ISBN: 978-81-19149-80-3

2. Novel Plant Breeding Techniques: A Solution to Climate Change

Purnima Ray

Department of Genetics and Plant Breeding,
NMCA, NAU,
Navsari, Gujarat.

Jatin Tanwar

Division of Genetics,
ICAR-Indian Agricultural Research Institute,
New Delhi.

H. E. Patil

Hill Millet Research Station,
NAU,
Waghai, The Dangs, Gujarat.

Deepak Saran

Department of Genetics and Plant Breeding,
IGKV,
Raipur, Chhattisgarh.

Abstract:

Climate change poses a significant threat to global food security, as it results in reduced crop productivity worldwide. As the global population is expected to exceed 10 billion in the coming years, ensuring food security has become a major concern. Meeting the ever-increasing demand for food production is a challenging task due to the continuously changing environment, crop diseases, and increasing population. Plant breeders are using conventional breeding methods based on plant biology to increase production.

However, these traditional approaches are time-consuming and require significant space and inputs to release improved crop varieties after making crosses. Recent advances in genomics, high-throughput phenomics, sequencing and breeding methodologies, and state-of-the-art genome-editing tools integrated with artificial intelligence offer new opportunities for accelerated development of climate-resilient crops. In recent years, researchers have made remarkable achievements using these technologies, leading to revolutionary developments. Using a comprehensive approach, novel breeding techniques show potential in addressing climate change and producing crop varieties that are better adapted to the changing environment.

Keywords:

Climate change, food security, next-generation breeding, genomics, genome editing, CRISPR/Cas, abiotic stress, crop improvement

2.1 Introduction:

As the world population grows at an alarming rate while available land is decreasing, modern agricultural practices are struggling to produce enough food to sustain approximately 10 billion people by 2050 (Hickey *et al.*, 2019). Climate change and the consequent rise in CO2 levels have had a negative impact on water-use efficiency, biosecurity, and crop quality, as well as increased the frequency of abiotic stressors like heat, salinity, and drought, as well as biotic stressors like pests and diseases. Furthermore, climate change is projected to cause biodiversity loss, particularly in less hospitable environments. Drought alone is expected to lower crop yield in half of the world's arable land by 50% over the next five decades (Dhankher *et al.*, 2018). It has been projected that, for each degree Celsius increase in the global average temperature, global yields of major crops like wheat, rice, maize, and soybean could decrease by 6.0%, 3.2%, 7.4%, and 3.1%, respectively (Zhao *et al.*, 2017). Plant breeding has played an important role in altering agriculture to fulfill the growing demand for food throughout history. **Figure 2.1** highlights some of the key milestones achieved in the history of plant breeding. The ultimate objective of plant breeding is to develop genetically superior genotypes/varieties that are suitable for specific environments, leading to higher production (Falconer and Mackay, 1996). The advent of molecular biology and biotechnology has resulted in a massive rise of genetic data connected to genes and QTLs (Quantitative Trait Loci), which can aid in the acceleration of breeding operations (Wang, 2007). Progress in precise phenotyping and genotyping provides enormous opportunity to establish crop varieties that can adjust to changing climatic circumstances, hence enhancing plant breeding activities to create climate-resilient cultivars (Gobu *et al.*, 2020). As a result, developing climate-resilient cultivars using novel breeding strategies is crucial for ensuring food security in harsh climatic situations.

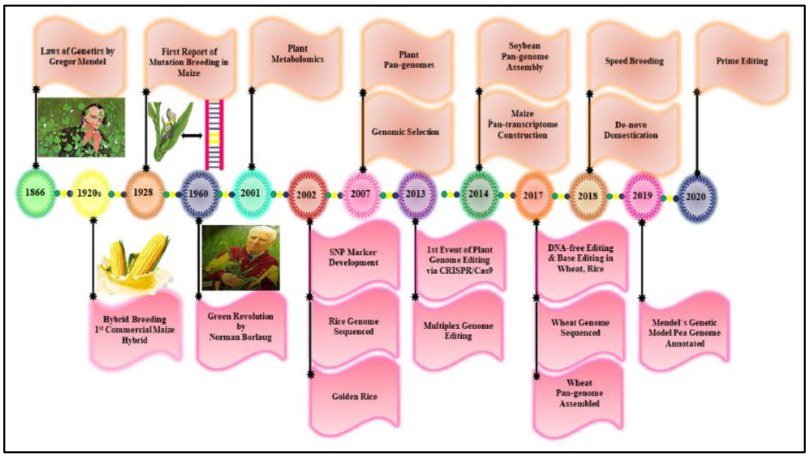

Figure 2.1 Representations of the key milestones achieved by conventional and modern plant breeding. (Razzaq *et al.*, 2021)

Classical plant breeding typically involves hybridization and rigorous screening to select elite crop varieties (Purugganan and Fuller, 2009). This method includes genetic diversity by intercrossing plants with novel agronomic traits, such as wild relatives or crop landraces, in order to select the best genotypes with extraordinary qualities. (Lavarenne *et al.,* 2018). Wild populations are a valuable resource for crop improvement because they offer greater genetic diversity and the opportunity to introduce desired traits. For example, hybridization among species can introduce diverse genetic recombination, which may provide an excellent chance to combat climate stresses (Becker *et al.,* 2013). Conventional breeding approaches, on the other hand, have limited utility due to genetic drag, genetic erosion, hybridization bottlenecks, and labarious selection processes. (Abberton *et al.,* 2016). Developing a crop variety with desirable qualities might take 10-20 years using traditional methods, making it a difficult and time-consuming process. (Fischer *et al.,* 2014). In contrast, modern breeding approaches have made significant progress in overcoming the obstacles posed by conventional methods over the last three decades.

The advancement of omics technologies has had a substantial impact on crop improvement efforts. These technologies have provided crop breeders with powerful tools and have played a vital role in omics-assisted breeding programs aiming at improving different agronomic traits. Omics technology is a modern molecular tool that helps us understand the functional genomic systems within organisms. The term "omics" is derived from the Greek word "ome," which means "whole." Omics disciplines study different kinds of biological molecules that make up whole biological systems. Some of the key omics disciplines include genomics (study of an organism's entire genome), transcriptomics (study of an organism's complete set of RNA transcripts), proteomics (study of set of proteins produced in an organism), metabolomics (study of complete set of small molecules/metabolites of an organism), and phenomics (study of complete set of observable traits of an organism). The integration of omics technologies into crop breeding programs has provided breeders with a wealth of information about the genetic and molecular basis of agronomic traits. This knowledge allows for more precise and efficient selection of desirable traits during the breeding process. Omics-assisted breeding programs can identify genetic markers associated with specific traits, accelerate the breeding process, and enhance the overall effectiveness of crop improvement efforts.

2.2 Marker Assisted Breeding:

Recent advances in biotechnology have opened up new avenues for crop improvement by offering a better understanding of the genetic variables that determine desired features. The use of molecular markers such as restriction fragment length polymorphism (RFLP), Rapid Amplified Polymorphic DNA (RAPD), Simple Sequence repeat (SSR), Cleaved amplified polymorphic sequence (CAPS), and Single Nucleotide Polymorphism (SNP) has significantly helped this process and revolutionized plant genetics (Nadeem *et al.,* 2018). Crop breeding's ultimate goal is to create high-performing varieties with numerous desirable features, such as high yield, higher quality, tolerance/resistance to biotic and abiotic stress, and good environmental adaptation. Traditional breeding, on the other hand, can be tough, time-consuming, and problematic in combining all of these qualities in a single genotype. Marker-assisted selection (MAS) provides an alternate method for combining all necessary qualities into a single variety by applying molecular markers connected to the genes/QTLs of interest in breeding operations. This strategy has become a vital component of genotype/germplasm improvement, with the ability to change from phenotype-based (conventional breeding) to a combination of phenotype and genotype-based selection, which is of great importance in modern breeding programs. (Tester *et al.,* 2010).

The integration of MAS strategies into breeding programs offers significant advantages over traditional phenotype-based breeding, mainly due to its efficiency and convenience in transferring the genes/QTLs of interest into the plant genome. By utilizing molecular markers linked to target traits, selection can be carried out specifically for these genotypes, reducing the breeding cycle by conducting off-season nurseries and enabling more generations to be grown per year (speed breeding). Furthermore, the technique minimizes the required population size because many lines can be removed in earlier breeding generations after MAS. The success of MAS techniques has been demonstrated in a variety of key crops, particularly for features with simple inheritance regulated by one or a few genes (Collard *et al.,* 2008).

Two major strategies are employed in MAS for breeding programs: (i) marker-assisted-backcrossing (MABC), which involves backcrossing of desirable alleles into elite germplasm, and (ii) marker-assisted gene pyramiding (MAGP), which involves stacking of multiple genes from different sources into elite breeding lines. The success of MAS is dependent on discovering major QTLs for complex traits, which account for an important portion of phenotypic variation. Major QTLs for important crops have been found and characterized, and successful applications of MABC and MAGP for yield or yield component traits have been reported. Marker assisted breeding allows for the introgression of genetic markers linked with agronomic traits into elite crop genetic backgrounds, resulting in the development of varieties that are better adaptable to climate changes.

2.3 Genome Sequencing Technology:

The rapid availability and falling cost of high-throughput genome sequencing technology is enabling the collection of massive amounts of genetic data. DNA samples can be preferentially enriched before sequencing to capture the diversity of specific gene families within big groups. Sanger sequencing is impractical for investigating plant genomes due to its low throughput and expensive sequencing costs. In 2005, Roche introduced the 454-pyrosequencing platform, which revolutionized genome sequencing. Since then, several sequencing platforms developed by companies such as Illumina, ABI, Life Technologies, PacBio, Oxford Nanopore, and Complete Genomics have been commercially released, changing the landscape of genome sequencing. Depending on their chemistry, second-generation sequencing (SGS) approaches are classified as ligation-based or synthesis-based approaches (Goodwin *et al.,* 2016). The rapidly falling cost of genome-wide genotyping enables large scale assessments of crop species diversity to capture traits related to the climate. There are two main strategies used: low SNP density strategies like reduced representation sequencing (RRS) and high SNP density strategies like whole-genome resequencing (WGR). However, high-density genotyping assays, such as SNP chips, allow for large-scale genotyping using SNP-specific oligonucleotide probes rather than direct sequencing. Genotyping by sequencing (GBS) variants can be used for traditional quantitative trait loci (QTL) analyses as well as modern approaches such as genome-wide association studies (GWAS). GWAS uses previous recombinations in different association panels to find genes associated with phenotypic traits (Yuan *et al.,* 2019). The widespread use of GWAS is improving our understanding of the genetics of important climate-specific traits like drought and heat tolerance (Yuan *et al.,* 2019).

2.4 Genome Editing: A Revolutionary Tool:

In recent years, advances in genome editing technology have enabled breeders to precisely add or remove any DNA sequence in the genome, which has shown substantial potential to alter crop improvement (Scheben *et al.,* 2017; Belhaj *et al.,* 2015). Some approaches have been used for more than two decades, such as transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs). However, type II clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein (Cas) system from Streptococcus pyogenes, which was developed in the last decade (Jinek *et al.,* 2012), has been the most versatile tool for introducing desirable or novel traits and expediting the development of climate-smart crop varieties.

Zinc finger nucleases (ZFN) and Transcription activator-like effector nucleases (TALENs) are gene-editing techniques that use restriction enzymes. ZFN functions by using a zinc finger DNA binding domain to locate a specific target DNA sequence and a restriction endonuclease domain to cut the DNA at that site. TALENs also use a DNA binding domain and restriction domain like ZFN, but their DNA binding domain has wider range of potential target sequences. However, the main challenges that researchers and manufacturers faced in both techniques were the difficulty of protein engineering, as well as the high cost and time consuming.

The CRISPR/Cas-9 system is the most effective, efficient, and accurate way of genome editing in all live cells, and it is widely used in various fields. It is made up of two important components: guide RNA (gRNA) and CRISPR-associated (Cas-9) proteins. CRISPR/Cas-9 genome editing comprises three steps: recognition, cleavage, and repair. The designed sgRNA recognizes the target sequence in the gene of interest via complementary base pairing. The Cas-9 nuclease then creates double-stranded breaks at a site located 3 base pairs upstream to protospacer adjacent motif. The double-stranded break is finally repaired by either non-homologous end joining or homology-directed repair cellular mechanisms. The utilization of CRISPR/Cas system has led to significant advancements in plant genome engineering, contributing to the development of climate-resilient crops (Puchta, 2017; Li *et al.,* 2013; Shan *et al.,* 2013). Several studies have shown that this technology has the ability to improve agronomic traits and increase stress tolerance to diverse abiotic and biotic stresses.

For instance, Ogata *et al.* (2020) employed CRISPR/Cas9-mediated frame shift mutations to generate rice mutant lines for the OsERA1 gene under drought stress, resulting in increased drought tolerance and promoting primary root development under normal conditions. In another study, Pan et al. (2020) showed that the ZmSRL5 gene is essential for the formation of cuticular wax structure in maize, which provides protection against different stressors. The loss of functional mutant maize progenies revealed that the ZmSRL5 gene is important in drought response by maintaining the cuticle wax structure.

2.4.1 Base Editing:

Recent study has demonstrated that a single base modification can have significant impact on desirable traits in plants (Henikoff *et al.,* 2003). Therefore, there is a need for an effective technology that can generate accurate and efficient point mutations in plants. CRISPR-Cas9-driven base editing is a revolutionary method for transforming one DNA base to another without the use of a repair template. (Komor *et al.*, 2016). For instance, cytidine deaminases can convert cytosine (C) to uracil (U), which is then treated as thymine (T) during DNA repair and replication, leading to the creation of a C•G to T•A substitution. This method has been applied successfully in crops such as wheat, maize, and tomato. (Zhang *et al.,* 2017), and it may also be beneficial in gene functional analysis and crop breeding to generate stress-tolerant cultivars.

2.4.2 Prime Editing:

Prime editing is a novel genome engineering approach that can introduce a wide range of base-to-base conversions as well as insertions and deletions using prime editing guide RNA (pegRNA) (Anzalone *et al.*, 2019). This innovative technology has enormous potential to precisely modify targeted genome sequences and facilitate functional genomics studies. It can also help with the introduction of genes for adaptation to various climatic conditions, which will speed up the breeding of climate-smart cultivars in the near future (Marzec & Hensel, 2020).

2.5 Next-Generation Plant Phenotyping Platforms:

Plant phenotyping has played a pivotal role in the successful domestication of crops over thousands of years. The term "phenome" refers to the entire phenotypic profile of a plant, and phenotype is a combination of observable genetic expression and its interaction with the environment (Houle *et al.*, 2010). Plant phenomics has developed as a rapidly growing research platform, comprising the multidimensional application of advanced tools and techniques for the detailed evaluation of plant growth, structure, function, and behavior in a given environment. This field involves the acquisition, organization, and analysis of large-scale phenotypic data sets, and the development of intelligent models for predicting plant growth under various scenarios (Houle *et al.*, 2010). Plant phenotyping is an important method for studying the relationship between plants and their environment. It can be undertaken at many levels of resolution, from the genome to the whole plant, in diverse climatic conditions, and in both field and controlled environments.

2.6 Mutation Breeding:

Mutation breeding is a technique used to induce mutations in plant genomes to generate genetic variation that can be used for crop improvement. The method involves treating plants with chemical or physical mutagens to create random mutations in their DNA, which can result in changes in various traits, such as yield, quality, and resistance to biotic and abiotic stresses. The mutated plants are then screened for desirable traits, and those with beneficial mutations are selected for further breeding. Targeting Induced Local Lesions in Genome (TILLING) is a powerful technique that has emerged from mutation breeding. It involves the creation of a large population of mutated plants through chemical mutagenesis, followed by the identification of desirable mutations through DNA screening. TILLING has revolutionized the identification of novel alleles in crops, enabling the discovery of new genes responsible for desirable traits such as drought tolerance, disease resistance, and increased yield. Mutation breeding and TILLING have been proven to be useful methods for identifying allelic variants responsible for crop adaptation to abiotic and biotic stresses caused by climate change. These techniques offer a fast and cost-effective way to introduce genetic diversity into crops and select for desirable traits, which can ultimately lead to the development of new varieties that are better adapted to changing environmental conditions.

2.7 Speed Breeding:

Speed breeding has become effective in producing more generations of crops in a year by using a pro-longed photoperiod of 22 hours of daylight followed by 2 hours of night light. Through a shorter breeding cycle, speed breeding has successfully increased the genetic gain of the crops (Watson et al., 2018). Today's globe needs more genetic progress to offset the effects of rising food demand and climatic change brought by rapid population growth. It has revolutionized agriculture by facilitating various activities like crossing, backcrossing, rapid gene identification, mapping populations, trait pyramiding, and transgenic pipeline development (Hickey *et al.,* 2019).

Speed breeding allows for up to four generations of *B. napus* and six generations of *Hordeum vulgare, Triticum aestivum, Pisum sativum, Cicer arietinum,* and *B. distachyon* in a single season, which is a remarkable improvement over conventional breeding (Watson et al., 2018). It is also an efficient and cost-effective platform for conducting crop improvement projects that integrate genomics and phenomics. It is possible to rapidly analyze the risks associated with gene-edited crops across multiple generations by integrating speed breeding with next-generation metabolomics technologies (Razzaq et al., 2019). Therefore, the integration of speed breeding technology with next-generation OMICS tools is an exciting way forward for accelerating crop breeding programs.

2.8 Conclusion:

The challenges that climate change poses to agricultural production and food security have have made the role of plant breeders even more crucial. As the impacts of climate change continue to affect food quality, availability, and accessibility, plant breeding has emerged as a powerful tool to combat these challenges in agriculture. To meet the demand for future food production, it has become crucial to develop new crop varieties, including novel crops. As a result, plant breeders are diligently exploring new tools and technologies to dealing with climate change challenges.

These technologies can accelerate the breeding process, facilitate the screening of elite germplasm under stress conditions, and enable the development of genome-edited non-transgenic plants that can adapt to environmental stress, thus ensuring food security. In comparison to traditional breeding approaches, novel plant breeding techniques can give a more sustainable strategy to boosting production in the face of biotic and abiotic stressors.

2.9 References:

1. Abberton, M., Batley, J., Bentley, A., Bryant, J., Cai, H., Cockram, J., de Oliveira, A. C., Cseke, L. J., Dempewolf, H., De Pace, C., Edwards, D., Gepts, P., Greenland, A., Hall, A. E., Henry, R., Hori, K., Howe, G. T., Hughes, S., Humphreys, M., Lightfoot, D., … Yano, M. (2016). Global agricultural intensification during climate change: a role for genomics. *Plant biotechnology journal*, 14(4), 1095–1098.
2. Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., Chen, P. J., Wilson, C., Newby, G. A., Raguram, A., & Liu, D. R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, 576(7785), 149–157.
3. Becker, M., Gruenheit, N., Steel, M., Voelckel, C., Deusch, O., Heenan, P. B., McLenachan, P. A., Kardailsky, O., Leigh, J. W., & Lockhart, P. J. (2013). Hybridization may facilitate in situ survival of endemic species through periods of climate change. *Nature Climate Change*, 3(12), 1039–1043.
4. Belhaj, K., Chaparro-Garcia, A., Kamoun, S., Patron, N. J., & Nekrasov, V. (2015). Editing plant genomes with CRISPR/Cas9. *Current opinion in biotechnology*, 32, 76–84.
5. Collard, B. C., & Mackill, D. J. (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 363(1491), 557–572
6. Dhankher, O. P., & Foyer, C. H. (2018). Climate resilient crops for improving global food security and safety. *Plant, cell & environment*, 41(5), 877–884.
7. Falconer, D. S., & Mackay, T. F. C. (1996). Introduction to quantitative genetics. Essex. UK: Longman Group, 12.
8. Fischer, R., Byerlee, D., and Edmeades, G. (2014). Crop Yields and Global Food Security. Canberra, ACT: ACIAR, 8–11.
9. Gobu, R., Shiv, A., Kumar, A.C., Basavaraj, P.S., Harish, D., Adhikari, S., Ramtekey, V., Hudedamani, U., Sujatha, M. (2020). Accelerated crop breeding towards development of climate resilient varieties. In *Climate change and Indian agriculture: challenges and adaptation strategies*. (pp. 49-69). ICAR-National Academy of Agricultural Research Management, Hyderabad, Telangana, India.
10. Goodwin, S., McPherson, J. D., & McCombie, W. R. (2016). Coming of age: ten years of next-generation sequencing technologies. *Nature reviews. Genetics*, 17(6), 333–351.
11. Henikoff, S., & Comai, L. (2003). Single-nucleotide mutations for plant functional genomics. *Annual review of plant biology*, 54, 375–401.
12. Hickey, L. T., N Hafeez, A., Robinson, H., Jackson, S. A., Leal-Bertioli, S. C. M., Tester, M., Gao, C., Godwin, I. D., Hayes, B. J., & Wulff, B. B. H. (2019). Breeding crops to feed 10 billion. *Nature biotechnology*, 37(7), 744–754.
13. Houle, D., Govindaraju, D. R., & Omholt, S. (2010). Phenomics: the next challenge. *Nature reviews. Genetics*, 11(12), 855–866.
14. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science (New York, N.Y.)*, 337(6096), 816–821
15. Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A., & Liu, D. R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*, 533(7603), 420–424.

16. Lavarenne, J., Guyomarc'h, S., Sallaud, C., Gantet, P., & Lucas, M. (2018). The Spring of Systems Biology-Driven Breeding. *Trends in plant science*, 23(8), 706–720.
17. Li, J. F., Norville, J. E., Aach, J., McCormack, M., Zhang, D., Bush, J., Church, G. M., & Sheen, J. (2013). Multiplex and homologous recombination-mediated genome editing in Arabidopsis and Nicotiana benthamiana using guide RNA and Cas9. *Nature biotechnology*, 31(8), 688–691.
18. Marzec, M., & Hensel, G. (2020). Prime Editing: Game Changer for Modifying Plant Genomes. *Trends in plant science*, 25(8), 722–724.
19. Nadeem, M. A., Nawaz, M. A., Shahid, M. Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., Labhane, N., Özkan, H., Chung, G., & Baloch, F. S. (2018). DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment*, 32(2), 261–285.
20. Ogata, T., Ishizaki, T., Fujita, M., & Fujita, Y. (2020). CRISPR/Cas9-targeted mutagenesis of OsERA1 confers enhanced responses to abscisic acid and drought stress and increased primary root growth under nonstressed conditions in rice. *PloS one*, 15(12), e0243376.
21. Pan, Z., Liu, M., Zhao, H., Tan, Z., Liang, K., Sun, Q., Gong, D., He, H., Zhou, W., & Qiu, F. (2020). ZmSRL5 is involved in drought tolerance by maintaining cuticular wax structure in maize. *Journal of integrative plant biology*, 62(12), 1895–1909.
22. Puchta H. (2017). Applying CRISPR/Cas for genome engineering in plants: the best is yet to come. *Current opinion in plant biology*, 36, 1–8.
23. Purugganan, M. D., & Fuller, D. Q. (2009). The nature of selection during plant domestication. *Nature*, 457(7231), 843–848.
24. Razzaq, A., Kaur, P., Akhter, N., Wani, S. H., & Saleem, F. (2021). Next-Generation Breeding Strategies for Climate-Ready Crops. *Frontiers in plant science*, 12, 620420.
25. Razzaq, A., Sadia, B., Raza, A., Khalid Hameed, M., & Saleem, F. (2019). Metabolomics: A Way Forward for Crop Improvement. *Metabolites,* 9(12), 303.
26. Scheben, A., Batley, J., & Edwards, D. (2017). Genotyping-by-sequencing approaches to characterize crop genomes: choosing the right tool for the right application. *Plant biotechnology journal*, 15(2), 149–161.
27. Shan, Q., Wang, Y., Li, J., Zhang, Y., Chen, K., Liang, Z., Zhang, K., Liu, J., Xi, J. J., Qiu, J. L., & Gao, C. (2013). Targeted genome modification of crop plants using a CRISPR-Cas system. *Nature biotechnology*, 31(8), 686–688.
28. Tester, M., & Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. *Science (New York, N.Y.),* 327(5967), 818–822
29. Wang, J. K. (2007). Simulation modeling in plant breeding: Principles and applications. *Agricultural sciences in China*, 6(8), 908-921.
30. 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., Adamski, N. M., Breakspear, A., Korolev, A., Rayner, T., Dixon, L. E., Riaz, A., Martin, W., Ryan, M., Edwards, D., Batley, J., … Hickey, L. T. (2018). Speed breeding is a powerful tool to accelerate crop research and breeding. *Nature plants*, 4(1), 23–29.
31. Yuan, Y., Scheben, A., Batley, J., & Edwards, D. (2019). Using genomics to adapt crops to climate change. In *Sustainable Solutions for Food Security: Combating Climate Change by Adaptation* (pp. 91-109). Springer International Publishing Switzerland.
32. Zhang, Y., & Gao, C. (2017). Recent advances in DNA-free editing and precise base editing in plants. *Emerging topics in life sciences,* 1(2), 161–168.

33. Zhao, C., Liu, B., Piao, S., Wang, X., Lobell, D. B., Huang, Y., Huang, M., Yao, Y., Bassu, S., Ciais, P., Durand, J. L., Elliott, J., Ewert, F., Janssens, I. A., Li, T., Lin, E., Liu, Q., Martre, P., Müller, C., Peng, S., … Asseng, S. (2017). Temperature increase reduces global yields of major crops in four independent estimates. *Proceedings of the National Academy of Sciences of the United States of America*, 114(35), 9326–9331.