PRECISION DESIGN BREEDING OF CROPS - INTELLIGENT COMBINATION OF NATURAL VARIATION AND INTELLIGENT CREATION OF ARTIFICIAL VARIATION

Muhammad Salman Iqbal[1], Abdul Ali Azam[1], Hai Wang[1,2,3,]*

[1] State Key Laboratory of Maize Bio-breeding, Frontiers Science Center for Molecular Design Breeding, National Maize Improvement Center, College of Agronomy and Biotechnology, China Agricultural University, Beijing, 100193, China

[2] Sanya Institute of China Agricultural University, Sanya, 572025, China

[3] Hainan Yazhou Bay Seed Laboratory, Sanya, 572025, China

* Correspondence: Hai Wang (wanghai@cau.edu.cn)

ABSTRACT

Crop breeding is shifting from traditional empirical breeding toward BT+IT-driven precision design breeding. Improving the capacity of precision design breeding is of great significance for addressing global food security in the future. Future precision design breeding of crops will feature a "two-wheel drive" mode: intelligent hybrid breeding, based on genotypic/phenotypic/environmental data and breeding models, uses genomic prediction/selection to precisely design optimal combinations of natural variations and realize them through the most efficient crossing schemes; biotechnology-assisted breeding, empowered by artificial intelligence and synthetic evolution technologies, designs DNA and protein sequences that can be applied in gene-editing and synthetic biology. This review discusses the theoretical basis, technical approaches, and development trends of the precision design breeding paradigm in crops.

Keywords: Precision design breeding; genomic selection; genome editing; synthetic biology; quantitative genetics

1. INTELLIGENT HYBRID BREEDING: INTELLIGENT COMBINATION OF NATURAL VARIATION

Hybrid breeding using natural variation as raw material remains the dominant breeding method at present. The genomes of crop populations contain abundant natural variations, such as single nucleotide polymorphism (SNPs), insertions/deletions (indels), presence/absence variations (PAVs), and structural variations (SVs). The maize HapMap3 database contains approximately 83 million variant loci from 1,218 maize accessions (Bukowski et al., 2018). According to the neutral theory of molecular evolution, proposed by geneticist Motoo Kimura (Kimura, 1968), most variations are neutral, and only a small fraction of loci affect phenotypes; these variations are called functional variations. Based on their effects on agronomic traits, functional variations can be divided into favorable alleles and deleterious alleles. The essence of hybrid breeding is to seek optimal combinations of favorable allelic variations through crossing parental materials and artificial selection of progeny populations under field conditions.

1.1. Large-Effect Natural Variations and Marker-Assisted Selection

Over the past three decades, crop genomics and population genetics have developed rapidly. Using natural or artificial populations, numerous quantitative trait loci (QTLs) controlling important agronomic traits during domestication and improvement have been cloned via association analysis or linkage analysis. Some QTLs have been fine-mapped to genes and functionally validated, and a few have even been resolved to functional variations, with

DOI: 10.37856/revistadea.v101i3.4417

detailed molecular mechanisms characterized (Chen et al., 2021; Liang et al., 2021; Jie Liu et al., 2020; Xiao et al., 2017). These studies have systematically elucidated the molecular mechanisms underlying crop domestication and improvement over thousands of years, providing a theoretical foundation and target loci for future crop genetic improvement.

In rice, Huang's team systematically summarized key functional variant loci, constructed the most comprehensive map of key variations for rice quantitative trait genes to date, and developed an intelligent rice breeding navigation program, providing technical support for the rapid development of new rice varieties (Wei et al., 2021). In maize, scientists have made original progress in the genetic dissection of important traits, including disease resistance (Zuo et al., 2015), latitudinal adaptation (Yang et al., 2013), leaf angle (Tian et al., 2019), and kernel row number (Chen et al., 2022).

The value of QTLs for future breeding may gradually decline. Wallace et al. (Wallace et al., 2018) pointed out that QTLs detectable by association or linkage analyses tend to have relatively large effects; such QTLs were often optimized and fixed in elite germplasm by early breeders, leaving later breeders to invest more resources to optimize minor-effect QTLs. QTLs are often pleiotropic, requiring breeders to achieve a robust balance among multiple conflicting breeding objectives (such as biomass vs. lodging resistance, yield vs. stress tolerance, yield vs. quality) and to select optimal combinations of natural variations according to specific water, fertilizer, and light conditions. Therefore, some seemingly unoptimized genomic loci in breeding materials may represent global optima when considered in a broader environmental context.

Among the massive number of QTLs cloned in rice, wheat, maize, and other crops, only a few can improve agronomic traits in the genetic background of current elite cultivars. Zhengdan 958 and Xianyu 335 are widely adapted maize hybrids with large planting areas in China, registered in 2000 and 2004, respectively, and are still widely grown across the country. This illustrates the difficulty of pyramiding minor favorable allelic variations after the fixation of large-effect loci. Nevertheless, crops with less advanced breeding systems or shorter breeding histories still harbor unoptimized large-effect loci that can be precisely improved through marker-assisted selection.

1.2. Minor-Effect Natural Variations and Genomic Selection

The basic concept, computational methods, and theoretical framework of deleterious variations originated in human genomics (Henn et al., 2015; Lynch, 2010), and have gradually been applied to crop genomics. Most deleterious variations in populations are recessive; inbreeding can expose the phenotypic effects of recessive deleterious variations, helping to reduce their frequency through natural and artificial selection (Roessler et al., 2019; Zhu et al., 2022). Vegetatively propagated crops carry severe deleterious variations because they do not undergo inbreeding. Some deleterious variations in potato and cassava genomes are even homozygous lethal, severely hindering hybrid breeding in these crops (Ramu et al., 2017; Zhou et al., 2020). Outcrossing crops (e.g., maize) retain more deleterious variations in their genomes than inbreeding crops (e.g., rice), and deleterious variations tend to be enriched in genomic regions with low recombination frequency, such as pericentromeric regions (Rodgers-Melnick et al., 2015). Despite strong long-term selection by breeders, modern elite maize inbred lines still carry numerous minor-effect deleterious variants.

How can these variants be efficiently mapped to guide hybrid breeding? Individual minor-effect deleterious variants exert extremely weak effects on agronomic traits and thus cannot be detected by conventional association or linkage analyses. Deleterious variants in coding regions can be indirectly inferred based on the conservation of variant loci and their population frequencies, using tools such as GERP and SIFT; deleterious variants in transcriptional regulatory regions often cause gene expression levels to deviate from the population median, thereby reducing individual fitness (Kremling et al., 2018). In practice, hybrids can be produced using two inbred lines carrying complementary deleterious alleles, allowing the two genomes to mutually mask each other's deleterious effects (i.e., heterosis) (Mezmouk & Ross-Ibarra, 2014).

In maize breeding, international seed companies widely adopt genomic selection to more precisely pyramid minor-effect favorable alleles and eliminate minor deleterious alleles. The principles and procedures of genomic selection have been comprehensively reviewed (Wang & Cai, 2019). No major unresolved scientific questions remain in the fundamental theory of genomic selection. The challenge in applying this technology to breeding lies in the engineering-oriented integration of human resources and financial resources through appropriate institutional mechanisms to achieve efficient and low-cost operation of breeding pipelines.

1.3. Growth Points and Breakthroughs for Future Intelligent Hybrid Breeding

The key goal of intelligent hybrid breeding is to design optimal combinations of natural variations more precisely according to climatic conditions and cultivation practices, and to achieve these optimal combinations through crossing and selection of existing breeding materials along the fastest path. To better achieve this goal, new growth points and breakthroughs are needed in three areas: data, models, and auxiliary breeding technologies. (i) Accumulation of genotypic, phenotypic, and environmental data is the key to intelligent hybrid breeding. Future development of high-throughput genotypic, environmental, and phenotypic data platforms will greatly improve data quantity and quality, making intelligent hybrid breeding decisions more accurate and giving it significant advantages over traditional empirical breeding. (ii) Establishment of statistical models to predict phenotypes from high-dimensional genotypic and environmental data is another key aspect of intelligent hybrid breeding. However, the mathematical and computational foundations for model construction are already mature, leaving limited room for improving breeding decision accuracy solely through algorithm optimization. (iii) Other auxiliary breeding technologies may also become future breakthroughs. For example, speed breeding (Watson et al., 2018), and doubled haploid technology (Li et al., 2021; Zhong et al., 2019), can greatly shorten the breeding cycle; targeted chromosome recombination (Kouranov et al., 2022; Taagen et al., 2020), can make trait introgression more precise; apomixis (Wang et al., 2019), and male sterility (Zhang et al., 2018), may substantially reduce breeding and seed production costs.

2. BIOTECHNOLOGY-ASSISTED BREEDING: INTELLIGENT CREATION OF ARTIFICIAL VARIATION

Evolution is imperfect. According to the neutral theory of molecular evolution (Kimura, 1968), most beneficial mutations were lost due to genetic drift. The metabolic control theory proposed by British biochemists Kacser and Burns (Kacser et al., 1995) states that since natural selection acts at the individual level rather than the molecular and cellular

DOI: 10.37856/revistadea.v101i3.4417

levels, there is enormous room for optimizing phenotypes at the molecular and cellular levels. In the long run, the number of elite alleles not yet discovered and utilized by breeders is decreasing and becoming increasingly exhausted. In recent years, some crops have faced shortages of usable elite alleles and severe varietal homogeneity, restricting the development of breakthrough new varieties. Therefore, it is urgent to carry out two lines of work to compensate for the inherent limitations of evolution: first, integrate population genetics and artificial intelligence to mine favorable alleles causally related to phenotypes from natural variations (especially low-frequency and rare variations inaccessible to association analysis), and introduce them into crop genomes using gene-editing to prevent their loss through random drift; second, for key genes controlling important agronomic traits, explore the variation space not yet occupied by natural variations through synthetic evolution or artificial intelligence, optimize or even *de novo* design their regulatory DNA sequences or protein sequences, and then modify crop genomes using gene-editing and transgenic technologies. These technologies will provide a useful supplement to hybrid breeding.

2.1. Mining Favorable Alleles from Natural Populations to Guide Genome Editing

Genome editing differs from hybrid breeding in that hybrid breeding can use variations in linkage disequilibrium with causal variants as molecular markers, whereas genome editing relies on the identification and utilization of causal variants themselves. Over the past few decades, numerous QTLs or genes controlling important traits have been resolved in animals and plants through linkage and association analyses. However, due to linkage disequilibrium, the causal variants in many loci remain unclear; in addition, the low statistical power for low-frequency/rare variants in association analysis has long prevented effective mining and utilization of this valuable resource.

To overcome these problems, deep learning has been widely used in human and animal genomics to mine functional variants from natural variations. Functional variants for final phenotypes (e.g., human diseases, livestock quality) influence molecular phenotypes at different levels (e.g., gene expression levels or protein biochemical activities). Therefore, deep learning models that predict molecular phenotypes from genomic sequences can be constructed, and these models can be used to scan natural variants to predict which ones may alter molecular phenotypes, and such variants are likely functional variants for the final phenotypes. In human genomics, this approach has been used to identify functional variants underlying human diseases such as autism (Zhou et al., 2019; Zhou et al., 2018). In plants, deep learning technologies that simulate molecular biological processes are also rapidly developing (Jianxiao Liu et al., 2020; H. Wang et al., 2020; Wang et al., 2021; Washburn et al., 2019). Computer simulations of livestock genomic selection indicate that introducing favorable alleles or removing deleterious alleles via genome editing during genomic selection can significantly improve genetic gain (Jenko et al., 2015; Johnsson et al., 2019).

2.2. Designing Non-Natural Favorable Alleles to Guide Genome Editing

How can favorable alleles (not found in nature) be designed in transcriptional regulatory regions? Over the past few decades, numerous favorable alleles located in cis-regulatory regions have been identified in crops (Chen et al., 2021; Liang et al., 2021; Jie Liu et al., 2020), indicating that optimization of

cis-regulatory regions plays an important role in crop domestication and improvement. The variation space of cis-regulatory regions is extremely large. For example, a 140-bp DNA segment has 4^{140} possible base combinations, exceeding the estimated number of atoms in the observable universe (Liang et al., 2021). This huge variation space makes it difficult to design favorable alleles in cis-regulatory regions. In crops, genome editing has been successfully used to generate favorable alleles in cis-regulatory regions. For example, saturated promoter editing of negative regulators of important agronomic traits followed by progeny screening has been used to knock down target genes (Liu et al., 2021; Rodríguez-Leal et al., 2017; Song et al., 2022), but this method is somewhat random and labor-intensive. Another strategy targets positive regulators of important agronomic traits, using genome editing to induce chromosomal inversions that exchange promoters between the target gene and a nearby highly expressed gene, achieving "knock-up" of the target gene (Lu et al., 2021); however, this strategy may disrupt the original spatiotemporal specificity of the gene and may affect the expression of other genes. Therefore, smarter, more targeted, and universal methods for creating favorable alleles in cis-regulatory regions are needed.

Deep learning is well-suited for designing favorable alleles in crop genomic DNA sequences because it excels at capturing the relationship between DNA sequences and molecular functions (H. Wang et al., 2020). Therefore, it is necessary to use deep learning to precisely design non-natural favorable alleles and introduce them into crop genomes via genome editing to improve key agronomic traits, complementing traditional hybrid breeding. How can non-natural favorable alleles be designed in protein sequences? For simple traits, design can mimic natural variants based on our understanding of protein function; for example, disrupting the coding region of the waxy gene via genome editing produces waxy maize (Gao et al., 2020). However, sophisticated protein design requires more advanced tools in two areas: First, artificial intelligence. Driven by protein structure prediction models such as AlphaFold and RoseTTAFold, AI-based protein design has become a rapidly developing field (Bepler & Berger, 2021). In the future, it will enable the precise design of protein sequences for transgenic or endogenous crop genes to directionally improve key agronomic traits. Second, synthetic evolution. Multiple synthetic evolution systems have been developed in microorganisms and animals, such as the PACE system in bacteria (Esvelt et al., 2011), the OrthoRep system in yeast (Ravikumar et al., 2018), and the VEGAS system in mammals (English et al., 2019). Using these systems, novel BT insecticidal proteins (Badran et al., 2016), artificial transcription factors (Inamoto et al., 2020; Popa et al., 2020), base editors (Richter et al., 2020; Thuronyi et al., 2019), RNA polymerases (Carlson et al., 2014), and SARS-CoV-2 antibodies (Wellner et al., 2021), have evolved.

Although comparable systems are still lacking in plants, high-throughput base-editing can be used to perform saturated mutagenesis on the coding regions of target genes, followed by screening for mutants with desirable phenotypes, such as herbicide-resistant proteins (Kuang et al., 2020; Li et al., 2020). In the future, synthetic evolution will also achieve breakthroughs in crops, and deep learning may form a "positive feedback loop" with synthetic evolution: synthetic evolution provides large-scale data for training and testing deep learning models, making them more "intelligent"; deep learning provides starting sequences and evolutionary directions for synthetic evolution, making it more "precise". Meanwhile, genome editing itself is still developing rapidly. Low-cost, high-throughput genome editing will surpass conventional breeding and become

DOI: 10.37856/revistadea.v101i3.4417

the mainstream breeding method. It can be widely used not only for improving existing crops but also for the de novo domestication of wild species (Yu & Li, 2022; Yu et al., 2021).

2.3. *De Novo* Design of Non-Natural Functional Elements Using Deep Learning to Guide Synthetic Biology

Synthetic biology is another revolutionary technology transforming breeding. As the cost of chemical synthesis of biological elements (e.g., DNA, proteins) decreases, *de novo* design of high-performance biological elements has become a bottleneck for synthetic biology. The application of transgenic technologies, especially insect-resistant and herbicide-resistant genes, has created a huge global market worth tens of billions of US dollars annually. However, existing transgenic technologies generally only use natural genomic elements or proteins, or perform limited optimization on them, with no examples of *de novo* design. In recent years, a new branch of deep learning - generative modeling - has emerged. This technology learns from large numbers of known biological sequences, and then *de novo* designs novel sequences with desirable biochemical properties. For example, by learning natural promoter sequences, generative models can design promoters that do not exist in nature (Y. Wang et al., 2020); by learning natural proteins, generative models can design non-natural proteins (Strokach & Kim, 2022; Wu et al., 2021)2022; Wu et al., 2021. Deep learning-based synthetic biology is expected to bring new technological innovations to crop breeding, enabling *de novo* design of key agricultural genomic elements and proteins (e.g., insecticidal proteins, herbicide-resistant proteins, gene-editing enzymes) for rapid breeding applications.

2.4. Biosecurity Considerations and Practical Challenges for Precision Design Breeding

Despite the enormous potential of precision design breeding, several scientific, technical, and regulatory challenges must be addressed before these technologies can be broadly implemented in agricultural production. Genome editing and synthetic biology substantially expand the range of genetic variation available for crop improvement, but they also introduce new biosecurity considerations. Potential unintended genetic modifications, off-target effects, ecological impacts following environmental release, and unintended gene flow to wild relatives or conventional cultivars require careful evaluation through rigorous risk assessment and long-term environmental monitoring (Brookes & Smyth, 2024; Movahedi et al., 2023). As increasingly sophisticated artificial intelligence models become involved in biological sequence design for crop improvement (Iqbal et al., 2026), establishing transparent evaluation standards and biosafety assessment frameworks will become increasingly important to ensure the safe development and responsible application of these technologies.

Practical implementation also remains constrained by several technical and economic factors. Precision design breeding relies heavily on the availability of large-scale, high-quality genotypic, phenotypic, and environmental datasets, which remain limited for many crops and production environments. Furthermore, the predictive performance of genomic selection and artificial intelligence models may decline when applied across diverse genetic backgrounds or variable environmental conditions because of complex genotype-by-environment interactions. Although genome editing technologies continue to improve rapidly, challenges including transformation efficiency, genotype dependence, editing efficiency, and regeneration capacity still

restrict their application in many economically important crops (Achary et al., 2026; Chen et al., 2024). In addition, the substantial investment required for advanced phenotyping platforms, computational infrastructure, and multidisciplinary expertise may limit the adoption of precision breeding technologies by smaller breeding programs.

Addressing these challenges will require coordinated advances in multiple disciplines. Continued improvements in sequencing technologies, high-throughput phenotyping, genome editing systems, computational modeling, and synthetic biology should gradually reduce technical limitations while improving breeding efficiency. Equally important are the establishment of internationally harmonized regulatory frameworks, standardized biosafety evaluation procedures, and open data-sharing platforms that facilitate collaboration among researchers, breeding companies, and regulatory agencies (Brookes & Smyth, 2024; Fernández Ríos et al., 2025). Such coordinated efforts will help ensure that precision design breeding technologies are developed in a scientifically robust, economically feasible, and socially responsible manner, enabling their sustainable contribution to global food security.

3. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Future precision design breeding of crops will enter a “two-wheel drive” era: on the one hand, through genomic selection and related technologies, favorable alleles in breeding populations can be pyramided more precisely and efficiently than relying solely on phenotypic selection; on the other hand, under the guidance of artificial intelligence models, non-natural favorable alleles and genomic elements can be designed, and genomic sequences can be precisely modified using genome editing, transgenic, and other technologies to improve field agronomic traits of crops.

REFERENCES

Achary, V. M. M. M., Syombua, E. D., Kaur, S., Katamreddy, S. C., Zou, D., Hearne, S. J. J., & Bandyopadhyay, A. (2026). Advances in the use of morphogenic regulators and peptide regenerating factors for boosting plant transformation and genome editing [Review]. *Frontiers in Plant Science*, *Volume 17 - 2026*. https://doi.org/10.3389/fpls.2026.1699984

Badran, A. H., Guzov, V. M., Huai, Q., Kemp, M. M., Vishwanath, P., Kain, W., Nance, A. M., Evdokimov, A., Moshiri, F., & Turner, K. H. (2016). Continuous evolution of Bacillus thuringiensis toxins overcomes insect resistance. *Nature*, *533*(7601), 58-63. https://doi.org/https://doi.org/10.1038/nature17938

Bepler, T., & Berger, B. (2021). Learning the protein language: Evolution, structure, and function. *Cell systems*, *12*(6), 654-669. e653. https://doi.org/https://doi.org/10.1016/j.cels.2021.05.017

Brookes, G., & Smyth, S. J. (2024). Risk-appropriate regulations for gene-editing technologies. *GM Crops & Food*, *15*(1), 1-14. https://doi.org/10.1080/21645698.2023.2293510

Bukowski, R., Guo, X., Lu, Y., Zou, C., He, B., Rong, Z., Wang, B., Xu, D., Yang, B., & Xie, C. (2018). Construction of the third-generation Zea mays haplotype map. *Gigascience*, *7*(4), gix134. https://doi.org/https://doi.org/10.1093/gigascience/gix134

Carlson, J. C., Badran, A. H., Guggiana-Nilo, D. A., & Liu, D. R. (2014). Negative selection and stringency modulation in

DOI: 10.37856/revistadea.v101i3.4417

phage-assisted continuous evolution. *Nature chemical biology*, *10*(3), 216-222. https://doi.org/https://doi.org/10.1038/nchembio.1453

Chen, F., Chen, L., Yan, Z., Xu, J., Feng, L., He, N., Guo, M., Zhao, J., Chen, Z., Chen, H., Yao, G., & Liu, C. (2024). Recent advances of CRISPR-based genome editing for enhancing staple crops [Review]. *Frontiers in Plant Science*, *Volume 15 - 2024*. https://doi.org/10.3389/fpls.2024.1478398

Chen, Q., Li, W., Tan, L., & Tian, F. (2021). Harnessing knowledge from maize and rice domestication for new crop breeding. *Molecular plant*, *14*(1), 9-26. https://doi.org/https://doi.org/10.1016/j.molp.2020.12.006

Chen, W., Chen, L., Zhang, X., Yang, N., Guo, J., Wang, M., Ji, S., Zhao, X., Yin, P., & Cai, L. (2022). Convergent selection of a WD40 protein that enhances grain yield in maize and rice. *Science*, *375*(6587), eabg7985. https://doi.org/https://doi.org/10.1126/science.abg7985

English, J. G., Olsen, R. H., Lansu, K., Patel, M., White, K., Cockrell, A. S., Singh, D., Strachan, R. T., Wacker, D., & Roth, B. L. (2019). VEGAS as a platform for facile directed evolution in mammalian cells. *Cell*, *178*(3), 748-761. e717. https://doi.org/https://doi.org/10.1016/j.cell.2019.05.051

Esvelt, K. M., Carlson, J. C., & Liu, D. R. (2011). A system for the continuous directed evolution of biomolecules. *Nature*, *472*(7344), 499-503. https://doi.org/https://doi.org/10.1038/nature09929

Fernández Ríos, D., Quintana, S. A. A., Gómez Paniagua, P., Arrúa, A. A., Brozón, G. R. R., Bertoni Hicar, M. S. S., Castro Alegría, A., & Goberna, M. F. F. (2025). Regulatory challenges and global trade implications of genome editing in agriculture [Perspective]. *Frontiers in Bioengineering and Biotechnology*, *Volume 13 - 2025*. https://doi.org/10.3389/fbioe.2025.1609110

Gao, H., Gadlage, M. J., Lafitte, H. R., Lenderts, B., Yang, M., Schroder, M., Farrell, J., Snopek, K., Peterson, D., & Feigenbutz, L. (2020). Superior field performance of waxy corn engineered using CRISPR–Cas9. *Nature biotechnology*, *38*(5), 579-581. https://doi.org/https://doi.org/10.1038/s41587-020-0444-0

Henn, B. M., Botigué, L. R., Bustamante, C. D., Clark, A. G., & Gravel, S. (2015). Estimating the mutation load in human genomes. *Nature Reviews Genetics*, *16*(6), 333-343. https://doi.org/https://doi.org/10.1038/nrg3931

Inamoto, I., Sheoran, I., Popa, S. C., Hussain, M., & Shin, J. A. (2020). Combining rational design and continuous evolution on minimalist proteins that target the E-box DNA site. *ACS Chemical Biology*, *16*(1), 35-44. https://doi.org/https://doi.org/10.1021/acschembio.0c00684

Iqbal, M. S., Bahitwa, R., Azam, A. A., Xu, H., & Wang, H. (2026). Deep learning–driven protein binder design for crop improvement. *aBIOTECH*, *7*(1), 100018. https://doi.org/https://doi.org/10.1016/j.abiote.2025.100018

Jenko, J., Gorjanc, G., Cleveland, M. A., Varshney, R. K., Whitelaw, C. B. A., Woolliams, J. A., & Hickey, J. M. (2015). Potential of promotion of alleles by genome editing to improve quantitative traits in livestock breeding programs. *Genetics Selection Evolution*, *47*(1), 55. https://doi.org/https://doi.org/10.1186/s12711-015-0135-3

Johnsson, M., Gaynor, R. C., Jenko, J., Gorjanc, G., De Koning, D.-J., & Hickey, J. M. (2019). Removal of alleles by genome editing (RAGE) against deleterious load. *Genetics Selection Evolution*, *51*(1), 14. https://doi.org/https://doi.org/10.1186/s12711-019-0456-8

Kacser, H., Burns, J. A., Kacser, H., & Fell, D. A. (1995). The control of flux. *Biochemical Society Transactions*, *23*(2), 341-366. https://doi.org/10.1042/bst0230341

Kimura, M. (1968). Evolutionary rate at the molecular level. *Nature*, *217*(5129), 624-626. https://doi.org/https://doi.org/10.1038/217624a0

Kouranov, A., Armstrong, C., Shrawat, A., Sidorov, V., Huesgen, S., Lemke, B., Boyle, T., Gasper, M., Lawrence, R., & Yang, S. (2022). Demonstration of targeted crossovers in hybrid maize using CRISPR technology. *Communications Biology*, *5*(1), 53. https://doi.org/https://doi.org/10.1038/s42003-022-03004-9

Kremling, K. A., Chen, S.-Y., Su, M.-H., Lepak, N. K., Romay, M. C., Swarts, K. L., Lu, F., Lorant, A., Bradbury, P. J., & Buckler, E. S. (2018). Dysregulation of expression correlates with rare-allele burden and fitness loss in maize. *Nature*, *555*(7697), 520-523. https://doi.org/https://doi.org/10.1038/nature25966

Kuang, Y., Li, S., Ren, B., Yan, F., Spetz, C., Li, X., Zhou, X., & Zhou, H. (2020). Base-editing-mediated artificial evolution of OsALS1 in planta to develop novel herbicide-tolerant rice germplasms. *Molecular plant*, *13*(4), 565-572. https://doi.org/https://doi.org/10.1016/j.molp.2020.01.010

Li, C., Zhang, R., Meng, X., Chen, S., Zong, Y., Lu, C., Qiu, J.-L., Chen, Y.-H., Li, J., & Gao, C. (2020). Targeted, random mutagenesis of plant genes with dual cytosine and adenine base editors. *Nature biotechnology*, *38*(7), 875-882. https://doi.org/https://doi.org/10.1038/s41587-019-0393-7

Li, Y., Lin, Z., Yue, Y., Zhao, H., Fei, X., E, L., Liu, C., Chen, S., Lai, J., & Song, W. (2021). Loss-of-function alleles of ZmPLD3 cause haploid induction in maize. *Nature plants*, *7*(12), 1579-1588. https://doi.org/https://doi.org/10.1038/s41477-021-01037-2

Liang, Y., Liu, H.-J., Yan, J., & Tian, F. (2021). Natural variation in crops: realized understanding, continuing promise. *Annual Review of Plant Biology*, *72*(1), 357-385. https://doi.org/https://doi.org/10.1146/annurev-arplant-080720-090632

Liu, J., Fernie, A. R., & Yan, J. (2020). The past, present, and future of maize improvement: domestication, genomics, and functional genomic routes toward crop enhancement. *Plant Communications*, *1*(1). https://doi.org/https://doi.org/10.1016/j.xplc.2019.100010

Liu, J., Li, J., Wang, H., & Yan, J. (2020). Application of deep learning in genomics. *Science China Life Sciences*, *63*(12), 1860-1878. https://doi.org/https://doi.org/10.1007/s11427-020-1804-5

Liu, L., Gallagher, J., Arevalo, E. D., Chen, R., Skopelitis, T., Wu, Q., Bartlett, M., & Jackson, D. (2021). Enhancing grain-yield-related traits by CRISPR–Cas9 promoter editing of maize CLE genes. *Nature plants*, *7*(3), 287-294. https://doi.org/https://doi.org/10.1038/s41477-021-00858-5

Lu, Y., Wang, J., Chen, B., Mo, S., Lian, L., Luo, Y., Ding, D., Ding, Y., Cao, Q., & Li,

DOI: 10.37856/revistadea.v101i3.4417

Y. (2021). A donor-DNA-free CRISPR/Cas-based approach to gene knock-up in rice. *Nature plants*, *7*(11), 1445-1452. https://doi.org/https://doi.org/10.1038/s41477-021-01019-4

Lynch, M. (2010). Rate, molecular spectrum, and consequences of human mutation. *Proceedings of the National Academy of Sciences*, *107*(3), 961-968. https://doi.org/https://doi.org/10.1073/pnas.0912629107

Mezmouk, S., & Ross-Ibarra, J. (2014). The pattern and distribution of deleterious mutations in maize. *G3: Genes, Genomes, Genetics*, *4*(1), 163-171. https://doi.org/https://doi.org/10.1534/g3.113.008870

Movahedi, A., Aghaei-Dargiri, S., Li, H., Zhuge, Q., & Sun, W. (2023). CRISPR Variants for Gene Editing in Plants: Biosafety Risks and Future Directions. *International Journal of Molecular Sciences*, *24*(22), 16241. https://www.mdpi.com/1422-0067/24/22/16241

Popa, S. C., Inamoto, I., Thuronyi, B. W., & Shin, J. A. (2020). Phage-assisted continuous evolution (PACE): A guide focused on evolving protein–DNA interactions. *ACS omega*, *5*(42), 26957-26966. https://doi.org/https://doi.org/10.1021/acsomega.0c03508

Ramu, P., Esuma, W., Kawuki, R., Rabbi, I. Y., Egesi, C., Bredeson, J. V., Bart, R. S., Verma, J., Buckler, E. S., & Lu, F. (2017). Cassava haplotype map highlights fixation of deleterious mutations during clonal propagation. *Nature Genetics*, *49*(6), 959-963. https://doi.org/https://doi.org/10.1038/ng.3845

Ravikumar, A., Arzumanyan, G. A., Obadi, M. K., Javanpour, A. A., & Liu, C. C. (2018). Scalable, continuous evolution of genes at mutation rates above genomic error thresholds. *Cell*, *175*(7), 1946-1957. e1913. https://doi.org/https://doi.org/10.1016/j.cell.2018.10.021

Richter, M. F., Zhao, K. T., Eton, E., Lapinaite, A., Newby, G. A., Thuronyi, B. W., Wilson, C., Koblan, L. W., Zeng, J., & Bauer, D. E. (2020). Phage-assisted evolution of an adenine base editor with improved Cas domain compatibility and activity. *Nature biotechnology*, *38*(7), 883-891. https://doi.org/https://doi.org/10.1038/s41587-020-0453-z

Rodgers-Melnick, E., Bradbury, P. J., Elshire, R. J., Glaubitz, J. C., Acharya, C. B., Mitchell, S. E., Li, C., Li, Y., & Buckler, E. S. (2015). Recombination in diverse maize is stable, predictable, and associated with genetic load. *Proceedings of the National Academy of Sciences*, *112*(12), 3823-3828. https://doi.org/https://doi.org/10.1073/pnas.1413864112

Rodríguez-Leal, D., Lemmon, Z. H., Man, J., Bartlett, M. E., & Lippman, Z. B. (2017). Engineering quantitative trait variation for crop improvement by genome editing. *Cell*, *171*(2), 470-480. e478. https://doi.org/https://doi.org/10.1016/j.cell.2017.08.030

Roessler, K., Muyle, A., Diez, C. M., Gaut, G. R., Bousios, A., Stitzer, M. C., Seymour, D. K., Doebley, J. F., Liu, Q., & Gaut, B. S. (2019). The genome-wide dynamics of purging during selfing in maize. *Nature plants*, *5*(9), 980-990. https://doi.org/https://doi.org/10.1038/s41477-019-0508-7

Song, X., Meng, X., Guo, H., Cheng, Q., Jing, Y., Chen, M., Liu, G., Wang, B., Wang, Y., & Li, J. (2022). Targeting a gene regulatory element enhances rice grain yield by decoupling panicle number

and size. *Nature biotechnology*, *40*(9), 1403-1411. https://doi.org/https://doi.org/10.1038/s41587-022-01281-7

Strokach, A., & Kim, P. M. (2022). Deep generative modeling for protein design. *Current opinion in structural biology*, *72*, 226-236. https://doi.org/https://doi.org/10.1016/j.sbi.2021.11.008

Taagen, E., Bogdanove, A. J., & Sorrells, M. E. (2020). Counting on crossovers: controlled recombination for plant breeding. *Trends in plant science*, *25*(5), 455-465. https://doi.org/https://doi.org/10.1016/j.tplants.2019.12.017

Thuronyi, B. W., Koblan, L. W., Levy, J. M., Yeh, W.-H., Zheng, C., Newby, G. A., Wilson, C., Bhaumik, M., Shubina-Oleinik, O., & Holt, J. R. (2019). Continuous evolution of base editors with expanded target compatibility and improved activity. *Nature biotechnology*, *37*(9), 1070-1079. https://doi.org/https://doi.org/10.1038/s41587-019-0193-0

Tian, J., Wang, C., Xia, J., Wu, L., Xu, G., Wu, W., Li, D., Qin, W., Han, X., & Chen, Q. (2019). Teosinte ligule allele narrows plant architecture and enhances high-density maize yields. *Science*, *365*(6454), 658-664. https://doi.org/https://doi.org/10.1126/science.aax5482

Wallace, J. G., Rodgers-Melnick, E., & Buckler, E. S. (2018). On the road to breeding 4.0: unraveling the good, the bad, and the boring of crop quantitative genomics. *Annual review of genetics*, *52*(1), 421-444. https://doi.org/https://doi.org/10.1146/annurev-genet-120116-024846

Wang, C., Liu, Q., Shen, Y., Hua, Y., Wang, J., Lin, J., Wu, M., Sun, T., Cheng, Z., & Mercier, R. (2019). Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes. *Nature biotechnology*, *37*(3), 283-286. https://doi.org/https://doi.org/10.1038/s41587-018-0003-0

Wang, H., Cimen, E., Singh, N., & Buckler, E. (2020). Deep learning for plant genomics and crop improvement. *Current opinion in plant biology*, *54*, 34-41. https://doi.org/https://doi.org/10.1016/j.pbi.2019.12.010

Wang, X., & Cai, Z. (2019). Era of maize breeding 4.0. *J. Maize Sci*, *27*(1), 1-9.

Wang, Y., Wang, H., Wei, L., Li, S., Liu, L., & Wang, X. (2020). Synthetic promoter design in Escherichia coli based on a deep generative network. *Nucleic Acids Research*, *48*(12), 6403-6412. https://doi.org/https://doi.org/10.1093/nar/gkaa325

Wang, Y., Zhang, P., Guo, W., Liu, H., Li, X., Zhang, Q., Du, Z., Hu, G., Han, X., & Pu, L. (2021). A deep learning approach to automate whole‐genome prediction of diverse epigenomic modifications in plants. *New Phytologist*, *232*(2), 880-897. https://doi.org/https://doi.org/10.1111/nph.17630

Washburn, J. D., Mejia-Guerra, M. K., Ramstein, G., Kremling, K. A., Valluru, R., Buckler, E. S., & Wang, H. (2019). Evolutionarily informed deep learning methods for predicting relative transcript abundance from DNA sequence. *Proceedings of the National Academy of Sciences*, *116*(12), 5542-5549. https://doi.org/https://doi.org/10.1073/pnas.1814551116

Watson, A., Ghosh, S., Williams, M. J., Cuddy, W. S., Simmonds, J., Rey, M.-D., Asyraf Md Hatta, M., Hinchliffe, A., Steed, A., & Reynolds, D. (2018). Speed breeding is a powerful tool to accelerate crop research and breeding. *Nature plants*,

DOI: 10.37856/revistadea.v101i3.4417

4(1), 23-29. https://doi.org/https://doi.org/10.1038/s41477-017-0083-8

Wei, X., Qiu, J., Yong, K., Fan, J., Zhang, Q., Hua, H., Liu, J., Wang, Q., Olsen, K. M., & Han, B. (2021). A quantitative genomics map of rice provides genetic insights and guides breeding. *Nature Genetics*, *53*(2), 243-253. https://doi.org/https://doi.org/10.1038/s41588-020-00769-9

Wellner, A., McMahon, C., Gilman, M. S., Clements, J. R., Clark, S., Nguyen, K. M., Ho, M. H., Hu, V. J., Shin, J.-E., & Feldman, J. (2021). Rapid generation of potent antibodies by autonomous hypermutation in yeast. *Nature chemical biology*, *17*(10), 1057-1064. https://doi.org/https://doi.org/10.1038/s41589-021-00832-4

Wu, Z., Johnston, K. E., Arnold, F. H., & Yang, K. K. (2021). Protein sequence design with deep generative models. *Current opinion in chemical biology*, *65*, 18-27. https://doi.org/https://doi.org/10.1016/j.cbpa.2021.04.004

Xiao, Y., Liu, H., Wu, L., Warburton, M., & Yan, J. (2017). Genome-wide association studies in maize: praise and stargaze. *Molecular plant*, *10*(3), 359-374. https://doi.org/https://doi.org/10.1016/j.molp.2016.12.008

Yang, Q., Li, Z., Li, W., Ku, L., Wang, C., Ye, J., Li, K., Yang, N., Li, Y., & Zhong, T. (2013). CACTA-like transposable element in ZmCCT attenuated photoperiod sensitivity and accelerated the postdomestication spread of maize. *Proceedings of the National Academy of Sciences*, *110*(42), 16969-16974. https://doi.org/https://doi.org/10.1073/pnas.1310949110

Yu, H., & Li, J. (2022). Breeding future crops to feed the world through de novo domestication. *Nature Communications*, *13*(1), 1171. https://doi.org/https://doi.org/10.1038/s41467-022-28732-8

Yu, H., Lin, T., Meng, X., Du, H., Zhang, J., Liu, G., Chen, M., Jing, Y., Kou, L., & Li, X. (2021). A route to de novo domestication of wild allotetraploid rice. *Cell*, *184*(5), 1156-1170. e1114. https://doi.org/https://doi.org/10.1016/j.cell.2021.01.013

Zhang, D., Wu, S., An, X., Xie, K., Dong, Z., Zhou, Y., Xu, L., Fang, W., Liu, S., & Liu, S. (2018). Construction of a multicontrol sterility system for a maize male-sterile line and hybrid seed production based on the ZmMs7 gene encoding a PHD-finger transcription factor. *Plant Biotechnology Journal*, *16*(2), 459-471. https://doi.org/https://doi.org/10.1111/pbi.12786

Zhong, Y., Liu, C., Qi, X., Jiao, Y., Wang, D., Wang, Y., Liu, Z., Chen, C., Chen, B., & Tian, X. (2019). Mutation of ZmDMP enhances haploid induction in maize. *Nature plants*, *5*(6), 575-580. https://doi.org/https://doi.org/10.1038/s41477-019-0443-7

Zhou, J., Park, C. Y., Theesfeld, C. L., Wong, A. K., Yuan, Y., Scheckel, C., Fak, J. J., Funk, J., Yao, K., & Tajima, Y. (2019). Whole-genome deep-learning analysis identifies contribution of noncoding mutations to autism risk. *Nature Genetics*, *51*(6), 973-980. https://doi.org/https://doi.org/10.1038/s41588-019-0420-0

Zhou, J., Theesfeld, C. L., Yao, K., Chen, K. M., Wong, A. K., & Troyanskaya, O. G. (2018). Deep learning sequence-based ab initio prediction of variant effects on expression and disease risk. *Nature Genetics*, *50*(8), 1171-1179. https://doi.

org/https://doi.org/10.1038/s41588-018-0160-6

Zhou, Q., Tang, D., Huang, W., Yang, Z., Zhang, Y., Hamilton, J. P., Visser, R. G., Bachem, C. W., Robin Buell, C., & Zhang, Z. (2020). Haplotype-resolved genome analyses of a heterozygous diploid potato. *Nature Genetics*, *52*(10), 1018-1023. https://doi.org/https://doi.org/10.1038/s41588-020-0699-x

Zhu, M., Cheng, Y., Wu, S., Huang, X., & Qiu, J. (2022). Deleterious mutations are characterized by higher genomic heterozygosity than other genic variants in plant genomes. *Genomics*, *114*(2), 110290. https://doi.org/https://doi.org/10.1016/j.ygeno.2022.110290

Zuo, W., Chao, Q., Zhang, N., Ye, J., Tan, G., Li, B., Xing, Y., Zhang, B., Liu, H., & Fengler, K. A. (2015). A maize wall-associated kinase confers quantitative resistance to head smut. *Nature Genetics*, *47*(2), 151-157. https://doi.org/https://doi.org/10.1038/ng.3170

Received in: mar, 10, 2026

Accepted in: jul, 06, 2026