

Chapter 9

Plant Breeding Strategies and Methods for Food Security: Review on the Technology



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9.1 Introduction

The primary necessity of human beings is food which needs to produce using diverse agricultural genetic resources from suitable environments for supplying balanced nutrients. Along with the advances in agricultural science, particularly, genetic manipulation, there is a hope to achieve food, nutrition and environmental security worldwide. However, meeting the expected increases in food and nutrition demand remains a big challenge. Crop breeding (i.e., genetic manipulation) had contributed to about half of the average global production increase in cereals that were achieved under the Green Revolution (Joshi 2017a, b; Tollenaar and Lee 2006). The other half came from other agricultural inputs such as the use of fertilizers, pesticides, irrigation and expansion of cultivated areas. Intensive agriculture relies on natural resources to a great extent and the degradation of the natural resource base could limit the scope of production ability for the future generations (Chaudhary et al. 2020; FAO 1999; Joshi et al. 2020a), emerging the sustainability issues in agricultural practices over the long run. This indicates the increased role of crop diversity and breeding in meeting the global demand for agricultural production.

FAO (2009) reported that the world's food production must increase by 70% in 2050 to feed a projected extra 2.3 billion people. The demand for cereals, both for food and animal feed, is projected to reach some 3 billion tons by 2050. To meet the

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production increment by 2050, only 10% is expected from an expansion in arable land and the remaining therefore should come from a high yield. In the past, the increased production of cereals enabled many countries to feed their populations (the doubling of yield per hectare was observed between 1960 and 1985) (Eliazer Nelson et al. 2019). In the current decade, the yield increment of major crops is relatively very low as compared to the population growth rate (Fig. 9.1). Land area increment and cereal productivity are given in Fig. 9.2. With an increase in arable land, cereal production is not increasing at the same rate (Fig. 9.2) which indicates the need for improving the crops for their higher yield than just increasing the

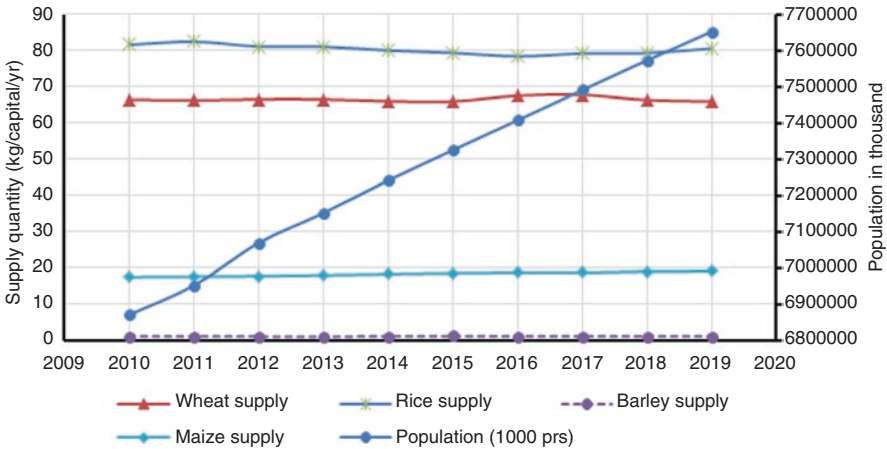


Fig. 9.1 Population and major crop production supply over the last decade. (Source: FAO (<https://www.fao.org/faostat/en/#home>))

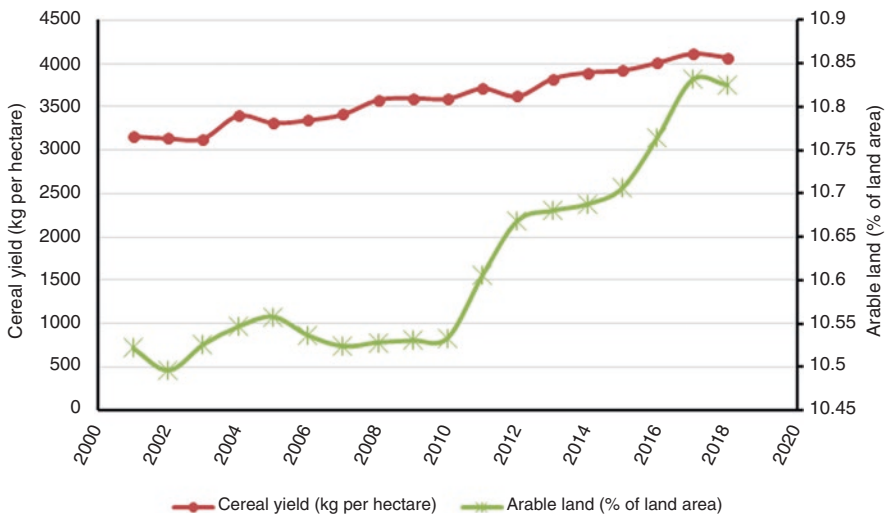


Fig. 9.2 Total arable land (% of land areas) and cereal yield (kg per hectare). (Source: World Bank Development Indicators <https://data.worldbank.org/indicator?tab=all>)

cultivated land area. Even the total production of major crops is not increased in this decade (Fig. 9.3). It indicates that the yield of these crops has reached a plateau level.

Among the three approaches (area expansion, management and genetic improvement) to increase food production (Fig. 9.4), genetic improvement is the best approach for food, nutrition and environmental security. However, genetic advancements introduced high-yielding varieties of mainly rice, wheat and maize to increase food production and alleviate hunger, and in contrast, several indigenous crops were largely ignored. This is because the human relies on just three crops (rice, wheat and

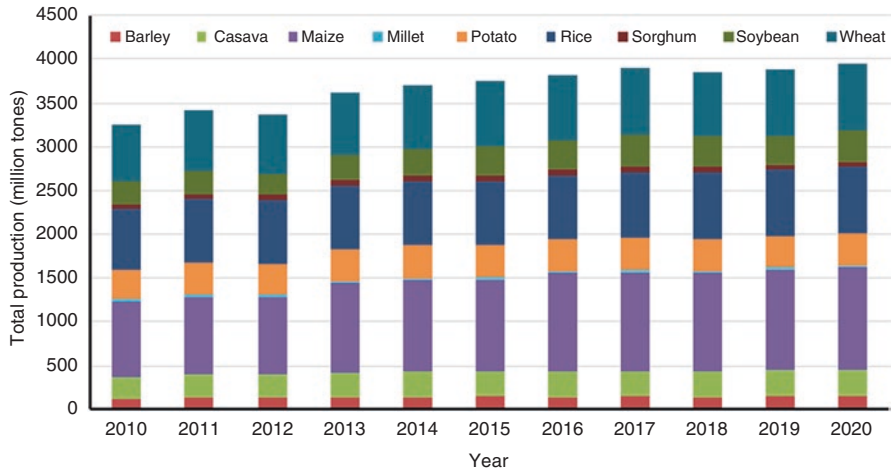


Fig. 9.3 Total production of major crops in the world. (Source: FAO (<https://www.fao.org/faostat/en/#data/QCL>))

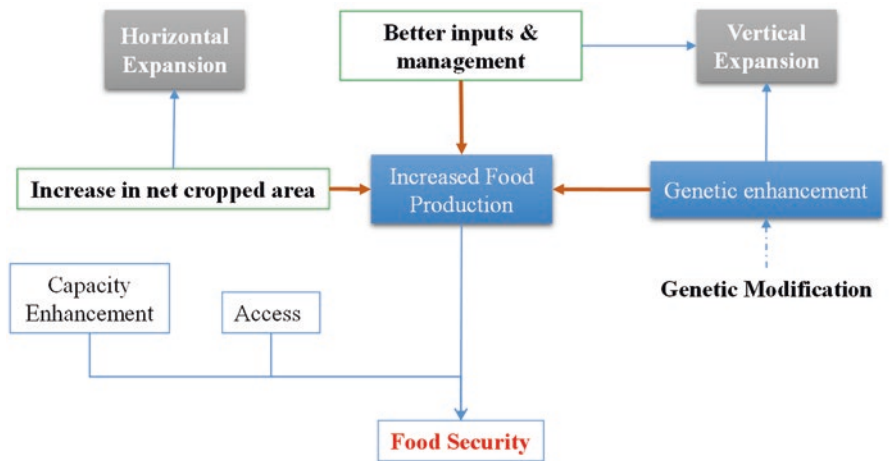


Fig. 9.4 Food security strategy and option

maize) for nearly 60% of their plant-derived calories (<https://www.fao.org/3/y5609e/y5609e02.htm>). The worldwide situation of food deficit and undernourishment are depicted in Figs. 9.5 and 9.6 respectively. The degree of food deficit is increasing each year. One in three people suffers from micronutrient deficiencies and almost 2 billion people are overweight or obese (<https://www.fao.org/news/story/en/item/455867/icode/>). Agricultural production is expected to reduce by 2% and demand will increase by 14% every decade till 2050 (<https://www.ipcc.ch/report/ar5/syr/>). In this situation, the food and nutritional supply will further worsen. Therefore, crop improvement by manipulating the genotypes is the most important strategy for food security across the world.

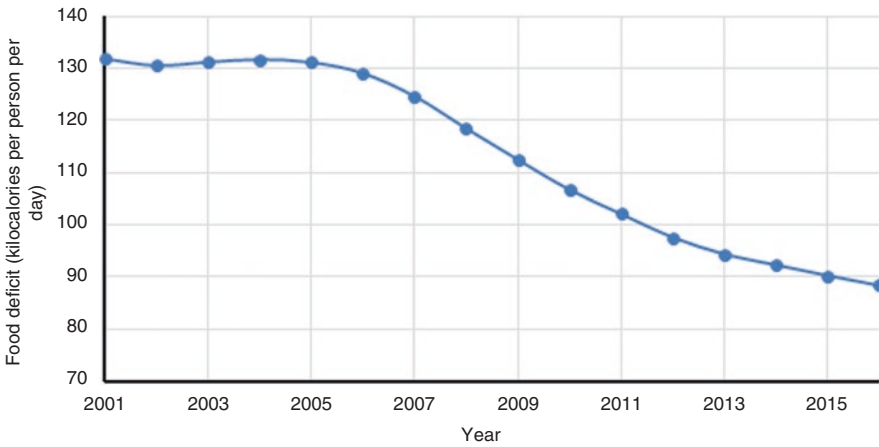


Fig. 9.5 Food deficit (kilocalories per person per day) worldwide over the years (Source: World Bank Development Indicators (<https://data.worldbank.org/>))

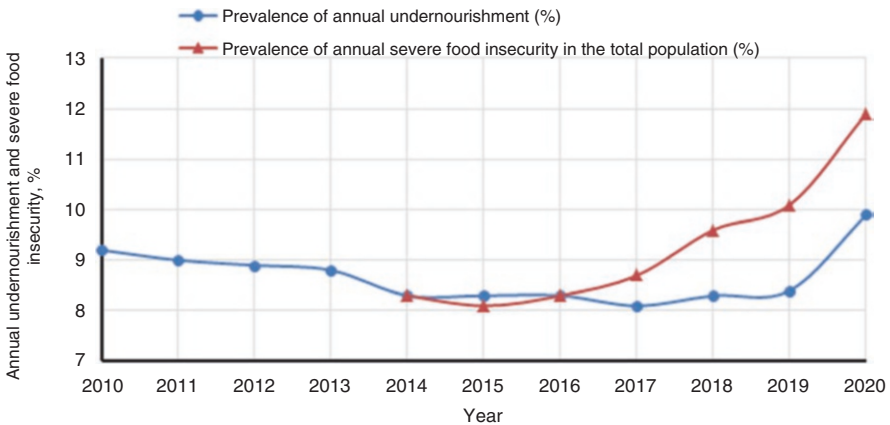


Fig. 9.6 The annual prevalence of undernourishment and severe food insecurity percentage. (Source: Food and Agriculture Organization (FAO) (<http://www.fao.org/faostat/en/#data/FS/visualize>))

9.2 Crop Biodiversity and Technological Advances

Genetic variation facilitates crop improvement the most. The higher the degree of genetic diversity, the more is the chance of getting superior genotypes concerning different traits. Natural variation is now almost fully exploited in major crops. If there is limited natural genetic variation, variations are being created artificially either through mutagens or crossing among varieties, species and even genera, or transferring the gene of interest. There are 250,000 to 300,000 known edible plant species in the world, however, only 4% i.e., 150 to 200 species are used by humans (FAO 1999). Among them, only rice, maize and wheat contribute about 60% of calories and proteins obtained by humans from plants. Crop improvement works also got priority to these three crops over the years and locations. Only 12 crops and five animal species provide 75% of the world's food. Advanced breeding techniques are only being used in a few crop species. Most of the natural crop diversity is conserved ex-situ in static conditions. A total of 63, 95, 166 accessions are available through ITPGFRA-MLS (known as the global gene pool) for utilization in breeding programs (<https://mls.planttreaty.org/itt/index.php?r=stats/pubStats>). Most of them are of rice, wheat and maize species. With due focus and priority to only a few crop species, only a few varieties have been grown in many countries. This resulted in the loss of about 75% of crop genetic diversity worldwide mainly because of growing and disseminating genetically uniform, high-yielding varieties (FAO 1999).

Many countries now have adapted strategies to minimize genetic erosion and diversify their food systems. The scope of crop breeding has extended to other species including both crops and plants. Crops include all domesticated species under the kingdom Plantae and plants cover wild species under the kingdom Plantae. These are collectively called agricultural plant genetic resources (APGRs). The classification of these resources has been represented in Fig. 9.7 based on their economic uses. All these groups need to consider in the breeding programs that help to secure food, nutrition, and the environment worldwide.

Natural and artificial selection remain as the most important practice to develop high-yielding varieties. To assist the selection process, breeders used only phenotype in the earliest period. With the progression of scientific understanding and technological advancement, breeders have started using genotype, metabolites, nutrient profiles as well as omics technology for developing desired genotypes (Fernie and Schauer 2009; Lenaerts et al. 2019). The key milestones related to crop improvement are represented in Fig. 9.8 along the year. All these advances have significant contributions to developing superior varieties for some economic traits. These are also equally important in creating genotypes with resilient to climate change.

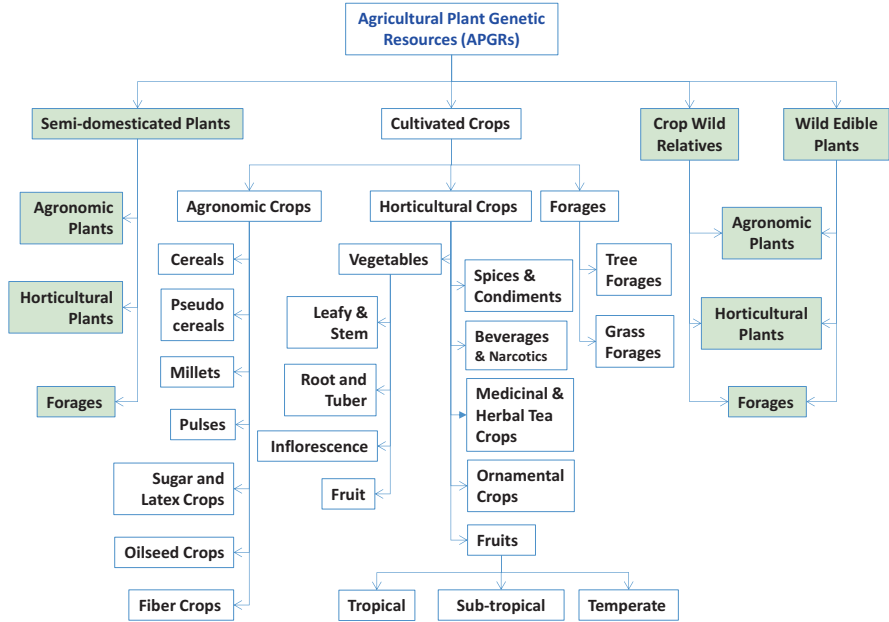


Fig. 9.7 Plant classification based on economic value and uses. (Adapted from Joshi and Shrestha 2017)

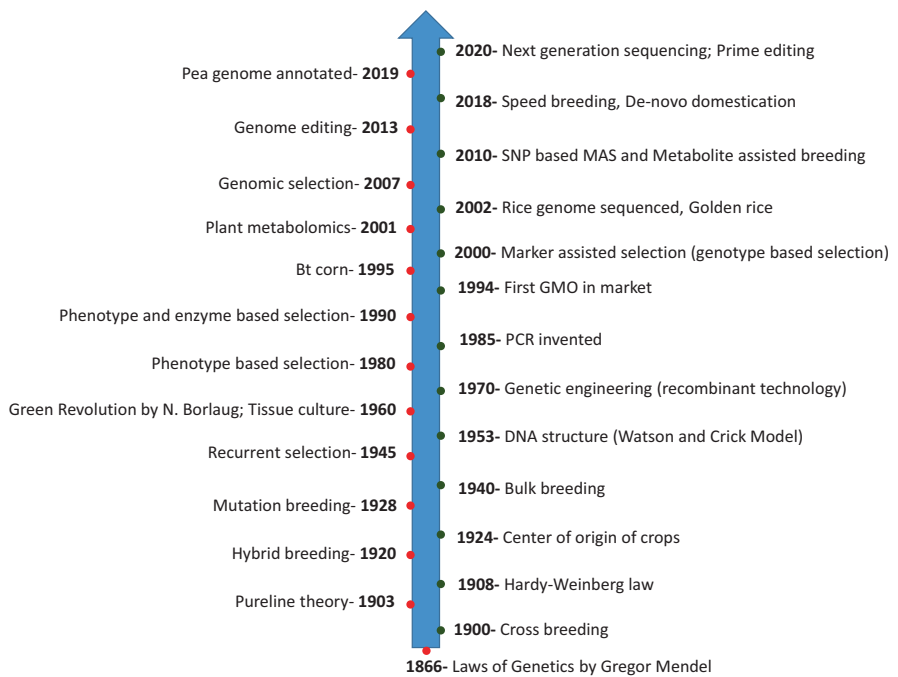


Fig. 9.8 Key milestones related to crop improvement

9.3 Crop Improvement Strategies and Methods

The strategy for crop improvement changes over time and differs among breeding institutes and countries. Developing an optimum strategy is very important to meet the objectives within a given time frame. A major aim of crop improvement is to increase economic yield; however, it depends on different factors. For example, in biotic (e.g., diseases and insect pests) and abiotic (e.g., temperature, drought, salinity and light) stresses, the major aim would be to develop resistance or tolerance varieties to these stresses. The strategic options for improving crops are wide vs specific adaptation, mono vs polymorphic variety, broad vs narrow genetic base variety, participatory vs non-participatory method, evolutionary vs static population, etc. In the past, the crop improvement strategy was to develop widely adapted monomorphic variety across the country, or for the region and even the world. Such varieties had a very narrow genetic base and were commonly developed by breeders with very limited involvement of farmers. The crop diversity in the field had been collected and conserved ex-situ. From this diversity, a single homozygotes variety was developed expecting to grow in large areas. This system ignored the importance of diversity in the field. These strategies increased the dependency for varietal seeds on other institutes and countries and replaced local crop diversity (Chaudhary et al. 2020; FAO 1999; Joshi 2017a; Joshi et al. 2020a, b). Many breeders focused on developing stable and high-yielding varieties across diverse environments. This limits the yield advantages of variety in a particular environment. A breeding strategy can also be either improvement on a single trait or multiple traits.

Then the strategy in many countries focused on developing site-specific polymorphic varieties with a broad genetic base. The involvement of farmers in the crop improvement process from the very beginning also got priority in many breeding programs (Joshi 2017a). This is called participatory plant breeding. Recently, the breeding strategy has been changed from a developing static population to an evolutionary population (Joshi et al. 2020a). With the multiple strategies, different approaches are being used by different breeding institutes, for example, conventional crop improvement approaches, fast track breeding, hybridization among close relatives as well as distantly related genotypes, phenotype-based selection, and genotype-based selection including gene editing. Crop improvement activities are carried out in the lab and field. During the field test, the trials are conducted both on-station and on-farm. Many improved varieties have a high amount of carbohydrates rather than balance nutrients (Joshi et al. 2020c) which trigger to focus on nutritionally balanced varieties.

Crop improvement is mainly concerned with the manipulation of the genetic makeup of existing landraces and varieties. Broadly four methods are being used to manipulate the genetic makeup (Fig. 9.9). Vertical gene transfer and mutation are old and very common methods of crop improvement globally used for many crop species. Horizontal gene transfer is an advanced technology through which genes of interest of any species can be transferred to the target genotype (Fig. 9.9). Grafting, also called organ transplant is a simple technique to change the genetics of rootstock in crop species. This is very useful for managing soil-borne diseases and insect pests along with better nutrient uptake.

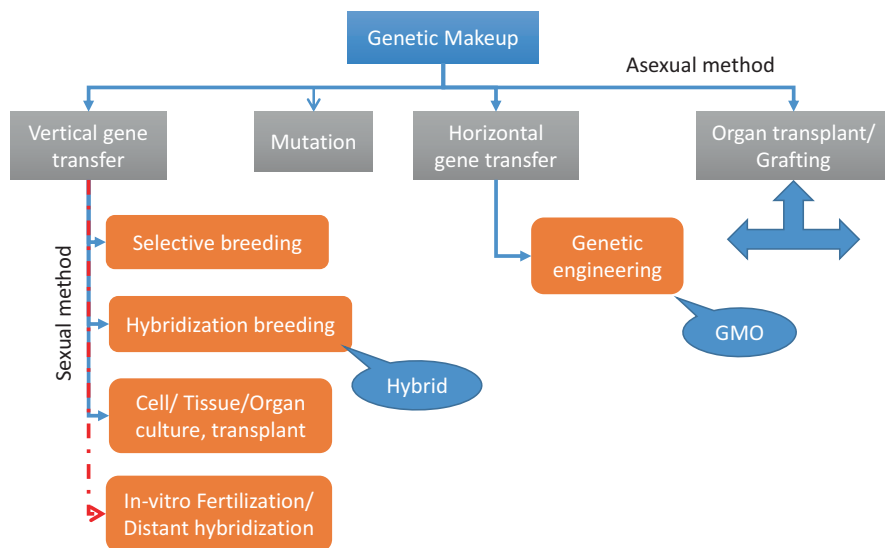


Fig. 9.9 The basic technology for genetic manipulation of crop genetic resources (red line means not commonly used, arrow below organ transplant means traits not inherited). (Adapted from Joshi 2017a, b)

Several different breeding methods have been developed and are used to improve the crops (Breseghello and Coelho 2013; Joshi 2017a, b; Lenaerts et al. 2019; Munaweera et al. 2022; Razzaq et al., 2021). The application of these methods depends on the types of crops used and localities e.g., in lab, glasshouse, on-station, or on-farm. Broadly breeding methods differ among three types of crops based on the mode of reproduction (self-pollinated, cross-pollinated and clonally propagated crops). The general scheme of crop improvement is provided in Fig. 9.10 for self and cross-pollinated crops. Clonal selection is a very common means of improving vegetatively propagated crops e.g. potato, apple, banana, etc. A brief description of Crop improvement methods is listed in Table 9.1 along with a brief description. The selection of methods depends on mainly the objectives of the breeding, crops and facilities available in the breeding center.

9.4 Biotechnological Advances

The current world population of 7.95 billion in 2022 is expected to reach 10 billion in 2057 (Worldometer 2021). Therefore, feeding the expected additional 2.1 billion people is a challenge in global plant breeding. Plant breeding is mainly concerned with the genetic improvement of crops through hybridization, screening, and selection of advanced lines (Adlak et al. 2019). During the last century, crop plants have been improved for increasing the yield in coordinated ways through classical plant

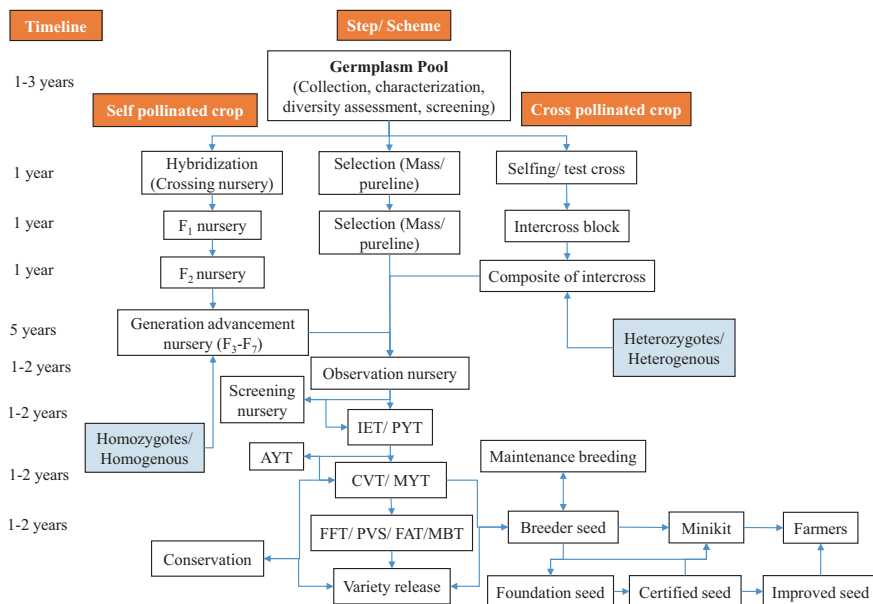


Fig. 9.10 Conventional methods of crop improvement and dissemination. Foundation seed is also called source seed, and certified seed as labeled seed. In another seed class, the foundation and certified seeds are equivalent to source and labeled seeds. (Adapted from Joshi 2017a, b)

Table 9.1 Crop improvement methods

SN	Crop improvement method	General definition	Important features
1	Allele and gene mining	Discovery of superior alleles, through ‘mining’ the gene of interest from diverse genetic resources	Genetic resources collections are screened for allelic variation by the ‘tiling strategy’ using DNA chip (microarray or biochip) technology
2	Anther culture	Regeneration of plant from anther	In-vitro technique for production of haploid and doubled haploid plantlets
3	Anti-sense RNA	One of the approaches that are used for the inhibition of gene expression or downregulation of a gene	Prevent translation of complementary RNA strands by binding to them. It plays an important role in generating high-yielding, disease and pest resistant, high nutritional value, and stress-tolerant crop varieties
4	Backcross breeding	A crossing of a hybrid with one of its parents	Transfer of single gene to target genotype
5	Biofortification	The process of developing a crop with higher levels of essential nutrients in its edible parts	Through selective breeding or biotechnological approaches

(continued)

Table 9.1 (continued)

SN	Crop improvement method	General definition	Important features
6	Bulk selection	Segregating population is grown in bulk with or without selection	A part of the bulk seed is used to grow the next generation and individual plant selection is practiced in F ₆ or later generations
7	Cultivar mixture	Growing more than one variety or landrace together by mixing seeds	Create high intra-varietal diversity and create buffering for many stresses
8	Domestication	Process of making wild plants responsive in a human management environment	Adaptation of a plant from a wild or natural state (as by selective breeding) to life in close association with humans
9	Double haploid breeding	Production of diploid plants from pollen or egg cells or other cells of the gametophyte, then by induced or spontaneous chromosome doubling	Pure homozygous lines require a shorter time to produce in comparison to classical <i>breeding</i>
10	Embryo rescue	An in-vitro technique to protect the weak, immature, and hybrid embryo and promote it to develop into a complete plant	Useful for distantly related plant species to cross
11	Evolutionary plant breeding	Development of crop populations with a high level of genetic diversity and subjected to natural selection	A large number of different genotypes can combine including segregating lines to create a resilient population
12	Gene (genome) editing	Technologies that allow genetic material to be added, removed, or altered at particular locations in the genome	Ability to make highly specific changes in the DNA sequence of a living organism, essentially customizing its genetic makeup
13	Genetic engineering	The artificial manipulation, modification, and recombination of DNA or other nucleic acid molecules to modify an organism or population of organisms	Uses of distantly related genes in species to modify their genetic makeup, which natural not possible to combine
14	Genome-wide association studies (GWAS)	Scanning several hundred thousand markers across the complete sets of DNA of many plants to find genetic variations associated with a particular trait.	An observational study of a genome-wide set of genetic variants in different individuals to see if any variant is associated with a trait
15	Genomic assisted breeding	The integration of genomic tools with high throughput phenotyping to assist breeding practices through molecular markers to facilitate the prediction of phenotype from genotype	The selection of beneficial alleles for multiple loci throughout the genome
16	Heterosis breeding	Development of hybrid showing hybrid vigor through using diverse parents	Hybrid performs better than either parent, hybrid is developed through heterosis breeding

(continued)

Table 9.1 (continued)

SN	Crop improvement method	General definition	Important features
17	Hybridization	Crossing of two or more genetically dissimilar parents	Artificial transfer of pollen from one parent to another parent to produce hybrids
18	Ideotype breeding	A method of crop improvement that is used to enhance genetic yield potential through genetic manipulation of individual plant character	Model plants or ideal plant types for a specific environment
19	Introduction	Taking a genotype or a group of genotypes into a new place or environment	High-yielding varieties developed elsewhere are brought to target new environment
20	Maintenance breeding	Principles and method of breeder/nucleus seed production and maintenance	a breeding procedure followed to maintain the genetic purity of the variety or parents of hybrid.
21	Marker-assisted selection	An indirect selection process where a trait of interest is selected based on a marker linked to a trait of interest, rather than on the trait itself	DNA marker closely linked to target trait is used to select at any crop stage
22	Mass selection	A large number of plants of different desirable phenotypes are selected and their seeds are mixed to constitute a new variety	The plants are selected based on their appearance or phenotype. The population obtained from the selected plants would be more uniform than the original population
23	Metabolomics assisted breeding	Selection of genotype based on metabolites, for developing variety which produces the higher and superior metabolites	A systematic screening of crops to know the metabolic and chemical footprints of plant regulatory processes
24	Micro-propagation	The artificial process of producing plants vegetatively through tissue culture or cell culture techniques	Produce virus-free plantlets, and a large number of plantlets within a short period
25	Molecular breeding	The use of genetic manipulation performed at DNA molecular levels to improve characters of interest in plants	It includes genetic engineering or gene manipulation, molecular marker-assisted selection, genomic selection, etc. application of molecular biotechnologies, specifically molecular markers, in combination with linkage maps and genomics, to alter and improve plant traits based on genotypic assays
26	Mutation breeding	The process of exposing crops to chemicals, radiation, or enzymes to generate mutants with desirable traits	Create variation in crops and many different new genotypes can be selected

(continued)

Table 9.1 (continued)

SN	Crop improvement method	General definition	Important features
27	Omics technology	Detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics) and metabolites (metabolomics) in a specific crop sample	Very advanced technology for understanding and manipulating the genotype of any crop plant
28	Participatory landrace enhancement	Mass selection approach involving both farmers and breeders for locally available landraces on-farm	Help improve local landrace genetically maintaining a broad genetic base and quality
29	Participatory plant breeding	Selection of segregating materials in farmer's field involving both farmers and breeders	Interaction among farmers and breeders helps to select suitable genotypes from the segregating populations
30	Participatory varietal selection	An approach that provides a wide choice of varieties to farmers to evaluate in their environment using their resources for increasing production.	Individual farmers can select different genotypes as per their choice from among the few fixed genotypes on-farm
31	Pedigree selection	A breeding method in which the breeder keeps records of the ancestry of the cultivar	Established by crossing selected parents, followed by handling an actively segregating population
32	Polyploidy breeding	Development of variety with multiple sets of chromosomes over the diploid number	Induced chromosome manipulation targeting different ploidy levels
33	Protoplast fusion	Two or more protoplasts bring in contact and adhere with one another either spontaneously or in presence of fusion inducing chemicals	Also called somatic fusion, is a type of genetic modification in plants by which two distinct species of plants are fused to form a new hybrid plant with the characteristics of both, a somatic hybrid
34	Pure line selection	Selection and breeding of progeny obtained from superior genotypes for several generations to attain a pure line having desired characters	Genetically same with almost zero genetic diversity
35	Recurrent selection	Repeated cycles of selection and breeding aimed at gradual genetic improvement of a few key traits	Population improvement involves reselection generation after generation with the interbreeding of selects to provide for genetic recombination
36	Resistance breeding	Development of resistance or tolerant varieties to disease and insect pests using suitable genes	Diseases and insect pests do not affect the crop stands
37	Shuttle breeding	An extra generation is advanced each year by using a different field location	Two generations are advanced growing two times in the same year

(continued)

Table 9.1 (continued)

SN	Crop improvement method	General definition	Important features
38	Single seed descent	Selection of a single seed from each plant, bulking the individual seeds and planting out the next generation	The advancement of one randomly selected seed per plant through the early segregating stages
39	Space breeding	Space-grown plants are exposed to cosmic radiation and microgravity, which led to the generation of crop varieties with diverse genotypes and phenotypes arising from different cellular, subcellular, genomic, chromosomal, and biochemical changes	Mutation breeding exposes seeds to the region beyond the earth's atmosphere or beyond the solar system (space) to create superior crop varieties
40	Speed breeding	The manipulation of environmental conditions under which crop genotypes are grown, aims to accelerate flowering and seed set, to advance to the next breeding generation as quickly as possible.	Growing plants under continuous light (20–22 h). This allows plants to photosynthesize for longer, resulting in faster growth. With this technique, four to six generations of wheat plants can be grown per year instead of two generations under normal growth conditions
41	Stress tolerance breeding	Breeding approaches to improve several traits simultaneously for stress tolerance	Using abiotic and biotic resistance/ tolerant genes
42	Transgenic breeding	Gene transfer between related or unrelated species and even between unrelated organisms	It permits gene transfer even between plants and/or animals, through genetic engineering

breeding techniques and numerous varieties of several crops have been developed across the world (Pandey et al. 2019). However, the conventional breeding approaches to improve crop varieties are considered a constraint due to genetic erosion, genetic drag, and reproductive obstacles, and take a longer time (6–12 years) (Adlak et al. 2019; Pandey et al. 2019). Therefore, considering the trends of current yield of crops, expected world population growth, and pressure on the environment, the yield-enhancing traits, including disease resistance, abiotic stress tolerance, biotic stress tolerance, and enhancing nutritional quality, and water use efficiency need to be developed stably and sustainably, which should be a major target of global plant breeding. Biotechnology tools make breeding methods more advanced by reducing the time to obtain improved varieties of desired traits. Therefore, there is an urgent need for the integration of both breeding and biotechnology tools to improve the yield, nutrition, and quality of crop varieties. Plant tissue culture, molecular breeding, and genetic engineering are the three major approaches to deal with crop improvement via biotechnology (Adlak et al. 2019). In addition, biotechnology has also greater role on conservation of agrobiodiversity (Joshi 2017b).

Plant tissue culture is a novel tool or an emerging field, which was invented by Gottlieb Harberlandt in 1902, is a culturing of any part of the plant (cells/tissues or organs) in the synthetic medium under an aseptic (sterile) environment or workplace, and controlled conditions of light, temperature and humidity in a laboratory or a greenhouse for improving the accessibility of existing germplasm and creating a new genetic variation for sustainable agricultural crop improvement, plant breeding, horticulture, fresh produce, medicinal or ornamental plants, industrial chemistry, forestry, and is a prerequisite for plant genetic engineering (Bonner 1936; Evans et al. 2003; Tazeb 2017; Loyola-Vargas and Ochoa-Alejo 2018; Vakhariya et al. 2019). The technique is also known as Micropropagation. The ability of plant cells or tissues to be totipotent is referred to as “totipotency”. It can be applied for the culture of callus, organ, cell, cell suspension, anther or pollen, somatic embryogenesis, protoplast, shoot tip/bud & meristem, embryo and seed for generating clumps of shoots or the whole plants that are useful to develop new crop varieties in agriculture (Bonner 1936; Joseph et al. 1996; Singh et al. 2011; Waghmare et al. 2017; Loyola-Vargas and Ochoa-Alejo 2018; Adlak et al. 2019; Efferth 2019; Vakhariya et al. 2019; PCT 2020; Motolinía-Alcántara et al. 2021).

Molecular marker-assisted breeding (MAB), particularly molecular (DNA) markers are the powerful, practical, and best indirect selection for target genes at the DNA level, which will greatly increase the efficiency and precision of plant breeding. It is the use of molecular technologies with linkage maps and genomic approaches, to edit and improve traits of interest based on genotypic analysis. It has significant advantages compared with conventional breeding methods such as improving the genetic quality of different plant species very fast; however, it is not a replacement conventional system. Therefore, the integration of MAB into conventional breeding programs represents an optimistic strategy for future crop improvement (Jiang 2013). Since the 1990s, MAB has received increasing attention and has been extensively used in different crop species. The reasons for using MAB are to (i) allow indirect selection of desirable gene alleles without the confounding effects of environment, pleiotropic, or epistatic gene effects; (ii) enable discrimination between plants homozygous or heterozygous for a given gene; (iii) monitor the introgression of a desirable allele in backcrossing; and (iv) identify of recombinants exhibiting the least amount of linkage drag (donor DNA) (Rajcan et al. 2011).

The use of DNA markers in plant breeding includes marker-assisted selection (MAS), marker-assisted or marker-based backcrossing (MABC), marker-assisted gene pyramiding (MAGP), marker-assisted recurrent selection (MARS) and genomic selection (GS) or genome-wide selection (GWS) plays a crucial role in the improvement of high yielding crop varieties (Castro et al. 2003; Collard et al. 2005; Francia et al. 2005; Dwivedi et al. 2007; Goddard and Hayes 2007; Li et al. 2010; Luo and Yin 2013; Al-Khayri et al. 2015; Osei et al. 2018; Pandey et al. 2019; Kumawat et al. 2020).

Genetic engineering, also known as genetic modification or genetic manipulation, is the engineering of an organism’s genes using a set of technologies for changing the genetic makeup of cells, including the transfer of gene(s) within the same species through the use of **cisgenic** approach or across species boundaries through

the use of **transgenic** approach to producing improved or novel organisms (Schouten et al. 2006; Jacobsen et al. 2008; Karthikeyan et al. 2012; Shrestha 2017; Pandey et al. 2019). In general, genetic transformation contains two steps, including genetic cargo delivery and regeneration. The new DNA can be inserted randomly or targeted to a specific part of the genome. In crops/plants, it is the modification of specific gene(s) into the crop(s)/plant(s) genomes, incorporating desired single or stacked genes (gene pyramiding and multigene) with a construct for achieving novel crop(s)/plant(s) more efficiently than the conventional breeding system (Shrestha 2017). The new DNA is obtained by either isolating and copying the genetic materials of interest using recombinant DNA methods or by artificially synthesizing the DNA. It has opened new avenues to modify crops and provided new solutions to solve specific needs. The system has a great opinion to improve crop genotype with significant commercial values that include increasing yield, protecting from environmental threats by developing biotic (e.g., resistance to disease, bacteria and pests) and abiotic (e.g., temperature, drought, salinity, light) stress tolerances, improving the nutritional quality of crops (e.g., vitamins A, Zinc, protein) and developing non-food crops (e.g., molecular pharming, biofuels, bioremediation) (Shrestha 2017).

The use of **plant genetic engineering** such as transgenic, cis-genic, and **genome editing** such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) have made it possible to transfer the gene from related, unrelated plant species and even from non-plant organisms to crop plants (Nemudryi et al. 2014; Bonawitz et al. 2018; Ei-Bassyouni and Mohammed 2018; Ran et al. 2018; Kumari et al. 2021; Zhang et al. 2021). The advances in the biotechnological plant breeding approaches have efficient options to improve target crop varieties with significant yield, nutrition, and quality attributes. In addition, conservation biotechnology has a greater role in making access to existing crop diversity, particularly endangered species, and landraces in the future (Joshi 2017a, b). Genetic engineering can also be used to remove genetic materials by knocking down a gene with RNAi to produce a desirable phenotype (Capecchi 2001). The term transgenic is favored by scientists but GM has been adopted most widely by non-specialists. Currently, it is a controversial public concern and reduces the market charm; therefore, the development of the marker-free system is a priority that can avoid selectable and marker genes in the target product(s).

9.5 Breeding for Food Security

Food security is one of the major global challenges that we face as access to the basic amount of food necessary for a healthy life must be achieved by the growing population. Food security, as defined by the United Nations Committee on World Food Security, means that all people, always, have physical, social, and economic access to sufficient, safe, and nutritious food that meets their food preferences and

dietary needs for an active and healthy life. Over the coming decades, a changing climate, growing global population, rising food prices, and environmental stressors will have significant yet uncertain impacts on food security (IFPRI 2022). Greater and more consistent crop production must be achieved against a backdrop of climatic stress that limits yields, owing to shifts in pests and pathogens, precipitation, heat waves and other weather extremes (Bailey-Serres et al. 2019).

As the current world population is increasing at a faster pace and so many mouths need to feed, trying to attain food security will exert significant pressure on arable lands. The current climatic trend and population growth are to further extend the gap between food demand and food production in the world. According to the predictions of FAO, agricultural productivity requires to be increased by 60% more in 2050 to feed the world population (Silva 2018; Bohn 2014). Future access to affordable and healthy food will be challenging, with malnutrition already affecting one out of three persons worldwide (Varshney et al. 2021a). Several studies have shown that global crop production needs to double by 2050 to meet the projected demands of the rising population, diet shifts, and increasing biofuel consumption. Boosting crop yields is a preferred solution to meet this goal of meeting the rising demands, rather than clearing more land for agriculture.

Globally, hunger levels remain alarmingly high (The World Bank 2022) and the number of people affected by hunger arrived to 828 million in 2021 with an increase of about 46 million since 2020 (FAO et al. 2022) indicating the acute food insecurity in near future. FAO predicts that crop yields will decline by 25% by 2050 if we do not adequately address climate change. Plant breeding and the seed sector can help to reduce global hunger (Europeanseed 2017). A world with zero hunger is possible only through a sustainable increase in food production and distribution and the elimination of poverty. Scientific, logistic, and humanitarian approaches must be employed simultaneously to ensure food security, starting with farmers and breeders and extending to policymakers and governments (Fiaz et al. 2021). At present when the availability of staple foods needs to be dramatically increased to meet the needs of a rising human population; abiotic stresses (drought, salinity, high temperature, cold) and climate changes have been established as severe threats to global crop production. Thus, increasing plant resistance to various abiotic stresses is one of the top-priority goals for the scientific community to meet the food security need (Choudhary et al. 2022).

Four key global crops maize, rice, wheat, and soybean that currently produce nearly two-thirds of global agricultural calories have increasing yields at the rate of 1.6%, 1.0%, 0.9%, and 1.3% per year, non-compounding rates, respectively, which is less than the 2.4% per year rate and is far below what is needed to meet projected demands in 2050 (Ray et al. 2013). However, climate change can dramatically reduce agricultural productivity and the improvement of cultivars with limited resources may be required for resolving the food security issues in the world. Farmers around the world have recently experienced significant crop losses due to severe heat and drought. Such extreme weather events and the need to feed a rapidly growing population have raised concerns for global food security. While plant breeding has been very successful and has delivered today's productive crop

varieties, the current rate of genetic improvement in terms of improved yield and quality trait performance must be doubled to meet the projected future demands (Voss-Fels et al. 2019). Thus, acceleration of the breeding process and advancement of breeding technologies are needed to make plant breeding more responsive to constantly moving targets and sustain its role as the principal provider of food security (Lenaerts et al. 2019).

The current agricultural production system is facing the challenge of sustainably increasing grain quality and yield and enhancing resistance to biotic and abiotic stress under the intensifying pressure of climate change. Under present circumstances, conventional breeding techniques are not sufficient. Innovation in plant breeding is critical in managing agricultural challenges and achieving sustainable crop production. Novel plant breeding techniques, involving a series of developments from genome editing techniques to speed breeding and the integration of omics technology, offer relevant, versatile, cost-effective, and less time-consuming ways of achieving precision in plant breeding and to attain global food security (Fiaz et al. 2021).

9.5.1 Role of Plant Breeding for Enhancing Crop Productivity

Plant breeding is the art and science of changing the genetic make-up of plants to produce desired characteristics like improved yield, taste, biotic and abiotic stress resistance, etc. for the benefit of mankind. Crop varieties play an essential role in improving the performance of the global agricultural industry; therefore, breeders are seeking new sustainable, efficient, and cost-effective methods to produce new varieties (Goncharov and Kosolapov 2021). It was found that almost 90% of the increase in average cereal yields over the past 25 years can be attributed to innovations in plant breeding with the development of new varieties with enhanced productivity. Typically, plant breeding contributes 1.0–1.5% yield improvement per annum (ISTA 2022).

Previously, conventional plant breeding through cross- and self-pollination strategies played a major role in improving agricultural productivity. Heterosis breeding can increase yield by more than 20% (Joshi 2003). New plant breeding approaches like evolutionary plant breeding can develop populations that have the potential to produce higher yields and perform better than their local or improved counterparts in adverse climatic conditions and thus can be utilized in developing improved populations of neglected and underutilized crops for food security (Joshi et al. 2020a). Similarly, another plant breeding approach like speed breeding is also under investigation which greatly shortens generation time and accelerates breeding and research programs. Speed breeding can be used to achieve up to 6 generations per year for spring wheat (*Triticum aestivum*), durum wheat (*Triticum durum*), barley (*Hordeum vulgare*), chickpea (*Cicer arietinum*) and pea (*Pisum sativum*), and 4 generations for canola (*Brassica napus*), instead of 2–3 under normal glasshouse conditions (Watson et al. 2018). These approaches can help in achieving the balance

between the improvement rate of crops and the growth rate of the population thus expected to ensure food security in near future. Moreover, careful deployment and scientifically informed regulation, new plant breeding technologies (NPBTs) such as genome editing and the adoption of genetically modified (GM) crops by small-holder farmers have led to higher yields, lower pesticide use, poverty reduction, and improved nutrition (Qaim 2016). Recent advancements in genetic engineering have revolutionized plant breeding and crop improvement. Biotechnology enables creating dramatic alterations on crops to withstand stress which is difficult to attain using conventional breeding approaches. The development of biotechnological approaches such as genetic engineering, genome editing, RNA-mediated gene silencing armored with next-generation sequencing, and genome mapping have paved the way for precise and faster genetic modifications of plants. Those tools are utilized in creating high-yielding better-adapted crop varieties that are resilient to climatic changes (Munaweera et al. 2022). Targeted and rapid assembly of beneficial alleles using optimized breeding strategies and precise genome editing techniques could deliver ideal crops for the future. The implementation of this knowledge in breeding strategies might accelerate the progress in obtaining high-yielding cultivars (Nadolska-Orczyk et al. 2017).

The world today relies on a small number of crops for food. Out of the most used 15 crops which provide 90% of the world's food energy intake, the four crops; rice, maize, wheat, and soybean are known to dominate the global food system (Vogel et al. 2019). In addition to those, potatoes, tomatoes, bananas, cassava, etc. also play a very important role in global food security in less productive developing regions of the world. It is also found that crops of temperate and sub-tropical zones are more prone to yield loss. From 1980 to 2015 the decline in global wheat and maize yields due to biotic stresses was found to be 20.6% and 39.3%, respectively (Ristaino et al. 2021; Daryanto et al. 2016). Drought stress alone is estimated to limit crop productivity by more than half the amount in the next 50 years (Castaño-Sánchez et al. 2020). When considering the impact of climate change on food security globally, climate change is a serious threat to global food security, sustainable development and poverty eradication. Genetic improvement of crops is considered one possible solution to the crisis (Joshi 2017a). Thus, the identification and efficient utilization of superior genes is crucial for crop improvement methods for ensuring global food security.

The current development of advanced biotechnology tools allows us to characterize the role of key genes in plant productivity. Scientists from the University of Cambridge and the University of Bordeaux have discovered the Phloem Unloading Modulator (PLM) gene that helps control the movement of nutrients throughout plants which could be modified to increase crop yields influencing the transport of sugars, proteins, and other key organic nutrients between different parts of the plant that could provide the key to boosting crop production (Yan et al. 2019). AaPEPC1 gene (from *Agave americana* into tobacco) overexpression enhanced proline biosynthesis, and improved salt and drought tolerance in the transgenic plants. Under salt and drought stress conditions, the dry weight of transgenic tobacco plants overexpressing AaPEPC1 was increased by up to 81.8% and 37.2%, respectively, in

comparison with wild-type plants. This finding has opened a new door to the simultaneous improvement of photosynthesis and stress tolerance in plants in the context of climate change (Liu et al. 2021). Such innovations in plant genomics and biotechnology will surely contribute to global food security. Several plant features and traits, such as overall plant architecture, leaf structure and morphological features, vascular architecture and flowering time are important determinants of photosynthetic efficiency and hence the overall performance of crop plants. The optimization of such developmental traits thus has great potential to increase biomass and crop yield (Mathan et al. 2016). The integration of genetic resources and transformative technologies, from genome editing to synthetic biology like improving metabolic pathways, and photosynthesis pathways, are necessary to capture traits could increase global food security and reduce the effects of agriculture on the environment (Bailey-Serres et al. 2019).

9.5.2 Role of Biotech in Improvement of Crops for Food Security

Conventional breeding has been transformed into molecular breeding, which then took the shape of genomics-assisted breeding with the aid of biotechnology. This advancement in plant breeding is precise and robust in developing new lines with enhanced agronomic as well as climate-resilient traits. Biotech crops are plants with DNA that have been modified through genetic engineering technology to introduce new characteristics to the plant which are not naturally present. Genetic engineering technique aims at developing biotech crops that could contribute to higher crop yields, lower use of chemical fertilizers and pesticides, better crop resilience to climate stress, reduced postharvest losses, and more nutritious foods (Qaim 2020). Farmers who have adopted genetically modified crops (GMCs) as a means of crop production have realized an approximately 22% increase in crop yield while pesticide use has been reduced significantly and farm income increased (WorldAtlas 2017). According to FAO (2015), 20–50% of yield losses worldwide are due to different biotic stresses or crop diseases. The other studies show that extreme heat and frequent droughts have resulted in about 10% reduction in the yield of cereal crops throughout the world from 1964 to 2007, and hence, the damage had been 8–11% more in developed than developing countries (Lesk et al. 2016). In 2019, the 24th year of commercialization of biotech crops, 190.4 million hectares of biotech crops were planted by up to 17 million farmers in 29 countries with an approximately ~112-fold increase from 1996 to 2019 (ISAAA 2020). Thus, biotech crops are considered the fastest adopted crop technology in the history of modern agriculture with global economic gains of US\$224.9 billion from 1996–2018 improving the socio-economic condition of 17 million farmers worldwide (ISAAA 2020). According to ISAAA (2020), aside from the economic benefits, biotech crops also contributed significantly to food security, sustainable development, and climate change mitigation from 1996 to 2018 by increasing crop productivity by 822

million tons, conserving biodiversity by saving 231 million hectares of land, providing a safer environment by saving 776 million kg of pesticides from being released into the environment and reducing CO₂ emissions by 23 billion kg, equivalent to taking 15.3 million cars off the road for 1 year (2018). Thus, the development of new crop varieties with the help of genomics and biotechnology can significantly contribute to ensuring food security, environmental security and economic security, globally.

Even though more than 20 years of research and commercial applications suggest that GMOs are not riskier than conventionally bred crops (Greenpeace 2015), the argument continues to be widespread concerns about the possible negative health and environmental consequences. GM crops could contribute to food production increases and higher food availability, especially at a country level, however, not necessarily to food security (Przezbórska-Skobiej and Siemiński 2020) because of poor social and regulatory acceptance by several developed countries concerning its health risk concerns. Thus, each country needs to analyze the conditions and incorporate strategies with improved policies on agricultural development for better food security (Pachapur et al. 2020). Before a GM crop is approved for commercial use, it must pass rigorous safety and risk assessment procedures but states introducing and developing the cultivation or import of GM products must take control over their safety and security. Although there are many technical challenges to overcome, the biggest potential obstacles to the adoption of genome-editing tools in agriculture are public acceptance of the technology and government regulatory policies (Hua et al. 2019).

9.5.2.1 Breeding Crops Using Plant Transformation for Food Security

Plant transformation is one of the core techniques in plant biotechnology used to introduce desirable traits into an existing genome while preserving the genetic identity of the germplasm. *Agrobacterium*-mediated gene transformation is preferred over other techniques like electroporation, or gene gun (particle bombardment) methods because of its simple operation, the capacity of the transfer of larger DNA fragments in size, and reproducibility (Krenek et al. 2015). Using several techniques, transgenic crops are designed to increase their production, increase the net profit per hectare, have easy crop management, less labor requirement, and ability to use safer management practices. The transformation system has been used in crop improvements by combining with several other techniques such as RNA interference (RNAi) and clustered regularly interspaced short palindromic repeats (CRISPR) genome editing (Hayta et al. 2021; Che et al. 2018). Successful genetic transformations have been reported on rice (*Oryza sativa*), wheat (*Triticum aestivum*), maize (*Zea mays*), barley (*Hordeum vulgare*), sorghum (*Sorghum bicolor*), and several other crops using optimized tissue culture techniques (Ishida et al. 2007; Zhao et al. 2000; Munaweera et al. 2022) which has shown improvements in the crop yield in those crops through transformation. Transgenic rice expressing

Capsicum annum methionine sulfoxide reductase B2 (*CaMsrb2*) gene has been developed which has shown drought tolerance at the reproductive stage (Dhungana et al. 2015).

The introduction of a gene conferring ability into rice through transformation to increase the biosynthesis of an iron chelator in rice has also resulted in improvement in yield and growth under low iron availability conditions (Takahashi et al. 2001). The transformation technique has been successfully used to manipulate the brassinosteroid levels of the rice plant, resulting in an enhancement in the crop yield (Datta 2013). In terms of resistance to biotic stresses, genetically modified rice lines have been designed incorporating the *Bt* gene from *Bacillus thuringiensis* in several countries (Wang et al. 2018b). Results indicated the ability of genetic improvement in minimizing losses due to lepidopteran pests in Asia (High et al. 2004), high tolerance against rice leaffolder in China field trials (Chen et al. 2011), insect resistance in Pakistan and Mediterranean regions (Rahman et al. 2007). And the potential effect of *Bt* proteins against humans, and other animals including non-targets are demonstrated to be negligible (Genetic Literacy Project 2022). Among the large array of isolated *Bt* genes, *CryIA(a)* and *CryIA(c)* proteins are the most used in developing transgenic plants resistant to lepidoptera. After successful results of the cotton plant, *cry* genes have been incorporated in several other crops such as maize, rice, soybean, chickpea, and tomato to develop resistance against lepidopteron insect pests (Perlak et al. 1991; ISAAA 2020). Gram-negative bacteria such as *Xanthomonas oryzae* pv. *oryzae* (Xoo) and *Xanthomonas oryzae* pv. *oryzicola* (Xoc) are considered to affect worldwide rice production by causing bacterial blight and bacterial leaf streak.

The dehydration-responsive element binding (DREB) gene is one of the transcription factor genes investigated for improving water stress and the DREB1A gene was placed under the control of a stress-inducible promoter from the *rd29A* gene and inserted via biolistic transformation into bread wheat (Izydorczyk et al. 2018; Pellegrineschi et al. 2004). Even though so far, a significant yield improvement was not observed in the transgenic wheat plants, they are known to demonstrate a considerable adaptation to water stress conditions when compared to controls (Bansal et al. 2021). It has been reported that salt-tolerant plants also often tolerate other stresses including chilling, freezing heat, and drought and such high-performing genetically modified wheat plants have been developed around the world (Tadesse et al. 2019). The strains have been improved with nitrogen-fixing characteristics and the roots of wheat crops have been induced to form nodular structures as a step enabling non-leguminous plants to fix nitrogen in the soil (Li et al. 2020).

The genetically modified “MON87460” transgenic maize is a popular variety against drought. This includes the expression of cold shock Protein B to maintain the cellular functions under water stress conditions, preserving the RNA stability and translation (Sammons et al. 2014). This is already approved in more than 13 countries including European Union, the United States and Japan due to its 20% increased yields under water-stressed conditions (Ortiz et al. 2014). Maize

homologs to the *Arabidopsis* Neurofibromatosis (NFB1) that confers resistance to drought have been used to engineer elite maize with enhanced adaptation to drought. The improved maize displayed reduced wilting, and maintenance of photosynthesis with a 50% increase in grain yield in drought conditions (Nelson et al. 2007).

Genetically modified soybean line transformed with *rd29A* showed overexpression of *AtDREB1A* gene and was found to enhance drought tolerance in soybean while increasing plant photosynthetic rate, plant chlorophyll content, and a higher stomatal conductance (Polizel et al. 2011). Several soybean varieties are currently commercialized and Roundup-ready (RR) crops were designed to tolerate the herbicide, glyphosate, through the expression of the *5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)* gene from *Agrobacterium* spp. strain CP4 (ISAAA 2020). The soybean Bt lines are approved for commercialization in Brazil producing more than 114-million-ton in 2019 (Bel et al. 2017). Thus, the development of such varieties has significantly contributed to global food security by increasing yield under adverse stress conditions.

Abscisic acid (ABA) is involved in several metabolic pathways that play role in drought, salinity, and cold tolerance the plant (Xiong 2007). ABA and different abiotic stresses are known to induce the *H. vulgare* abundant protein 1 (HVA1) and the overexpression of HVA1 in different cereal plants was found to improve tolerance against different abiotic stresses (Nguyen and Sticklen 2013) without compromising the grain yield. Similarly, overexpression of HvSNAC1, a stress-responsive transcription factor, improves drought tolerance without any reduction in the plant's yield (Visioni et al. 2019). Several such mechanisms underlying stress-related gene expression have been evaluated and are known to have a major impact on the improvement of barley (*H. vulgare*) productivity with the increasing changes in climatic conditions (Gürel et al. 2016).

The tuber-specific gene *AmA1* is associated with increased protein content in potatoes (Hameed et al. 2018). Transgenic potato tubers with this gene contained more than a 60% protein than that of controls (Chakraborty et al. 2010), contributing to nutritional food security. Transformations have been done using *cry1Ac9* and *cry9Aa2* genes against several insect pests and such Bt-based transgenic approaches are considered highly successful practices in potato crops for increasing yield under biotic stress conditions (Meiyalaghan et al. 2006). Potato transformation experiments have shown that controlling biotic and abiotic stress can increase potato production for meeting global food demands. Overexpression of *Musa acuminata* plasma membrane intrinsic protein gene 1;1 (MaPIP1;1) in bananas has improved tolerance to numerous stressors. Various transcription factor genes, such as *MaERF14*, *MaDREB1G*, *MaMYB1R1*, *MaERF1/39*, *MabZIP53*, and *MaMYB22*, with similar expression patterns to MaPIP1;1 under salt or cold stress, have been identified which contributed for the development of stress resistance in banana (Sreedharan et al. 2013) and enhanced yield and quality.

9.5.2.2 Breeding Crops Using Next-Generation Sequencing for Food Security

Analysis of whole genomes is performed in next-generation sequencing (NGS) while allow to determine the genetic basis of important phenotypic differences and novel useful variations. Genomes of several important crops have been sequenced including rice (*Oryza sativa*), chickpea (*Cicer arietinum*), soybean (*Glycine max*), pigeon pea (*Cajanus cajan*), foxtail millet (*Setaria italica*), pearl millet (*Pennisetum glaucum*), etc., identifying their genotype-phenotype relationships (Li et al. 2015; Varshney et al. 2019). Several other plant genomes have been sequenced including wheat (*Triticum aestivum*) (Appels et al. 2018), and rye (*Secale cereale*) (Li et al. 2021). Re-sequencing of plant genomes and transcriptomes is aided by NGS techniques and the information has been used to create new, modified reference genome maps on crops such as rice (Sasaki 2005), maize (Schnable et al. 2009), and soybean (Schmutz et al. 2010). Information obtained from such studies can contribute to improving crops for their yield, quality and other agronomic traits with precision. The development of next-generation sequencing has accelerated the quantitative trait loci (QTL) mapping and is successfully used in the identification of genes conferring defense mechanisms against biotic and abiotic stresses (Vlk and Řepková 2017). Sequencing techniques have been successfully employed in the identification of effector proteins that could be useful in breeding wheat varieties resistant to pathogens, *Puccinia striiformis* (Garnica et al. 2013) and increasing productivity.

Similarly, drought-tolerant genes have been revealed in *Populus* sp. (poplar) and *Trifolium pratense* (red clover) plants using NGS. Illumina sequencing has been used for the identification of copper tolerance genes in plants (Ando et al. 2012), herbicide-resistant genes in *Lolium rigidum* (Gaines et al. 2014), and identification of transcription factor family of soybean, during development and dehydration stress (Le et al. 2011). Incorporation of such biotic and abiotic stress resistant/tolerant genes in staple food crops can significantly increase food production in drought conditions with the advent of new genomics-based biotechnological approaches.

9.5.2.3 Breeding Crops Using Omics Technologies for Food Security

Genomics technology has made it possible to sequence all the genomes of different plants and animals and has successfully identified several candidate genes playing role in different growth and development of plants and animals. After the development of next-generation sequencing, several advanced technologies have been developed like genomics, transcriptomics, proteomics, metabolomics, etc. which have contributed to identify several QTLs, candidate genes, transcription factors, protein-coding sequences and metabolites in model crops as well as in their related species.

Transcriptomics is one of the most popular omics (study of the whole) technologies used widely in crop improvement these days. Transcriptomics studies the functional genome of living organisms including a total number of transcripts, their abundance in a specific cell, and post-transcriptional modifications. With the

development of new techniques, RNA sequencing and transcriptome level profiling have been used to understand the molecular basis of abiotic stress responses of maize such as salinity, heat, drought, etc. (Qian et al. 2019). Several regulatory regions in transcripts involved in plant stress responses are identified using transcriptome data (Juntawong et al. 2014). The combination of published RNA sequence data, also known as transcriptome, and meta-QTL analysis has been used for the identification of candidate genes involved in kernel row number in maize (Taheri et al. 2018) and novel salt tolerance genes in soybean (Qi et al. 2014) for improved yield in abiotic stress conditions. In hybrid rice, the differentially expressed genes (DEGs) involved in metabolic activities, regulation of signal transduction, and photosynthesis in response to heat stress have been identified using these techniques. This baseline information could be successfully used in crop improvement programs afterward for improving yield in those crops under adverse climatic stresses. Root transcriptome analysis at different growth stages of the plant has revealed the molecular mechanism of root growth and development of maize (Zhang et al. 2015).

Transcriptome analysis has been used to reveal nearly 130 drought-responsive genes in tomatoes and transcription factors regulating stress-responsive genes have also been identified (Kim et al. 2015). Tomato's resistance to chilling conditions has been revealed using Brassinosteroids (BS) mediated regulation (Xia et al. 2021). Revealing enhanced tolerance to water deficit, salt stress, and chilling conditions using the transcription factor CBF driven by ABTC1 is considered a great finding as results were not affecting plant growth and yield under normal growing conditions (Foolad and Panthee 2012).

Proteomics, the study of protein profiles, of the plant could reflect the currently occurring processes in the biosystem and could be used for crop improvement with much more precision. For example, protein profiling carried out on soybean has revealed that more than 141 proteins were significantly upregulated in salinity conditions, and at moderate salinity levels, embryo proteins were found to be protected from degradation. However, in high salinity conditions, the protection seemed to be reduced (Varshney et al. 2021b). A thorough analysis of these pathways could be important for improving plants' resistance to stress conditions. Similarly in *Verticillium dahliae* inoculated cotton, two proteins 1-aminocyclopropane-1-carboxylate oxidase (ethylene biosynthesis) and ethylene-responsive transcription factor (ERF060), were found in high amounts and found to be involved in the defense response of the plant against stress conditions. That information can be utilized in improving crops for stress tolerance.

9.5.2.4 Breeding Crops Using Marker-Assisted Selection for Food Security

Marker-assisted selection (MAS) has been utilized for the introgression of several candidate QTLs into elite cultivars successfully to improve crops such as maize, rice, wheat, cowpea, etc. (Kumar et al. 2019). Molecular markers closely associated

with major QTLs in different crops are used for identifying the crop species with those QTLs and genotyping them for crop improvement. The pleiotropic effect of SCM2 QTL was found to enhance the number of spikelets per tiller in rice (Kumar et al. 2017). Identification of the gene *SUBMERGENCE 1 (SUB1)*, which is a major QTL conferring tolerance to submergence in rice, is one of the most successful examples of QTL utilization for increasing rice grain yield under submerged conditions (Nair and Shylaraj 2021). Genome-wide studies have identified some submergence-related genes *Sub1A* and *SNORKEL* (Hattori et al. 2009) of rice and *Rhg1* in soybean which confers resistance to cyst nematode (Cook et al. 2012). These identified genes can be utilized to further improve the crops for better productivity under biotic and abiotic stress conditions.

Potassium homeostasis in plants is regulated by *SKC1* in chromosome 1, providing it the salt-tolerant ability, while acting as a molecular marker suitable for selecting salt-tolerant cultivars (Luo et al. 2019) for saline conditions. When compared with other crops, maize is considered a crop relatively sensitive to flooding, and interestingly there are several maize relatives such as *Zea nicaraguensis*, and *Z. luxurians* with a higher flood resistance. Finding out marker genes for salt and flood tolerance can identify maize varieties suitable for flood-affected and saline areas and increase food production. Multiple QTL-related studies were carried out on these crops to locate the flood and salinity resistant genes (Mustroph 2018).

Several markers associated with QTLs which govern several abiotic stress tolerance characteristics such as drought tolerance, submergence tolerance (*SUB1*), and salinity tolerance in barley (Singh et al. 2013) have been identified for improving barley productivity. Molecular markers have been developed for major resistance genes and QTLs against many pathogens in barley such as rust and powdery mildew for increasing yield under biotic stress conditions (Perovic et al. 2019). In tomatoes, more than 100 loci responsible for the resistance to 30 major diseases have been mapped and molecular markers associated with those traits have been reported (Lapidot et al. 2015).

9.5.2.5 Breeding Crops Using Gene Silencing for Food Security

The RNA interference (RNAi) technique, considerably old, used in the creation of the “Flavr Savr®” tomato, is now applied on several crops. Currently, endeavours are taken in developing climate-ready crops, resistance to several abiotic factors such as salinity, drought, temperature, and biotic stresses such as insect and pest spread (Borges and Martienssen 2015) so that already popular crops and their varieties can be further improved for yield, quality and other agronomic traits. RNAi silencing of *RACK1* gene expression in rice has been utilized for improving drought tolerance and the resulting plants have shown higher growth even under water stress conditions (Li et al. 2009). RNAi technology applied in the putative V-ATPaseA coding region in maize has shown corn rootworm pest resistance making the plant biotic stress resistance with improved yield (Shaffer 2020). These innovations have

a significant impact on improving the crop yield in stress conditions although further research is necessary.

RNA is the transcribed copies of functional regions of DNA present in an organism's genome which produces protein translation. There is another class of RNA called micro-RNA (miRNA) which plays the role as a key regulator in plant growth, development, and metabolism, especially in root development architecture targeting auxin response transcription factors, regulation of fruit growth, development of leaves, apical dominance, and production of plant biomass (Zhang and Wang 2015). miRNA technology in plant biotechnology is an innovative approach for gene silencing. When miRNA is inserted in a plant cell, it can mimic the endogenous RNA and shows translational inhibition of certain genes which may be useful in many cases (Wang 2009). Several miRNAs such as miR319, miR393a, and miR5144, have been recognized in numerous crops including wheat (Gupta and Huang 2014), rice (Mittal et al. 2016) for salinity stress-tolerance and several other miRNAs were found to be involved in plant drought-related mechanisms. For example, miR319 is known to be linked with salt stress tolerance, drought tolerance, and the tolerance to chilling conditions in rice (Sircaik et al. 2021) which can be optimized to improve crops to resistant against several stress conditions. Therefore, the single attempt at using the miRNA technique seems to be enough to improve crops against several stress conditions thus improving crop productivity.

Both abiotic and biotic stress-related plant responses are regulated by using WRKY transcription factors superfamily genes in soybean. Virus-induced gene silencing technique has been used to silence 64 soybean WRKY transcription factors. Silencing of GmWRKY36, GmWRKY40, and GmWRKY45 genes was highly related to plant stress resistance (Pandey et al. 2011). Such genes can be incorporated into cultivated soybean varieties for developing stress resistance varieties for increasing yield even under stress conditions due to climate change. RNAi approach was also used in potatoes for increasing beta-carotene and lutein content by silencing the *bch* gene (Van Eck et al. 2007) for ensuring nutritional security. Flavr Savr™ tomato, which was created using antisense RNA technology to increase the shelf life, can be considered the first transgenic variety approved by FDA (USA) and commercialized in 1994 (Bruening and Lyons 2000). However, with the introduction of several other transgenic products and biosafety concerns, it was withdrawn from the market in 1999.

9.5.2.6 Breeding Crops Using Genome Editing Technology for Food Security

Genome editing is predicted to be a powerful addition to the fight against hunger and poverty. The global community should seize this opportunity by developing conducive regulatory frameworks and support mechanisms (Zaidi et al. 2019). Genome editing tools are a few of the most used techniques now to overcome the adverse effects of climate change and to compensate for the increased demand for food in the future. It is a novel, highly used technique and, a fast crop improvement

method (Ahmar et al. 2020). Genome editing technologies have an advantage over other older transgenic techniques or plant transformation techniques in that the genome of any crop can be easily modified without introgression of the transgene (a gene from other unrelated organisms) which may have negative impacts and thus, can be taken as an alternative for overcoming the biosafety concerns of transgenic crops or GMOs. Engineered homing endonucleases/meganucleases, Zinc Finger Nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) 9 are some highlighted gene-editing tools in plant research recently (Bilichak et al. 2020).

ZFN was commonly used for several genome modifications of plants such as *Arabidopsis thaliana*, tobacco (*Nicotiana tabacum*), and maize (*Zea mays*) (Petolino et al. 2010). In *Brassica napus*, activation of transcriptional machinery of the b-ketoacyl-ACP Synthase II gene (Gupta et al. 2012), edition of herbicide transmission-related *DCL* gene, and *PAT* gene of soybean and maize (Shukla et al. 2009) are a few of the instances of effective use of ZFN. This technique has allowed precise integration of the transgene to the target site with no disruption to the gene coding elements while segregating together in meiosis as a single locus. Therefore, it has also facilitated obtaining the progeny with all added traits. ZFN has been used to edit maize gene *ZMIPK1*, improving it with herbicide tolerance characteristics (Shaffer 2020; Liu et al. 2020). The development of herbicide tolerance maize can reduce labor costs and farmers will be more interested to cultivate such varieties for stable yield on a commercial scale.

Successful utilization of TALENs was performed in several crops including soybean, barley, wheat, and tomato (Becker and Boch 2021). TALENs have been used to target three TaMLO homologous alleles in making the resistant to powdery mildew. In addition, the nutrition profiles of several crops have been modified with the use of TALENs. Soybeans with high oleic acid content and tubers containing reduced sugars are some examples developed using TALENs (Becker and Boch 2021).

CRISPRCas9-mediated gene knockout is widely used for a variety of applications in crop improvement—for example, high-yield rice, disease-resistant bread wheat, and flavor-enhanced tomato (Zaidi et al. 2019). CRISPR has been developed as a versatile genome editing tool that can be applied for several applications due to its ability to perform on multiple target sites, efficacy, simplicity, and low cost compared to ZFN and TALENs (Bortesi and Fischer 2015). Research reports from 2014 to 2017 suggest that CRISPR-mediated genome editing has been applied to several fruits and crop genomes, and rice (*Oryza sativa*) was the most studied and nearly 40% of them were successful with an improvement in yield (Cong et al. 2013). Improvement of rice seedling salt tolerance using transcription factor SST, cold tolerance using editing the yield-related genes (*OsPIN5b* and *GS3*), and the cold stress-related gene (*OsMYB30*) are recent uses of the CRISPR technique (Sircaik et al. 2021). Modification of the gene *CsLOB1* has resulted in plants' resistance to the severe disease called "citrus canker". Many genomes including rice, maize, wheat, and soybean have been modified using CRISPR in making plant genomes

“climate-ready” or climate change resilient. Such crops have improved traits for yield, quality and other agronomic traits. The ability of this technique to avoid tedious screening and its less tricky nature has advantages over other methods. However, compared with its use on animal cells, the success rate of plant cells is considered low (Jia et al. 2016) and is still under experimentation.

CRISPR has been used in conferring resistance to rice blast in both seedling and tillering stages by silencing the ERF transcription factor gene *OsERF922* (Perlak et al. 1991). Other than that, CRISPR has been successfully used to knock out the *Os8n3* or the *xal* gene in rice which makes it resistant to *Xanthomonas oryzae* (xoo) infection (Yang et al. 2006). Thus, such advancements in genomic tools have contributed to stable and higher yield of rice varieties under biotic and abiotic stresses ensuring food security in stress-prone regions. CRISPR-mediated knockdown of *gna1a*, *dep1*, and *gs3* genes are found to be involved in the development of climate-ready rice (Li et al. 2016). The GRAIN NUMBER 1a (Gn1a) allele in the Indian rice Habataki has a mutation in the gene encoding cytokinin (CK) oxidase/dehydrogenase; the (OsCKX2) catalyzing the degradation of CK. The resulting accumulation of CK in the inflorescence is found to increase the grain production of the rice plant (Wang et al. 2020; Joshi et al. 2018).

Similarly, the dense and erect panicle gene (*dep1*) was also found associated with increasing yield. A mutation in grain Size (GS3), the gene which is co-located with a major QTL for grain characteristics was also found to result in long grains and an increase in grain weight. CRISPR edition of OsLOGL5 coding sequence of rice cytokinin activation enzyme-like gene that affects root growth, tiller number, and yield has successfully resulted in an increase in grain yield under drought conditions (Li et al. 2016). Similarly, CRISPR/Cas9 system was found highly efficient for the knockout of negative regulating loci, e.g., GS3, DEP1, GS5, GW2, Gna1, IPA1 and TGW6, which control grain yield in *O. sativa* L., resulted in a significantly improved grain yield in mutant plants (Li et al. 2016). TALEN and CRISPR-mediated genome editing was applied in wheat for manipulation of the transgene *Pinellia pedatisecta* agglutinin (PPA) for improvement of aphid resistance, and powdery mildew resistance by manipulating the target gene TaMLO which can contribute to improve wheat yield under biotic stress conditions (Duan et al. 2018). CRISPR/Cas9-based genome editing of wheat for achieving powdery mildew resistance has shown reductions in fungal structures and microcolonies in edited lines (Wang et al. 2018a). CRISPR-mediated editing of the gene *TaGASR7* for the improvement of grain length and weight (Zhang et al. 2016), and phytoene desaturase (PDS) gene for the improvement of plant chlorophyll synthesis (Cram et al. 2019) are a few of the successful modifications in the world for wheat productivity improvement. Further research is necessary for wheat for developing varieties with improved performance.

CRISPR/Cas9-mediated editing of MORC1, a defense-related gene previously identified in *Arabidopsis thaliana*, has been used to increase the resistance to both *Blumeria graminis* f. sp. *Hordei*; the cause of barley powdery mildew, and *Fusarium graminearum* in barley. Genes responsible for several abiotic and biotic stress resistances have been identified in the genome of wild species of barley (Galli et al.

2022) paving the pathway to several improvements in a barley crop. Identification of such biotic and abiotic stress resistance genes can be useful in developing the varieties for improved yield in barley and other related species for food security. In addition, a semi-dwarf banana variety has been developed by generating knockouts of genes for the biosynthesis of gibberellins using CRISPR/Cas 9 technology, which can withstand lodging conditions that occur due to intense winds, typhoons, and storms (Xu et al. 2021). Studies showed that CRISPR/Cas9 editing tools have been efficiently utilized in several horticultural crops including petunia, citrus, grape, and apple for gene mutation, repression, activation, and epigenome editing (Munaweera et al. 2022).

Following rice and maize, cassava is considered the third largest source of dietary carbohydrates, especially starch, in the tropics. It is one of the most drought-tolerant crops, able to thrive in even arid conditions and ensure food security in those regions. Gene editing has been used to combat the brown streak virus, which can cause yield losses of up to 70% in cassava (Munaweera et al. 2022). Potyviridae viruses require eIF4E isoforms encoded by the cassava genome for the infection, similar to rice. The simultaneous targeting of two such eIF4E genes, *ncbp1* and *ncbp2*, using CRISPR/ Cas9 has improved plant resistance in cassava (Gomez et al. 2019). These findings can contribute to increasing the yield of cassava under biotic and abiotic stress conditions in food insecure countries.

9.5.3 Breeding Neglected and Underutilized crops for Food Security

Neglected and underutilized species (NUS) are those crop species that are not included in formal research, education, and development. These crops are known for their high potential for food and nutritional security, and high adaptability to changing climates (Joshi et al. 2020b). Several underexploited crops and local landraces are the major sources of food security in high hills and marginal lands. Some of them are defined as future smart food crops based on their nutritional value (Joshi et al., 2019). Most of the hybrid and genetically modified crops can't perform well under poor management as is done by remote poor farmers in marginal lands across the world. There are several traditional crops that play an important role in household food security and livelihood needs of mountain communities, while at the same time safeguarding crop biodiversity for future generations (Gauchan et al. 2020). Thus, breeding in those crops for biotic and abiotic stress resistance thus increasing crop yield is a major option to ensure food security in less accessible remote areas worldwide. New plant breeding approaches like evolutionary plant breeding can be applied successfully in improving neglected and underutilized local landraces of crops and developing climate resilient crop populations which have an important role in ensuring food security in less productive regions of the world (Joshi et al. 2020a). Several field experiments have also been conducted in Nepal for identifying

biotic and abiotic stress resistance/tolerance in local landraces which have an important role in rural food security. There are some local landraces of naked barley (*Hordeum vulgare*) (Karkee et al. 2022), finger millet (*Eleusine coracana*) (Gurung et al. 2020), mountain rice (*Oryza sativa*) (Ghimire et al. 2018), etc. which are resistant to disease with higher yield as compared to other improved varieties and have played a key role in ensuring food security in mountain regions of Nepal. There are several other crops that need breeders' attention for identification and improvement for meeting global food as well as nutritional demands. Taro (*Colocasia esculenta*) is one underutilized crop that has been identified as a potential source of antioxidants and to mitigate chronic malnutrition and hunger (Munaweera et al. 2022). Recently genomic sequencing of Taro has revealed that 17,097 genes could potentially be functional proteins. Meanwhile, 26 genes were associated with the starch biosynthetic pathway followed by validation through RT-PCR analysis. Since Taro is distributed worldwide, it needs much attention towards modern biotechnology techniques to become one of the "climate-ready crops" (Matthews and Ghanem 2021; Kapoor et al. 2022).

Similarly, crops such as cassava, cowpea, and yams with a relatively low commercial potential are considered to grow in most developing countries to meet the food requirement. Despite their importance, they are less addressed in biotechnological efforts. That could be one of the reasons for less usage of GM crops because the crop of the target in research is a non-target in multiple regions of the world (Munaweera et al. 2022). Yam (*Dioscorea* spp.) is another good example of a neglected but important crop for food security. It is the staple food crop in many African countries such as Ghana and is also considered to have several nutritional properties along with medicinal importance (Epping and Laibach 2020). However, utilization of yams in crop improvement is negligible as the research work on yams is mostly limited to its characterization based on phenotypic and molecular markers. Efforts can be targeted to improve their use as there are some yams with high medicinal properties but are not edible (Munaweera et al. 2022). They could be modified to contain no toxins, so more germplasm becomes available for utilization and major food crop improvement. A similar condition remains true for the crop *Lathyrus*, which is a protein-rich legume, known to use after soaking overnight to clear the toxins contained in split seeds. Identification of genes for removing toxins and utilizing them as minor food crops can also pave way for ensuring food security in marginal areas.

As marker assistance and genomics continue to speed up conventional breeding, agricultural researchers should extend attention to the ignored crops often called "orphan crops". Orphan crops include sorghum, millet, taro, faba bean, etc. all of which are important food sources for many people in food-insecure regions across the world. Marker assistance and genomics made it easier to achieve quick yield improvements in these less-studied crops by increasing the pace of breeding programs and by understanding the gene combinations that have already led to yield gains in more intensely studied crops such as maize, rice, and wheat. Breeders can then select these advantageous gene combinations in the orphan crops to achieve yield gains and can be utilized for securing food demand (World Resources Institute 2014).

Recent advances in genome editing allow the alteration of endogenous genes to improve traits in crops without transferring transgenes across species boundaries. In particular, CRISPR-Cas has emerged as one of the foremost systems with which to edit the crop genome, with rapidly increasing agricultural applications in major cereals such as rice, wheat, and maize and other food security crops such as banana and cassava (Bull et al. 2018). Because of its low cost, genome editing can also be used to improve orphan crops such as local fruits, vegetables, and staple crops that can play an important role in healthy diets (Zaidi et al. 2019). Further research in the omics and gene editing field is still needed to identify useful candidate genes and QTLs in different crops so that they can be utilized to accelerate the rate of crop improvement in less time and ensure global food security.

9.6 Current Challenges and Prospects

There is an urgency to double the crop production to feed the ever-rising human population which is projected to reach 10 billion by 2057. With the existing availability of food resources and crop varieties, the current food production system would not be sufficient to reach future food demand. Thus, urgent actions need to be executed to improve the global food system. Proper identification of the remaining challenges in crop improvement and the introduction of novel plant breeding techniques for improving crop yield and other agronomic traits is mandatory. Cereal, fruit, vegetable, legume and several underutilized crops also need attention towards genomic assisted breeding to combat abiotic and biotic stresses so that global food demand can be met. Genomic-assisted breeding techniques have been directed towards major cereal crops, however, the accomplishment of sustainable development goals (SDG) by 2030 needs immense production of staple food crops and other minor food crops to ensure global food security. Realizing the potential of marker-assisted and genomics-assisted breeding of major food crops as well as of orphan crops, plant breeders will require substantially more and consistent investment in research and development. Thus, it is important to increase and stabilize crop breeding programs budget by the government and private sectors in near future by leveraging technologies through a public-private partnership. The research attention should also be given to neglected and orphan crops for ensuring food security worldwide. Breeders should not only focus on improving higher yielding traits but also on the health and environmentally advantageous traits like pesticide and herbicide resistance, improved photosynthesis rate and reduced toxins, etc.

9.7 Conclusion

Greater and more consistent crop production must be achieved against a backdrop of climatic stress that limits yields, owing to shifts in pests and pathogens, precipitation, heat waves and other weather extremes to feed the global population of 9.6

billion by 2050 and 10 billion by 2057 for which above 70% more food must be increased. Crop improvement through genetic manipulation is the only viable option to meet the global food and nutrition demands in addition to minimizing the other environmental problems. Site-specific staple crops or varieties need to be developed not only focusing on only a few major crops but also focusing on underexploited/local crop diversification which has an important role in ensuring site-specific food and nutrition security. However, the conventional breeding system is considered as not effective due to consuming a long time (6–12 years) and tedious to develop new varieties.

Therefore, multiple strategies are needed to accelerate crop performance using advanced biotechnological tools such as transformations, next-generation sequencing, omics technologies, marker-assisted selections and a novel genome editing technology for accelerated improvement of food crops for getting higher yield as demanded by the growing population. Several genes have been identified and utilized in different crops like rice, wheat, maize, soybean, tomato, banana, etc. which have a major role in food security, thus those crops must be improved to cope with the adverse impact of climate change, biotic and abiotic stresses so that food security can be ensured. Crop improvement should not only be limited to major staple food crops, breeders must also study the potentiality of underutilized crops which have a small but significant role in meeting global food security. Breeding of wide adaptability varieties of only a few crops needs to reorient to develop site-specific and dynamic populations.

For the ornamental and horticultural crops and plants propagated vegetatively, plant tissue culture plays a great role to improve crops. More recently, molecular breeding and genetic engineering systems have been used successfully in many crop species. The genetically engineering system using cisgenic technology would be a good option for the upcoming breeding system, where genetic material from the same species or a species that is naturally bred with the host is used. Among different technologies adopted for crop improvement, the CRISPR/Cas9 system can be adopted and modified for precisely editing, regulating, and monitoring an individual gene in plants and animals as well as microbes for a wide range of purposes, including improvement of crop growth, development and further yield and quality as well as tolerance to various environmental biotic and abiotic stresses would be the best option. However, it must be modified to make it more efficient, and robust with few off-target effects should be of priorities of these days.

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