

PERSPECTIVE

Genome Editing-accelerated Re-Domestication (GEaReD) – A new major direction in plant breeding

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Background: The effects of climate change, soil depletion, a growing world population putting pressure on food safety and security are major challenges for agriculture in the 21st century. The breeding success of the green revolution has decelerated and current programs can only offset the yield affecting factors.

Purpose and scope: New approaches are urgently needed and “Genome Editing-accelerated Re-Domestication” (GEaReD) is proposed as a major new direction in plant breeding. By combining the upcoming technologies for phenotyping, omics, and artificial intelligence with the promising new CRISPR-toolkits, this approach is closer than ever.

Summary and conclusion: Wild relatives of current crops are often adapted to harsh environments and have a high genetic diversity. Redomestication of wild barley or *teosinte* could generate new cultivars adapted to environmental changes. De novo domestication of perennial relatives such as *Hordeum bulbosum* could counter soil depletion and increase soil carbon. Recent research already proved the principle of redomestication in tomato and rice and therefore laid the foundation for GEaReD.

KEYWORDS

de novo domestication, genome editing, resilient crops

1 | CHALLENGES AND EMERGING SOLUTIONS

Agriculture is faced with an urgent need to evolve in response to the global challenges of the 21st century. The recently released IPCC climate report indicates a faster progression of climate change than previously expected. Climate models predict severe impact for agriculture at a global level. In Europe, a severe impact on yield due to the temperature increase and changes in precipitation is imminent.^[1] Globally major impact of soil erosion in tropical regions will reduce the farmable

area.^[2] Currently, classical breeding for spring wheat can barely mitigate the existing yield affecting factors, and the situation is projected to get worse.^[1] Looking into other aspects that agriculture needs to fight, such as the impact of pests, biodiversity, soil depletion, and extensive fertilizer and chemical use, it is evident that a second green revolution of agriculture and plant breeding is urgently needed.

The advent of site-specific nuclease(SSN)-based genome editing technologies has resulted in a great interest in their applications in plant breeding. Much emphasis has been placed on the ability of genome editing technologies to introduce beneficial mutations in an elite genetic background without the high number of background mutations or linkage drag associated with conventional approaches. However, this “precision breeding” strategy has limitations: 1) it

Abbreviations: AI, artificial intelligence; Cas, CRISPR-associated; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; GEaReD, Genome Editing accelerated Re-Domestication; IPCC, Intergovernmental Panel on Climate Change; PAM, Protospacer adjacent motif; SSN, site-specific nuclease

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requires knowledge about the identity and function of the target gene and 2) it only applies to traits that are already described and which can be modified through one or a few genes. However, many genes or pathways leading to stress adaptation are not fully understood yet, or like drought stress extremely complex.^[3] A different strategy is needed to exploit the full diversity of adaptation found in nature. We propose that the answer may lie in turning the concept of precision breeding upside down. Rather than attempting to first understand and then re-engineer the complex genetic networks that result in environmental adaptation to wild plants, it may be more feasible to acquire these networks for agriculture by domesticating the wild plant itself.

The major staple crops of the modern world were domesticated in prehistoric times and perfected over millennia. Although there are some examples of modern domestication such as blueberries, blackberries, or strawberries, these novelty crops do not compete directly with the staples.^[4–6] It is undoubtedly a bold proposal to re- or de novo-domesticate staple crops, as already in depth described.^[7] However, we do so because we see the synergy of progress in two areas: genome editing technology and molecular characterization of the genes behind the domestication syndrome in major crops. Thus, it should be feasible to accomplish or repeat domestication by the application of genome editing to wild plants by using already domesticated relatives as a roadmap. This idea is, however, not entirely new; the idea of re- or de novo-domestication in the context of marker-assisted breeding has been around for at least 20 years.^[8] Moreover, with the recent breakthroughs in SSN technology, more and more people propose this idea.^[9,10] To differentiate from other forms of re- or de novo-domestication, and to incorporate the extensive use of new technology from interdisciplinary fields, we propose to call that approach “**Genome Editing-accelerated Re-Domestication**” (GEaReD) (see Figure 1).

GEaReD relies on natural selection occurring over millions of years to secure adaptation and resilience. Agronomic and possibly food safety traits are then introduced with techniques such as CRISPR/Cas9, CRISPR/Cas9 Integrase/Isomerase, or CRISPR prime editing to create highly adapted plants with yield and quality that can compete with current cultivars or be used in existing breeding pipelines (see Figure 1). Since Genome Editing can accelerate breeding substantially, it would be possible to generate new cultivars in 2–4 years compared to the current much longer period. The road to de novo domestication of wild plants for new breeding material or even new cultivars has never been shorter. The first examples include the de novo domestication of a ground cherry (*Physalis pruinosa*) and wild tomatoes.^[11,12] These first reports were shortly after followed by reports on cereal de novo domestication. Recently, work was published on African rice landraces and their ability to accelerate domestication and development by CRISPR-mediated genome editing.^[13] Furthermore, a roadmap including the necessary tools and examples of their application for the domestication of allotetraploid rice has been provided.^[14]

2 | TOOLKITS FOR GEARED

The successful implementation of GEaReD consists of four pillars as a foundation. 1) Efficient phenotyping technologies are required. Wild plants can be more phenotypically diverse and they are not well described in terms of agronomic traits compared to domesticated crops. It is crucial that the right representatives with the desirable traits within the species are selected for GEaReD. This also accounts for the need for new phenotyping approaches and the creation of fully automatic AI-managed phenotyping facilities for high throughput. 2) DNA sequence information of wild plants is also often sparse. Genome sequencing efforts of candidate species such as wild relatives of current crops are a prerequisite for releasing the full potential of GEaReD. The combination of these improvements will lead to a further increase in data and requires new data processing solutions. Artificial Intelligence will be a key technology for data processing, and first-generation AI is already employed for genomic data processing. In the future, AI will further support scientists with acquiring and connecting phenotyping data with omics data, enabling the construction of large databases.^[15] We regard this step as one of the most crucial for GEaReD. 3) Transformation methods, usually involving tissue culture, are needed to facilitate genome editing in candidate species. Even in many of our crops, tissue culture technologies are limited to be efficient in only a few cultivars of the crop. Ideally, there should be no such constraints in a candidate species for GEaReD. 4) A platform of molecular tools for precision genome editing over a wide range of species is essential for releasing the full potential of genome editing technologies. Among these, the most frequently used tool is CRISPR/Cas9-mediated genome editing. However, recent progress has already provided many new methodologies for targeted mutagenesis in the plant genome. First, concurrent mutagenesis of multiple genes was made possible through multiplex genome editing. This development was further accentuated through the development of alternatives to the canonical PAM site (NCC). Second, the function of the endonuclease was modulated to provide a nickase activity that creates single-strand breaks and allows site-specific genomic integration.^[16] Base editing of specific nucleotides is one of the latest developments. By introducing deaminases or so-called base editors, together with for example a nickase, specific C-G base pairs can be changed to T-A base pairs and vice versa.^[17] In another technology, the fusion of a transposase/recombinase to hijack a transposon system enables the introduction of sequences at a predetermined location.^[17] A similar approach to these transposon systems, but a more elegant way is CRISPR prime editing. With that, reverse transcriptase is fused to an endonuclease, enabling the integration of small sequences at specific target sites in the genome.^[17] These two new techniques are perfectly suited to alter the activity of promoters but could also be used to redesign proteins. Changes in the amino acid sequence could be used to alter the activity, phosphorylation, or localization of proteins.

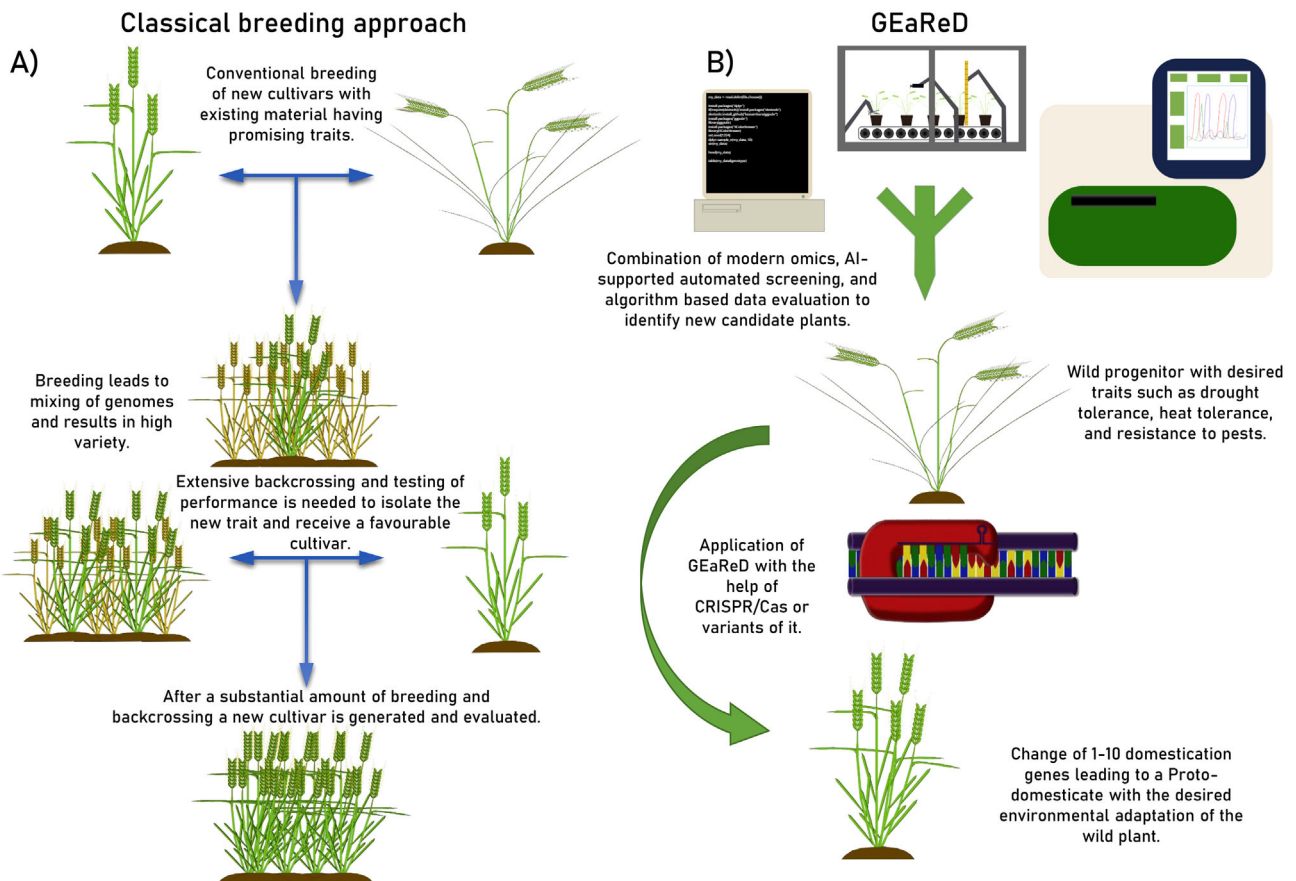


FIGURE 1 Application of GEARED compared to a classical breeding approach and its beneficial time-saving aspect. A) Classical breeding approach. A high yielding cultivar is used as one parent with another, usually a domesticated cultivar with interesting traits. The resulting plants will then be screened for the desired trait and backcrossed with the high-yielding parent until the desired trait is fixed in the high-performance cultivar. This can take several generations of crossing and limits the parental material to be breedable with the cultivar. B) GEARED as an outlook to future breeding. Wild progenitors will be screened for desired traits in a highly automated environment. An automated screening facility will be coupled with an omics facility and the resulting data will be analyzed by AI algorithms to identify interesting traits. Then, the most promising candidates will be used for the genome editing, and after altering major domestication genes a new cultivar is created with formerly not existing properties

3 | GEARED EXAMPLES

In the following, we describe two major challenges and put them briefly in the context of GEARED.

3.1 | Challenge I: Temperature-dependent yield loss in agriculture

For crops such as wheat, it is already evident that climate change and higher temperatures will severely negatively affect their yields in different areas on earth.^[1] To increase or maintain yields while temperatures are rising, climate-ready crops adapted to high temperatures and possible drought stress are needed. Drought tolerance in plants has been extensively studied and several approaches for improving the drought tolerance of plants were identified. However, drought or stress responses are complex and exploitation of the identified traits in our crops is limited so far. GEARED provides a new way of

introducing complex traits for handling drought and other stresses in new breeding materials. For example barley, instead of extensive breeding of cultivated barley cultivars that are adapted to optimal climate conditions, a redomestication of wild barley *Hordeum vulgare* ssp. *spontaneum* from hot climates in the Middle East could be done. Wild barley is already adapted to drought stress and may be armed to combat heat stress.^[18] To redomesticate wild barley, multiplex genome editing on key domestication genes could already create new breeding material. Three groups of genes in barley would need to be adapted (Table 1). 1) Thresh ability which incorporates genes like *Btr1/2* or *Thresh-1*. The *Btr* genes are responsible for seed dispersion, and a loss of function in either of them eliminates brittleness.^[19] *Thresh-1* is responsible for the thresh ability of the seeds.^[20] 2) Plant and spike morphology; this group would contain genes such as *Vrs-1*, *APETALA2*, or *Dep1*. Changes to *Vrs-1* would enable conversion from two- to six-row barley, while *APETALA2* and *Dep1* are known genes responsible for plant height.^[21-23] 3) Grain morphology. Here the *Nud* gene would be a good candidate.^[24] It results not only in

TABLE 1 Overview of some candidate domestication genes from different crops

Domestication gene	Crop plant	Function	References
<i>Br1 Brt2</i>	Barley	Non-brittle rachis	[19]
<i>thresh-1</i>	Barley	Free threshing	[20]
<i>Nud1</i>	Barley	Naked grain	[24]
<i>Vrs-1</i>	Barley	Six row	[22]
<i>APETALA1</i>	Barley	Plant height	[21]
<i>Dep1</i>	Barley	Plant height	[23]
<i>emp5</i>	Barley	Grain size	[25]
<i>TGW6-3</i>	Barley	Grain weight	[25]

naked seeds, but also has positive effects on different grain traits. Two other possible candidates could be *emp5* and *TGW6-3*; however, *emp5* and *TGW6-3* are just candidate genes from QTL analysis.^[25] Redomestication could therefore be a key way to quickly generate adapted cultivars for agriculture sustaining the upcoming climate conditions.

3.2 | Challenge II: Extensive soil use and fertilizer use in agriculture

Soil degradation threatens roughly 20% of the global area and has a severe impact on food safety and security.^[26] Different strategies are deployed to combat soil degradation, either by physical methods, chemical methods, or biological methods. These methods range from No-tilling cultivation, use of fertilizer, or using organisms for bioremediation, respectively.^[26] The depletion of nutrients from the soil and the extensive use of fertilizers and tilling/plowing or the use of cover crops have a further drawback. They increase the effort required by the farmer and result in an increased release of greenhouse gasses. A way to solve this would be to use perennial crops instead of annual crops. Perennial crops would support higher carbon binding in the soil, less fertilizer use, less use of farming equipment, and less soil erosion. Unfortunately, perennial grains developed by conventional breeding result in lower grain yields decreasing for each consecutive year of growth.^[27,28] For many current annual crops, wild perennial relatives exist. Among the Triticeae, barley has several close perennial relatives including *H. bulbosum*, *H. chilense*, and *H. brevisubulatum*.^[29] Wheat does not have as close perennial relative however, wide crosses with *Thinopyrum* spp. have been used in previous attempts to breed perennial wheat.^[30] In addition, maize and rice have perennial relatives *Zea diploperennis* and *Oryza longistaminata*, respectively. Research with perennial rice, wheatgrass, and other perennial plants clearly indicates the benefits of the use of annual alternatives. Even though the yield is usually not competitive, with exception of the perennial rice PR23 in its first year,^[28] the benefits for the soil, the farmer, and the environment are pointing toward the use of perennial crops. A GEaReD approach to the challenge of developing competitive perennial crops could be to strive for analogs of the current major crops by the domestication

of their closest perennial relatives with the annual crop as a genetic roadmap.

4 | CONCLUSIVE REMARKS

We will never be entirely sure how agriculture and plant breeding will develop throughout the years; however, here in this perspective article, we offered a view about how plant breeding might develop in the not-too-distant future. Many ideas have already been proposed and classical approaches of breeding or de novo and redomestication are already applied. However, the concept of GEaReD takes those ideas into a perspective of what is important to establish, and which toolkits we currently have. In general, much more emphasis will be needed to streamline the whole process of identification, evaluation, and modification. Moreover, technological progress in all the areas will help achieve this.

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CONFLICT OF INTEREST


The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Not applicable.

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