [23, 24].

While this type of irregularity could be invoked as a technological flaw and used as a recourse against the technology, it is rare and, in some cases, can be avoided altogether. For example, variants of the Cas proteins have been developed and different guide-RNAs (gRNAs) designed, which allow for more efficient targeting and higher degrees of precision [25]. In other designs, mutation is achieved through the use of two gRNAs and a dimeric Clo51 nuclease (Cas-Clover system). This design promotes the encasement of the target site to be mutated in a highly specific manner that no off-target binding is permitted [26]. Moreover, with the increasing ease of DNA sequence analysis, new GenEd crop varieties - harbouring only targeted edits - need be released [14].

3.2 Political Views

One of the most detrimental political views in Europe is the relentless attitude that unlike conventionally bred crops, those obtained through new breeding techniques (NBTs), e.g., CRISPR-GenEd, should be subject to the same rigorous rules and regulations laid out in the context of the EU GMO directive [27, 28] as applied to transgenics [29, 30]. Conceptually, the scientific merits of such notion are highly contestable both from a process-based and a phenotype-based perspectives.

From a process-based viewpoint, the genetic changes produced by the CRISPR-GenEd technologies can be molecularly indistinguishable from those accomplished through conventional or natural mutation. CRISPR-triggered modifications are also far more specific, with much less hazard potentials, than those induced by mutation breeding, where the mutation is elicited unnaturally through the use of harmful radiation or carcinogenic chemicals. However, while the aftereffects from all treatments are filtered out through multiple generational rounds before arriving at the final seed stock, the GMO products obtained by the use of harmful radiation and (or) chemical carcinogens are ironically not subjected to the same restrictive biosafety regulations being applied to those obtained through GenEd approaches. It is argued, however, that plants subjected to radiation or harmful mutagenic chemicals, are considered to have a history of substantial application and record of safety and are, therefore, excluded from the debilitating regulations imposed on transgenic GMOs as well as GenEd-produced crops. Such argument is flawed, since it overlooks the fact that this manner of harsh genetic mutagenesis was never met with the same degree of opposition in the early years of its adoption. If the standards being applied to transgenic-and GenEd-crops today were applied with equal rigour to the results of radiation and chemical breeding then, only few new cultivars would have survived and become useful, and the world would have been in a worse shape with respect to food abundance than it is now. Therefore, that the GenEd technology should be allowed the chance to establish a similar history of safety to parallel its already proven beneficial applications is only fair-minded and in order.

It seems that much of the ongoing debates in Europe have slowly taken the semblance of a prolonged workshop for finding new interpretations and definitions of what may or may not constitute a GMO [31] and coining more acronyms to add to the confusion. The political uncertainties created by such a drawn-out process, debating each crop-case individually to determine whether or not it should be regulated, is highly counterproductive, tedious and hindering.

The inflexible political atmosphere, insisting on complete and full regulation of GenEd crops of all types, as dictated by the EU Directive 2001/18, and the inability of member countries to decide individually on the legal status of GenEd products as, for example, in the UK [32] and Sweden [31, 33], and whether or not they should be judged in the same way as transgenics [33, 34, 35] is markedly at variance with the US position, which is more defined and straightforward. In the US, transgene-free CRISPR products are prevalently assessed by the US Department of Agriculture (USDA) on the basis of their phenotype. Under this accord, GenEd plants are treated as equivalent to plants obtained by conventional mutations, which means that they will not be evaluated in the same way as recombinant transgenics [14, 36]. This is further elaborated in the recently updated document on the Federal Coordinated Framework which comprises the USDA, the US Environmental Protection Agency (EPA) and the US Food and Drug Administration (FDA) [37]. A more recent comprehensive revision of the regulations has also been issued by the Department of Agriculture with the aim of further reducing the regulatory burden for developers (https://www.aphis.usda.gov/brs/fedregister/BRS_20200518.pdf). Similarly, in Canada, GenEd-produced crops are categorized under the existing umbrella of 'Plants expressing Novel Traits (PNT)', which denotes phenotype-based products and are, therefore, exempt from restrictive biosafety regulations [38].

In a recently issued draft document, the Government of India recognised that "Genome Editing Technology offers to increase yield and productivity of agricultural crops to meet constantly increasing demand for food and food security optimally by protecting them from various biotic and abiotic stresses and various other traits" (Draft Document on Genome Edited Organisms: Regulatory Framework and Guidelines for Risk Assessment. (2020) dbtindia.gov.in/sites/default/files/Draft Regulatory Framework Genome Editing 9jan2020a.pdf). For GenEd plants, animals and human cells the draft prescribes three distinct groups, forming the basis for the degree of safety evaluation. Groups I, II and III differ in the extent of genome modification and the editing techniques employed. Group I, being the simplest, includes single or few base pair edits or small deletions, while group II includes few to several base pair edits. Group III encompasses edits involving synthetic or foreign DNA incorporation. In the case of plants and animals, groups I and II follow similar regulatory pathways with group II requiring case by case assessment, while group III requires full assessment to establish absence of unintended changes. The regulatory pathway for edited human cells is determined on the basis of the type of cells used, e.g., somatic or germline cells with the latter being restricted in humans. Edits in both types of cells are subject to ethical clearance.

The degree of off-target edits, among other considerations, is being used in the draft as justification to dictate the mode of evaluation of GenEd crops, which means that for the most part these will continue to be heavily regulated. However, the draft's heavy emphasis on off-target mutations may be a blessing in disguise since it is conditional on the frequency of occurrence of nonspecific edits. Therefore, the ability to minimise or eliminate these as the technology evolves [26], sets the expectations for lesser regulatory measures. In other countries, e.g., Australia, Israel, South America, Asian and developing countries, similar considerations apply [33]. Of note, Argentina has adopted an exemplary guideline resolution (Resolution No. 173 of 2015) which enables applicants (scientists and industry) to obtain, beforehand, an assessment of whether or not their intended product, to be modified through NBTs, will be allowed to bypass hindering regulatory restrictions, in an "Instance of Prior Consultation" [39]. Such a brilliant move not only is it commendable as it proactively provides much needed saving of time and funds, but also necessary for the continuation of research and development of novel ideas and solutions. The initiative was also updated and maintained in Resolution No. 36 of 2019 [39]. It follows the guides and definitions outlined in the document, "Cartagena Protocol on Biosafety (CPB) to the Convention on Biological Diversity", regarding safe handling and use of "living modified organisms" (LMOs) developed through so-called modern biotechnology, including NBTs [40]. It is an example that should be considered of great benefit for other countries to follow.

In spite of all, whereas the GenEd technology is accepted, and the concept adopted, Argentina seems to still be unable to effectively penetrate the market - local or international - with novel crops. This is not a case of politics and counterproductive arguments. Rather, it is a paradoxical effect of an equally important set of common local issues, which require essential coordination of the various elements involved, e.g., scientists, funders, farmers, developers, and other authorities in a closely concerted manner to effectively achieve the business objectives. The "paradox" in Argentina is elegantly discussed by Lewi and Vicién [39].

In socioeconomic terms, the frustrating regulation policies of GenEd products in Europe [33, 34], compared to other parts of the world [41], will have many negative ramifications and impose potential trade problems [42, 43]. In the worst scenario, antithetical activists will continue to produce erroneous propaganda which will keep the public opinion and various committees' decisions entrapped in an endless loop of futility. Scientists and investors alike will be discouraged from keeping abreast with the technology, except from a purely academic interest, if they continue to be unable to sustain research funds or market their products competitively and in a timely manner [39]. Farmers, industrialists and commercial developers, in absence of clear regulatory processes and guidelines, will have less vested interest to venture into long term commitments, knowing that it will cost more in terms of time and money for their products to reach the market place than the anticipated returns.

In the meantime, where the technology and its marketable products are more readily accepted, commercialization of such products will be limited to local markets, and exports will be hampered or restricted to countries that approve of the technology [39, 43]. Moreover, it will still be difficult to prevent some GenEd crops from finding their way into restricted markets (e.g., EU) if they cannot be distinguished molecularly from GMOs produced by conventional means. Such is a possible overall scenario where progress toward sustainable agriculture future and global food security remains under siege thanks to misguided, uncompromising laws and legislations.

4. Concluding Remarks

The term genetically-modified-organisms (and its problematic acronym, GMOs) have been linked in many minds to meddling with the genetic makeup of an organism, e.g., a plant, by inserting foreign genes into its chromosomes (transgenesis) and generating an unnatural mutant or derivative. Given the fear of the unknown, this may create an apprehension in the layperson's mind that such a plant may not be safe to consume or may pose an ecological hazard. As such, GMO skeptics would have confusedly managed to promote the assumption that a genetically-unmodified-organism (GUMO), e.g., the 'Organic' gimmick, would be considered safer and more acceptable to the victimised consumer, because natural [44]. The question is: Is the average consumer aware that conventionally bred crops are also GMOs? Further, it is interesting to caution that transgenesis through foreign-gene-insertion can also occur naturally in some food crops, as is the case with sweet potato, for example [45]. The paradox is that such crops seem to be transparent on the GMO radar, and are not considered GMOs, even though they are caused by the plant pathogen *Agrobacterium tumefaciens*, the very same bacterium which has been, and still is, in use as a tool in plant biotechnology research for the past 40 years or so. The next question is, how would the consumer react if this became widely known [46]? Therefore, being natural, and hence, more acceptable to consumers is, of course, frequently untrue. In addition, this train of logic is self-defeating, in that GenEd may be used in a way which creates changes that resemble naturally produced mutations in every respect, including having no foreign DNA incorporation. That is, the CRISPR GenEd technology can be employed to produce crops which are difficult to distinguish from those produced by conventional mutation [30, 47]. Thus, the persistent counterproductive arguments insisted upon by anti-GMO/biotechnology activists become generally meaningless, unsubstantiated and should, therefore, be dismissed [48]. Furthermore, the controversial health and environmental hazards, claimed to be linked to plant biotechnology in general, should best be left to be substantiated by solid scientific evidence, as defined and prescribed by the Food and Agriculture Organization (FAO) [44], free from any political agendas, negative opinions or unproven theories.

Logic notwithstanding, experts predict that the impact GenEd-produced crops will have on the enhancement of consumer confidence in the technology may remain below optimal [49]. However, it is refreshing to see contrasting trends in terms of consumer awareness, as exemplified by a recent survey conducted in Costa Rica [50]. The study showed a high percentage of local consumers willing to accept the use of GenEd for crop improvement. Optimism was also evident among the surveyed group that CRISPR foods would increase crop production in the country and improve the economy. Almost 50% of the interviewees perceived low or no risk to the quality of life, health, and environment. This type of consumer confidence needs to be nurtured and developed further throughout the world. It would ultimately allow the public to play an active role in determining what is good for local societies and for humanity at large.

On the other hand, if the political charade of interpretation games continues to cloud the thoughts of policy makers, then the past forty years of scientific progress will have been of little or no practical avail.

5. Food for Thought

In the wake of the current world calamity of COVID-19, the race for developing an effective vaccine against the virus has become the main preoccupation of science around the globe. All manner of technological knowhow is being recruited in the fight to protect and preserve humanity and the planet as a whole. This sobering reality warns us of how fragile a species we can be in the face of overpowering out-of-hand circumstances. In this race, humanity stands grateful for the scientific tools of genomics, GMO, genetic engineering and genome editing, all of which are in the forefront of trying to understand and combat the threat of COVID-19. The very same technologies political activists have been aggressively battling to thwart without sound reasons.

In conclusion, I cannot emphasize forcibly enough the danger of reducing a significant scientific achievement such as the CRISPR crop improvement technology to the level of mere academic knowledge with no permissible practical benefits to humanity. We must face the challenge and strike a reasonable balance between fear and high hope before too late.

Acknowledgement

I gratefully acknowledge Dr. Adrian Cutler for agreeing to read through the manuscript for accuracy.

Author Contributions

Single-author contribution.

Competing Interests

The author has declared that no competing interests exist.

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