

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

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Abstract

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. New approaches are urgently needed and we propose, “Genome Editing accelerated Re-Domestication” (GEaReD) 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. Wild relatives of current crops are often adapted to harsh environments and have a high genetic diversity. Re-domestication of wild barley or teosinte could generate new cultivars adapted to environmental changes. De novo domestication of perennial relatives like *Hordeum bulbosum* could counter with soil depletion and increase soil carbon. Recent research already proved the principle of re-domestication in tomato and rice and therefore laid the foundation for GEaReD.

Genome **E**diting **a**ccelerated**Re -D**omestication” (**GEaReD**) – a new major direction in plant breeding

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Abstract

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. New approaches are urgently needed and we propose, “**G**enome **E**diting **a**ccelerated**Re -D**omestication” (GEaReD) 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. Wild relatives of current crops are often adapted to harsh environments and have a high genetic diversity. Re-domestication of wild barley or *teosintecould* generate new cultivars adapted to environmental changes. *De novo* domestication of perennial relatives like *Hordeum bulbosum* could counter with soil depletion and increase soil carbon. Recent research already proved the principle of re-domestication in tomato and rice and therefore laid the foundation for GEaReD.

Challenges and emerging solutions

Agriculture is faced with an urgent need to evolve in response to the global challenges of the 21 century. The recently released IPCC climate report indicates a faster progression of climate change than previously expected. Climate models predict severe impacts for agriculture on a global level. In Europe, severe impact on yield due to temperature increase and changes in precipitation is imminent [1]. Globally major impacts 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 agriculture needs to battle, like 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 have 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 requires knowledge about the identity and function of the target gene and 2) it only applies to traits which can be modified through one or a few genes. A different strategy is clearly in need for exploiting the full diversity of adaption 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 confer environmental adaptation to wild plants, it may be more feasible to acquire these networks for agriculture by domesticating the wild plant itself.

The major stable crops of the modern world were domesticated in prehistoric times and perfected over millennia. Although there are some examples of modern domestication like blueberries, blackberries or strawberries, these novelty crops do not compete directly with the staples [3-5]. It is undoubtedly a bold proposal to re- or *de novo* - domesticate staple crops. 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. We propose to call that approach “**G**enome **E**ding a ccelerated **Re**-**D**omestication” (**GEaReD**) (see Fig. 1).

GEaReD rely on the natural selection happened 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 PRIME-CRISPR to create highly adapted plants with yield and quality that can compete with current cultivars (see Fig. 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 timeframe. 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 [6, 7]. These first reports were shortly followed by reports on cereal *de novo* domestication. Recently work on African rice landraces, and their ability to accelerate domestication and development by CRISPR-mediated genome editing was published [8]. Furthermore, a roadmap including the necessary tools and examples of their application for the domestication of allotetraploid rice has been provided [9].

Toolkits for GEaReD

For the successful implementation of GEaReD, technological improvements at several stages are needed. 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. 2) DNA sequence information of wild plants is also often sparse. Genome sequencing efforts of candidate species such as wild relatives of current crops is 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 this, and first generation AI is already employed for genomic data processing. In the future, AI will further support scientist with acquiring and connecting phenotyping data

with omic data enabling the construction of large databases. 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 the genome editing technologies. Among these, the most frequently used tool is the 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 were made possible through multiplex genome editing. This development was further accentuated through the development of alternatives to the canonical PAM site (NCC). Secondly, the function of the endonuclease were modulated to provide a nickase activity that creates single strand breaks and allow site specific genomic integration [10]. 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 [11]. In another technology, the fusion of a transposase/recombinase to hijack a transposon system, enables the introduction of sequences at a predetermined location [11]. A similar approach to these transposon systems, but more elegant way is prime-CRISPR. Hereby a reverse transcriptase is fused to an endonuclease, enabling to integrate small sequences at specific target sites in the genome [11]. These two new techniques are perfectly suited to alter the activity of promoters, but could also be used to re-design proteins. Changes in the amino acid sequence could be used to alter activity, phosphorylation or localization of proteins.

GEaReD-Examples

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

Challenge I: Temperature dependent yield loss in agriculture

For crops like 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 keep yields while temperature is 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 plants drought tolerance were identified. However, drought or stress responses are complex and exploitation of the identified traits in our crops is limited so far. GEaReD provide a new way to introduce complex traits for handling drought and other stresses in new breeding materials. Taking for example barley, instead of extensive breeding of cultivated barley cultivars which are adapted to optimal climate conditions, a re-domestication of wild barley *Hordeum vulgare ssp. spontaneum* from hot climates in The Middle East could be done. Introducing the major important domestication genes like *btr*, *thresh-1* or the photoperiod gene family *ppd-H* could be done with genome editing techniques (see Table I). Wild barley is already adapted to drought stress and may be armed to combat heat stress [12]. Re-Domestication could be a key way to quickly generate adapted cultivars for agriculture sustaining the coming climate conditions. In addition, a *de novo* domestication of example *teosinte* could be valuable. *Teosinte* originates from high temperate areas and is already adapted to high temperatures. Re-domestication via Genome Editing could potentially lead to new maize plants adapted to higher temperatures.

Challenge II: Extensive Soil use and fertilizer use in agriculture

Soil degradation threatens roughly 20% of global area and is a severe impact for food safety and security [13]. 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 [13]. The depletion of nutrients from the soil and the extensive use of fertilizers and tilling/plowing or the use of cover crops has a further drawback. It increases the effort required by the farmer and results 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 soil erosion. Unfortunately, perennial grains developed by conventional breeding delivers lower grain yields decreasing for each consecutive year of growth [14, 15]. 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* [16]. Wheat does not have as close perennial relatives however wide crosses with *Thinopyrum* spp. has been used in previous attempts to breed perennial wheat [17]. In addition, maize and rice has perennial relatives *Zea diploperennis* and *Oryza longistaminata* respectively. Research with perennial rice, wheatgrass and other perennial plants clearly indicate the benefits over the use of annual alternatives. Even though the yield is usually not competitive, with exception for the perennial rice PR23 in its first year [15], the benefits for the soil, the farmer and the environment are clearly 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 domestication their closest perennial relatives with the annual crop as a genetic roadmap.

Table I: Overview of some candidate domestication genes from different crops.

Domestication gene	Crop plant	Function	Reference
Brt1 Brt2	Barley	Non-brittle rachis	[18]
<i>thresh-1</i>	Barley	Free threshing	[19]
Nud1	Barley	Naked grain	[20]
Tb1	Maize	Plant/tiller/comb architecture	[21]
Ra1	Maize	Ear morphology	[22]
Abphyl1	Maize	Phyllotaxy	[23]
Q	Wheat	Free threshing, spike architecture	[24]
Sog	Wheat	Free threshing	[25]
Tg	Wheat	Free threshing	[25]
Btr1-A/B	Wheat	Non-brittle rachis	[26]
TaGW2-A1	Wheat	Grain morphology	[27]
Laba1	Rice	Long, barbed awns	[28]
Prog1	Rice	Erect growth	[29]
qSH1	Rice	Free threshing	[30]

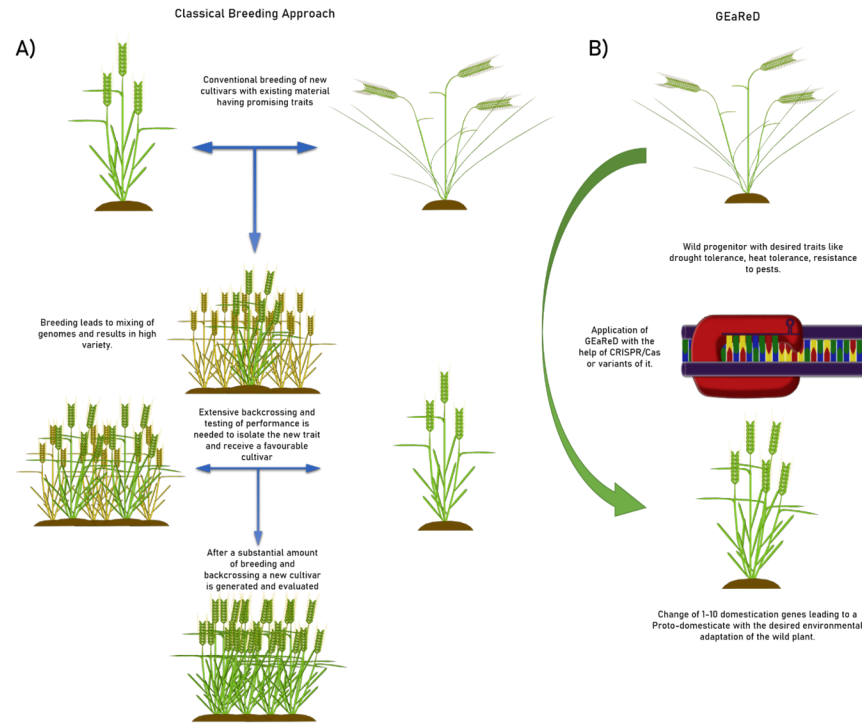


Figure 1: Application of GEaReD compared to a classical breeding approach and its beneficial time saving aspect.

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Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study

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