

REVIEW

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Modern plant biotechnology as a strategy in addressing climate change and attaining food security

T. I. K. Munaweera^{1*} , N. U. Jayawardana², Rathiverni Rajaratnam² and Nipunika Dissanayake²

Abstract

Global warming causes a range of negative impacts on plants especially due to rapid changes in temperatures, alterations of rainfall patterns, floods or drought conditions, and outbreaks of pests and diseases. These, in turn, affect crop production reducing the quality and quantity of agricultural produce. Climatic extremes and high population growth significantly increase the world's food demand. Therefore, fulfilling the goal of attaining food security for the present and future generations is of prime importance. Biotechnology enables creating dramatic alterations on crops to withstand stress which is difficult to attain using conventional breeding approaches. It is a viable tool used to improve agricultural production. 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. Such intensive efforts are currently underway creating desirable crop cultivars to meet the food demand and to support sustainable agricultural productivity for climate change adaptation.

Keywords: Biotechnology, Crop improvement, Food security, Genetic engineering

Introduction

A statistically significant change in the climate state over a long time (more than 30 years) is defined as “climate change” [1]. In 1938, rather astonishing evidence by Callendar revealed that atmospheric CO₂ levels are increasing mainly due to anthropogenic activities and as a consequence, the earth's surface began warming up [2]. Perusing over 200 meteorological records over 50 years, Callendar has predicted an annual increment of 0.005 °C in mean global temperatures. Combustion of fossil fuels and changes in the land use pattern has led to a hike of CO₂ to 400 ppm in the present day from 280 ppm prevalent during pre-industrial levels. The climate changes that

may occur due to elevated CO₂, could lead to pest and disease outbreaks often disregarded in enrichment studies [3]. This phenomenon naturally accompanies changes in temperature and rainfall causing extreme weather events [4]. The most alarming prediction for Sri Lanka and the tropics is that even with warming less than 1 °C, these regions will experience extreme weather events much sooner than several other regions of the world [5]. For instance, rainfall has significantly increased in Madagascar with an increase of 23% compared to the last four decades [6]. Changes in climatic events are further exacerbated by natural events such as ENSO (El Niño-Southern Oscillation). Such activities are significantly affected by even minute changes in driving forces. These activities are difficult to be simulated in climate models causing predictions to be uncertain [7].

Since agricultural productions mostly depend on the selection of crops suitable for the climatic condition of

*Correspondence: kalhari@molbiotech-nagoya.org

¹ Department of Applied BioSciences, Graduate School of Bio Agricultural Sciences, Nagoya University, Nagoya, Japan

Full list of author information is available at the end of the article



specific regions, agriculture is always considered a climate-dependent bio-industry with some regional characteristics. The recent report on crop prospects and food situation by the Food and Agriculture Organization (FAO) shows if current GHG emissions and climate change trends persist, there will be an increasing frequency of extreme weather conditions leading to increased pest and pathogen incidence, abiotic stress, and reduction of crop yield of many major crops [8]. Therefore, innovative solutions using intensive research efforts have to be unraveled to improve productivity, nutritional status, and resilience of crops [9, 10]. Attempts are being taken to increase the adaptive capacity of crops by developing tolerant crop varieties using several methods. Conventional plant breeding techniques are practiced using the capacity of plants to acquire resilient traits from wild relatives [11]. Comparatively, as a faster and more precise method, biotechnology is used to create genetically improved climate-ready crops utilizing modern genetic engineering techniques [12]. Recent advancements in genetic engineering have revolutionized plant breeding and crop improvement. Genomics-assisted breeding, next-generation sequencing methods, and genome editing are some of the tools utilized in creating high-yielding better-adapted crop varieties that are resilient to climatic changes. This review mainly aims to catalog the biotechnological advancements that had been made towards producing climate-ready crops that are better adapted and resilient to changing climate conditions.

Impacts of climate change and adaptation plan

With increasing issues related to climate change and global warming, crops significantly encounter severe incidence of both abiotic and biotic stresses compromising the plant yield [13]. A decade ago, it was widely accepted that CO₂ enrichment positively influences plant growth. Several studies have provided evidence to the contrary. The responses of crops attain a plateau when the CO₂ levels reach saturation. An increase in CO₂ concentration is found to drop the Ca, Mg, S, and N content in wheat while increasing the *Oryza* sp. (rice) foliage glucose content making the foliage more vulnerable to insect attacks [14]. Elevated CO₂ levels increase the susceptibility of soybean to insect pathogens [15]. Elevated temperature levels are problematic in the tropics as the evaporation causes plants to stress. Excessive heat is found to decrease the grain number in *Triticum* sp. (wheat) while increasing the grain filling time. Frost is found to induce sterility and abortion of developed wheat grains [16]. Heat is also known to reduce the starch quality in *Zea mays* (maize) and also cause granules to shrink in size, reducing the yield [17]. Experimental evidence suggests that under temperate climates, increasing

temperature up to 30 °C promotes seed germination of Soybean. However, beyond a threshold level, germination is considered to be adversely affected [18]. Extreme temperatures can cause severe damage to crop plants. The stress is considered crucial at the flowering stage due to the high sensitivity of plant reproductive organs reducing the pollen viability and shortening grain filling period [19]. An increase of 3–4 °C increase in temperature was found to reduce crop yield by 15–35% in Africa and Asia and by 25–35% in the Middle East in 2007 [20].

According to [21, 22], a considerable decline in the yield of the world's major crops is now happening due to heat stress. It is also found that crops of temperate and sub-tropical zones are more prone to yield loss.

Studies reveal that reduced water availability decreases yield and surges soil moisture deficit, thus reducing agricultural arable lands [23, 24]. From 1980 to 2015 the decline in global wheat and maize yields was found to be 20.6% and 39.3%, respectively [25, 26]. An increase in demand for food will create a problem for water availability as 70% of the water is utilized for agriculture. Drought stress alone is estimated to limit crop productivity by more than half the amount in the next 50 years [27]. In rice, extended drought yield losses are calculated to exceed 40% while it is expected to be particularly severe in the south and south-east Asia, where 23 Mha of rice is rain-fed and requires nearly 3000–5000 L of water to produce 1 kg of rice seeds [28, 29]. Similarly, a review of 60 primary studies has identified that the hardest hit from climate change is on countries that are at greater risk of undernutrition, such as sub-Saharan Africa and South Asia [30].

Groundwater salinization is considered to be increased by global warming. Seawater intrusion into coastal aquifers and rising sea levels can be pointed as the main reasons behind the phenomena [31, 32]. Climate change is expected to double salt-contaminated areas by 2050 while increasing the sea levels. Salinity is considered to affect more than 20% of the world's agricultural soils. Plants generally exhibit several negative responses to salt stress conditions such as stomatal closure, inhibition of shoot elongation, and increased leaf temperature [33]. In extended stress conditions, plant growth will frequently decline, affecting the seed formation process of crops [34].

Being a major issue in both academic and international debates, the impact of food security is known to have a global impact on economic fundamentals. Among the vast number of definitions and indicators proposed, several suggest using anthropogenic measurements for the monitoring of food security. Almost half of the world's population live in rural areas and a vast majority of them depend on agriculture as their livelihood, including

50–90% of the population in developing countries [35, 36]. Therefore, in most developing economies, economic growth is spearheaded by agriculture. For instance, in the west African country, Liberia's gross domestic product (GDP) depends on agriculture which accounts for about 76.9% making it the highest in the world. However, it is recorded to be about 30% in most developing countries. Therefore, agriculture can be a very effective buffering against economic and financial crises in many parts of the world [37, 38]. The FAO highlights the importance of efforts to build resilience to the adverse impacts. In 2019, there were more than 750 million people or nearly one in ten people around the world, exposed to severe starvation. However according to FAO, estimation of 800–900 million undernourished people is just a gross underestimation of the prevalence of food insecurity, and even if the number count is correct, that should be considered as the lower bound to get the “big picture” [36]. According to the report, the impact of natural disasters on the agricultural sector of only developing countries is estimated at over USD 108 billion, and Asia was the most affected region, with an overall economic loss of USD 49 billion followed by Africa at USD 30 billion, and Latin America and the Caribbean at USD 29 billion [36]. The crop and livestock production loss in the least developed and middle developed countries between 2008 and 2018 was equivalent to a loss of 6.9 trillion kilocalories per year. By 2055, it is forecasted that maize production in Latin America will decline by 10% which will cause a huge threat to global food security. Even in Northern America and Europe, where the lowest rates of food insecurity are found, the prevalence of food insecurity increases. At the same time, it is noteworthy that the population will reach an astonishing 8.6 billion by 2030, and at the end of 2050, it's expected to exceed 9.7 billion [38] with several million “undernourished” [39]. With that many mouths 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 of the world. According to the predictions of FAO, agricultural productivity should be increased by 60% more in 2050 to feed the world population [40, 41]. It has been reported that over 2 billion people worldwide are deficient in one or more micronutrients. More than 160 million children under 5 years of age are not having a height according to their age and more than 50 million are severely thin for their height. This has resulted in more than 3 million annual child deaths due to undernutrition and nearly 790 million people suffering due to insufficient daily dietary energy intake [42, 43]. The global demand for crop calories is expected to increase by $100\% \pm 11\%$ whereas global crop protein demand is expected to increase by $110\% \pm 7\%$

(mean \pm SE) from 2005 to 2050 [43, 44]. The prices of these commodities are predicted to increase by 31–106% by 2050 [45].

When considering the impact of climate change on food security globally, developing countries, especially South Asian countries would face a great decline in food production rather than most of the other regions. Table 1 summarizes the impact of climate change in the agriculture sector and adaptation strategies of several countries globally. 126 out of 154 developing countries are undertaking the process to formulate and implement National Action Plans (NAPs) [46].

Biotechnology for climate change adaptation of crops

Agriculture plays a major role in climate change by contributing more than 10–14% of the global anthropogenic greenhouse gas (GHG) emissions [58–60]. Biodiversity has been already threatened due to land clearing and fragmentation of habitats for cultivation purposes [61, 62]. Therefore, agricultural expansions to increase global food production seem not always possible. Currently, there is of higher importance to safeguard a person's right to adequate and nutritious food. FAO introduced the “climate-smart agriculture (CSA)” approach in 2010 at The Hague Conference on Agriculture, defining it as the ‘Agriculture that sustainably increases productivity, enhances resilience (adaptation), reduces/removes greenhouse gases (mitigation) where possible, and enhances achievement of national food security and development goals [61]. Utilizing sustainable agricultural applications and food production using proper policies, and investments in the environment are some of the main aspects of CSA [23].

Genetic improvement of crops is considered one possible solution for the crisis. Identification of superior alleles or haplotypes is crucial for crop improvement methods. There are more than 80,000 plant species conserved at about 3400 gardens all over the world and more than 17,000 institutes worldwide dealing with the conservation and sustainable use of plant genetic resources [23]. Currently, nearly 711 gene banks, 16 regional centers in 90 countries are retaining more than 5.4 million accessions from more than 7051 genera for the conservation of crop species, their wild relatives, cultivars, and breeding materials [62]. Passport data stored in gene banks could be used to identify genotypes that harbor important characteristics such as abiotic and biotic stress tolerance, high yield, etc., which in turn enhances the genetic gain of crops by incorporating novel superior alleles and new sources of genetic variation into the gene pool [61].

Table 1 Impact of climate change in food security and adaptation plan

| Region/country | Impact on the agriculture sector | Adaptation strategies for food security | References |
|----------------|--|---|------------|
| Albania | Increased temperatures and the threat of waterlogging of fields due to intense rainfall and/or flooding may also result in an increased presence of pests and diseases | According to Albania National Adaptation Plan 2021, Agricultural resilience against droughts is enhanced | [47] |
| Argentina | Climate change-related threats are estimated to potentially impact between 4.5–7% of Argentina's GDP | Enacted National Adaptation Plan for climate change 2021 | [48] |
| Armenia | By the 2070s, potato crop yields will decrease by 21% due to climate change | Use disease- and pest-resistant crop species; research and use drought- and heat-resistant species and hybrids, especially local species | [49] |
| Bangladesh | The salinity threshold by the 2050s as a result of sea-level rise could result in a 15.6% reduction in rice output | Nationally determined contribution (updated) 2020 National Agricultural Technology Program-Phase II Project (NATP-2), support to seaweed cultivation | [50] |
| Brazil | Food insecure population 66 million and cotton crops are expected to be moderately impacted by climatic changes, but maize and wheat yields will decline significantly | Enacted National Adaptation Plan for climate change 2016, genetic improvement to develop drought-resistant varieties, Biofuels | [51] |
| Chile | Climate change models predict that the average daily maximum temperatures in Chile will increase by around 2 °C by the 2050s and fruit loss increase by 50% | National Adaptation Plan enacted 2017 | [52] |
| Ethiopia | Estimates suggest climate change may reduce Ethiopia's GDP up to 10% by 2045, largely through drought-induced impacts on agricultural productivity | Ethiopia's climate-resilient green economy national adaptation plan-2019 | [53] |
| India | Wheat and maize yield in central India is expected to decline significantly even at lower carbon emission pathways | National Adaptation Plan for climate change in the process includes an adaptation of new technology to produce resilient crop varieties | [54] |
| Nigeria | Studies show that increased levels of CO ₂ will lead to nutrient declines in rice of up to 17%, with increased rainfall variability and higher temperatures likely to also further reduce rice yields | Enacted National Adaptation Plan for climate change in 2021 | [55] |
| Sri Lanka | By the 2060s, crop yields are projected to decline in the range of 12–19% and 27–41% in Maha and Yala season, respectively. The poverty rate could increase in the range of 12–26% | The National Adaptation Plan for climate change has been enacted in 2016 | [56] |
| Thailand | Their study projects rain-fed rice yields to reduce 10% by 2080 under the RCP 8.5 emissions pathway and crop water productivity reducing 29% by 2080 under the same emissions pathway | Thailand's third national communication; Climate change adaptation plan | [57] |

Such genes are identified in crop-wild relatives and efficiently transferred into several crops making them “climate-ready” [62].

High heterozygosity, auto incompatibility, long life cycles, labor requirement, and extended juvenile periods are a few of the causes which make conventional breeding, a slow and difficult process [63]. Therefore, new biotechnological tools are considered to result in more efficient and robust genetic improvement [64]. Organismal genomes are manipulated using several new biotechnological approaches such as RNA-mediated gene silencing, genome editing, and techniques such as next-generation sequencing (NGS), omics are discussed in this review.

Plant transformations

The transfer of genes among plant species plays an important role in crop improvement and it is acquired by recombinant DNA (rDNA) technology. Here, genes responsible for useful traits such as high yield, resistance to diseases, and stress conditions are extracted from related or unrelated organisms and transferred to the crop of interest.

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. That is mainly carried out through the *Agrobacterium*-mediated gene transformation, electroporation, or gene gun (particle bombardment) methods. The simple operation, the capacity of the transfer of larger DNA fragments in size, and reproducibility are some of the characteristics that have made the *Agrobacterium*-mediated method preferred over the others [65]. *Agrobacterium tumefaciens* is a proteobacterium that causes the crown gall disease in several plant families such as Rosaceae, Vitaceae, and Juglans [66]. The ability of the bacterium to transfer the T-DNA to plant cells is used in transformation as later the T-DNA is being integrated into the plant genome, so the gene of interest can be expressed. Initially, the technique was used on dicotyledonous plants and later extended to monocotyledonous plants because they were not natural hosts for the bacterium [67, 68].

The design of transgenic crops is considered a major solution for several climate change-related issues as their commercialization is known to contribute to reducing the CO₂ emission in the world. They are expected to adapt to increasingly changing climatic factors, temperature, and rainfall fluctuations, increasing pest attacks [69]. Also, they are known to be satisfied with less amount of fertilizer and energy requirements using environmentally friendly practices. For example, insect pest-resistant crops are requiring fewer amounts of insecticides or

pesticides. Using several techniques, transgenic crops are designed to increase their production, increase the net profit per hectare, easy crop management, less labor requirement, ability to use safer management practices, are the major advantages of transgenic crops. The laborious nature, issues related to introgression of traits, trait deregulation, the requirement of time, and substantial cost, are some of the drawbacks of this technique [70].

These highlights the importance of optimizing the *Agrobacterium*-mediated gene transfer method with strategies that precisely identify the target site and well-characterized integration of transgene, gene expression elimination of unintended gene modifications. The *Agrobacterium*-mediated site-specific integration (Agro-SSI) used as an efficient technology to improve maize crop [71] and investigation of high-throughput transformation systems in *Setaria italica* (foxtail millet) are some milestones in the pathway of improving the technology into a commercial scale. Among the efforts to improve the technique, the gene gun method was developed. But the poor transformation efficiency, limited availability of plant regeneration protocols hindered its commercial use over other techniques.

The technique has been successfully used to manipulate the brassinosteroid levels of the rice plant, resulting in an enhancement in the crop yield [72]. The introduction of gene conferring ability to increase the biosynthesis of an iron chelator in rice has also resulted in improvement in yield and growth under low iron availability conditions [73].

Similarly, the phosphorus absorbance of plants has been enhanced using genetic transformation [74]. Engineering plants to enhance their ability to efficiently absorb soil nutrients can reduce the use of harmful agrochemicals, in turn reducing the environmental pollution.

Also increasing the tolerance of the crops to withstand high metal levels in the soil is also practiced in *Nicotiana tabacum* (tobacco) and *Carica papaya* (papaya) plants in making them tolerant to aluminum [75].

Even though efforts are there in creating transgenic crops, only a few are being commercialized. Mostly commercial crops such as maize, *Glycine max* (soybean), *Gossypium* sp. (cotton) are used in research due to the high cost of research work. In terms of wheat and rice, even though several transgenic traits are produced such as glyphosate-tolerant wheat [76] and Golden Rice [77], they seem to be absent from the commercial scale.

The release of herbicide-tolerant glyphosate-tolerant Roundup Ready[®] from Monsanto and glufosinate-tolerant Liberty Link[®] from Bayer is known as the first commercialized transgenic crop in the USA in the 1990s [78]. Even though it was accepted and cultivated in several areas in the world, the adoption of glyphosate-tolerant

traits has resulted in weed species developing resistance to the herbicide [79]. With the higher commercial application of these plants, later several commercial products have been available with multiple herbicide-tolerant traits. The use of single lepidopteran insect resistance genes derived from *Bacillus thuringiensis* in *Bt* cotton and *Bt* maize are the best examples known to save the Pakistan cotton industry 2017–2018. The combination of insect resistance and herbicide-resistant traits is considered a turning point in transgenic crops. The percentage of the planted area has been found to grow with this especially in USA and Brazil [80].

In terms of drought tolerance, only two crops have been successfully commercialized to date. One is Genuity® DroughtGard® (MON87460) that has incorporated cold shock protein from *B. subtilis* (CspB) and the other is brought to market by FuturaGene Group which is a *Eucalyptus* spp. expressing anendo-1,4-β-glucanase from *Arabidopsis thaliana* (cell) to increase woody biomass [81].

The transformation system has been used in crop improvements by combining with several other techniques such as RNA interference and clustered regularly interspaced short palindromic repeats (CRISPR) genome editing [82, 83]. Successful genetic transformations have been reported on rice, maize, *Hordeum vulgare* (barley), wheat, *Sorghum bicolor* (sorghum), and several other crops using optimized tissue culture techniques [68, 84–86], with improvements in the crop yield. A few of the commercialized crops are mentioned in Table 2.

Next-generation sequencing (NGS) and genomics

Harnessing the potential of a gene pool of crops and their wild relatives facilitates their improvement by combining genes to design plants with superior performance in agriculture, and food industries [87]. Analysis of whole genomes is performed in NGS while allowing to determine the genetic basis of important phenotypic differences and novel useful variations. Assembly of sequence information and its annotation is performed in most cases and genomes of several important crops have been sequenced including rice, *Cicer arietinum* (chickpea), soybean, *Cajanus cajan* (pigeon pea), foxtail millet, *Pennisetum glaucum* (pearl millet), identifying their genotype–phenotype relationships [88, 89]. The rapidly developed second-generation sequencing methods have facilitated the assemblage of several other plant genomes including wheat in 2018 [90], *Digitaria exilis* in 2020 [91], and *Secale cereale* (rye) in 2021 [92]. Re-sequencing of plant genomes and transcriptomes are aided by NGS techniques and the information has been used to create new, modified reference genome maps on crops such as rice [93], maize [94], and soybean [95]. The characterization of the sorghum genome has identified several important features, such as ~29.6 Mb additional sequence, 24% increase in annotated genes, increased gene length, and narrowed error frequency by tenfold [96]. NGS techniques are continuing to be developed covering complex plant genomes using platforms such as Roche/454, Illumina/Solexa sequencing, Ion Torrent, PacBio, and Oxford Nanopore

Table 2 Commercialized GM crops. Source: [87]

| Trait | Crop | Developer |
|-------------------------|-------------------------------------|---|
| Glyphosate resistance | <i>Medicago sativa</i> (Alfalfa) | Monsanto |
| | <i>Brassica napus</i> (Canola) | Monsanto |
| | Maize | Monsanto |
| | Soybean | Monsanto |
| Glyphosate resistance | Canola | Bayer |
| | Cotton | Bayer |
| | Maize | Bayer |
| | Rice | Bayer |
| Virus resistance | Papaya | Cornell University, South China agricultural university |
| | <i>Solanum tuberosum</i> (Potato) | Simplot, Monsanto |
| Lepidopteran resistance | <i>Solanum melongena</i> (Eggplant) | MAHYCO |
| | Cotton | Dow AgroSciences, DuPont, Monsanto, Syngenta |
| | Maize | Dow AgroSciences, DuPont, Monsanto, Syngenta |
| | Potato | Monsanto |
| | Soybean | Monsanto |
| Drought tolerance | Maize | Monsanto |
| High lycopene | <i>Ananas comosus</i> (Pineapple) | Del Monte |
| Modified oil/fatty acid | Soybean | DuPont |

which are a few of the most used [97]. Among them, the third-generation approaches; Nanopore and PacBio, have facilitated the high-quality assemblies for complex genomes [98]. These techniques are also being used in RNA sequencing; a relatively new method helpful for quantifying and mapping transcriptomes, especially for non-model plants such as olive during fruit development [99], bread wheat cultivar Yunong 201 [100].

Quantitative trait loci (QTL) that are regulating quantitative traits are used to estimate the number of loci controlling genetic variation in species [101]. The development of NGS has accelerated the QTL mapping and is successfully used in the identification of genes conferring defense mechanisms against biotic and abiotic stresses [102]. Wheat, being one of the most cultivated crops in the world, gets severely affected by *Puccinia striiformis*. Sequencing techniques have been successfully used in the identification of effector proteins that could be useful in breeding wheat varieties resistant to pathogens [103]. 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 [104], herbicide-resistant genes in *Lolium rigidum* [105], and identification of transcription factor family of soybean, during development and dehydration stress [106].

NGS is one of the most important platforms for several molecular biological studies. Successful profiling of the transcriptome of plants, analysis of gene expression, and functional gene detection are a few of them [107]. Transcriptomics includes the functional genome of living organisms dealing with the total number of transcripts, their abundance in a specific cell, and post-transcriptional modifications. Several regulatory hubs involved in plant stress responses are identified using transcriptome data [108]. The combination of published RNA seq data (transcriptome) and meta-QTL analysis has been used for the identification of candidate genes involved in kernel row number in maize [109, 110] and novel salt tolerance genes in soybean [111]. Several regulatory hubs involved in plant stress responses have also been identified using transcriptome data [112, 113]. 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.

Marker-assisted selection (MAS) has been utilized for the introgression of this QTL into elite cultivars, successfully improving crops such as maize, rice, wheat, *Vigna unguiculata* (cowpea) [112]. In rice, a large number of QTLs controlling different agronomic traits have

been identified so far [113]. The *SCM2* QTL which regulates the culm strength and a gene encoding the panicle strength is found in the same genomic region. The pleiotropic effect of *SCM2* was found to enhance the number of spikelets per tiller [114]. 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. The main QTL responsible for plant submergence tolerance is designated as the *SUB1* gene [115], and is also it is identified to be an ERF transcription factor [115]. Among the numerous available markers developed, single nucleotide polymorphism (SNP), being cost-effective and accurate is highly used for the identification of plant genes through positional cloning [116]. The whole-genome regression (WGR) approach is also used for the detection of SNPs and insertions–deletions, structural variants of target crops by comparing the sequence information with a completely different genotype [117]. Even though sequencing techniques are used for the identification of SNPs, problems have been encountered regarding the ability to read long reads. Therefore, optical mapping has been used along with genotyping by sequencing (GBS) coupled with NGS to overcome this problem, generating long read maps [118] in crops with larger genomes such as wheat, rice [119, 120], maize, and barley [121, 122]. GBS has been used to identify several SNPs linked with several desired traits in rice, including leaf width and aluminum tolerance [123]. In maize and barley, several traits have been identified with more than 200,000 maize markers using this approach [122]. Linkage maps for wild relatives of Triticale crops [119] and crop-wild cross in *Solanum lycopersicum* (tomato) [124] have been constructed in recent years. Also, GBS has been utilized to characterize wheat breeding lines [119] and 20,000 wild and domesticated barley accessions [121, 122]. Information on several genome-wide studies is utilized for the creation of pangomes and has been used in the identification of several important genes. Some of them are the presence or absence of submergence-related genes *Sub1A* and *SNORKEL* [125] of rice and identification of soybean locus *Rhg1* which confers resistance to cyst nematode [126].

GWAS is a staple method for trait association mapping. It is composed of a high mapping resolution and compatibility with a wide range of populations across genomes. Bioinformatics tools such as GWAS including R packages such as GAPIT [127], PLINK [128], QTCAT [129] are some that have been developed to conduct GWAS. Thermo-tolerance in maize has been identified using GWAS of 261 near-isogenic populations [130] and it was used in increasing wheat precision breeding efficiency. Wheat haplotypes were identified recently with the use

of SNPs, integrated with QTL, and characterized using results of GWAS to be used in breeding programs [131]. Also, a similar GWAS tool called, PAV GWAS identified structural variations associated with silique length, seed weight, and flowering time in *Brassica napus* (rapeseed) [132].

Even though the theoretical possibility of crop improvement can be achieved using genomics and gene expression analysis, those data always do not correlate with the ultimate protein quality of the production [133]. Therefore, proteomics is considered a powerful tool in crop improvement. The cells mostly depend upon the available metabolic and regulatory pathways for survival. Only the proteome analysis of the plant could reflect the currently occurring processes in the biosystem. For example, protein profiling carried out on soybean has revealed that the production of about 141 proteins was 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 [134]. 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 *ERF060* (ethylene-responsive transcription factor), were found in high amounts and found to be involved in the defense response of the plant. In terms of rice and several other major crops, since the current pathogen control is mainly carried out using chemicals [135], an alternative strategy based on the innate defensive mechanisms using proteins of the plants is essential for sustainable improvement in production.

Despite the progress in plant proteome studies, it is known to have a low pace, especially when compared with human and yeast proteomics studies. Since the latest technologies have reduced errors in sequencing and other assessments, it is high time to reach new advancements of high-throughput proteome analyses. Accurate identification and characterization efforts of crop genetic and phenotypic variations enable the successful target introduction of adaptive traits in making plants climate-ready [136]. Lately, genome-wide association studies have revealed salt-related traits in 306 rice cultivars. Similarly, a new k member-based genome-wide association approach has been created and applied on 2000 phenotypes in *Arabidopsis thaliana*, tomato, and maize, detecting new associations with structural variants and with regions missing from reference genomes [137]. The advancement of genomic resources creates naturally unprecedented opportunities by enabling the incorporation of various important genes into crops in a lesser time and increased precision [138]. For this several

omics platforms are utilized and a proper alignment of each becomes important to increase accuracy [139]. Even though several achievements related to sequencing are there, still gaps are found in the number of properly characterized genes in the most studied model organism *Arabidopsis thaliana*.

RNA-mediated gene silencing

RNAi technology is a technique used to turn off expressing certain genes or regulate gene expression before translation by overexpressing RNA sequences [140]. Small RNA (sRNA), short interfering RNAs (siRNA), and micro RNAs (miRNAs) are employed in the technique. Even though different mechanisms of RNAi have been used in organisms, both plants and animals utilize dsRNA to trigger the detection of homologous mRNAs to negatively regulate its transcription. Currently in plants, post-translational gene silencing (PTGS) [141], Transcriptional gene silencing [142], and miRNA silencing [143, 144] have been identified as popular gene silencing routes. These mechanisms of regulating endogenous plant gene expression are considered evolutionarily conserved [145] and also considered as methods that help to witness plant improvements in terms of yield, nutrition, abiotic and biotic stress resilience, in several major crops including rice, maize, wheat, tomato and cotton [141–144].

Even though the RNAi technique is a considerably old technique, used in the creation of "Flavr save tomato®", it now experiments on several crops. Currently, efforts are done in creating climate-ready crops, resistance to several abiotic factors such as salinity, drought, temperature, and biotic stresses such as insect and pest spread [140]. miRNA's role as a key regulator in plant growth, development, and metabolism especially in root development architecture targeting Auxin response transcription factors [144], regulation of fruit growth in *Arabidopsis* [145], development of leaves, apical dominance, and production of plant biomass [146] have been already identified. Differences in the behavior of small RNAs are observed concerning their salt-tolerant potential since especially in stress conditions, plant miRNA expression levels are altered in a considerable amount [147]. MiRNA families showing such expressional behavior include miR156, miR159, miR168, miR169, miR393, and miR398. Salinity stress-tolerant miRNAs such as miR319, miR393a, miR5144, have been recognized in numerous crops including poplar [148], wheat [149], *Arabidopsis thaliana* [150], rice [151]. Similarly, due to the presence of drought-responsive elements in promoter regions [152], several miRNAs involved in plant drought-related mechanisms have been identified [153]. miRNA expression profiles such as miR166, miR159, miR408, miR319,

miP169 have been observed in *Gossypium hirsutum* [154], potato [155], *Oryza rufipogon* [156], *Triticum turgidum* [157], *Elettaria cardamomum* [158] and have been equipped them with drought-responsive mechanisms. The increase of atmospheric temperature with climate change naturally prefers crops designed to adopt high temperatures using the presence and absence of temperature-responsive miRNA species [159]. These efforts have been already carried out with *Triticum aestivum* [160], maize, and rice [161] crops. It can be identified that the same miRNAs can be optimized to improve crops to be resistant against several stress conditions. For example, miR319 is known to be linked with salt stress tolerance, drought tolerance, and also the tolerance to chilling conditions in rice [162]. Therefore, the single attempt in using the technique seems to be enough to improve crops against several stress conditions.

Plants could be made resistant to pathogens by turning the receptors off or by specific gene targeting [163, 164]. The dsRNA targeting a specific insect gene is inserted into the plant or into the bacteria that spread onto plants as insecticides [165]. Insertion of dsRNA is performed by spraying or injecting into the target plant [166]. Such protection efforts are demonstrated for a range of crops against multiple insect pests [167, 168]. Recently, two regulatory authorities, the Canadian Food Inspection Agency, and US Environmental Protection Agency have declared the approval of the release and commercialization of RNAi-based corn event Monsanto MON87411, the “SmartStax PRO”. Similar crops are engineered containing dsRNA constructs targeting the *SUCROSE-NON-FERMENTING7* gene of western corn worm (WCR) (*DvSnf7*), along with two insecticidal proteins *Cry3Bb1* and *Cry34Ab1/Cry35Ab1* [168]. Approval has been granted for a crop such as an apple and potato with modified dsRNA for enhanced fruit quality [169, 170], since the molting stage is vital to regulate insect development, genes associated with molting are targeted and have got promising results related to *Helicoverpa armigera* and *Spodoptera exigua* [171, 172]. Hormone receptor 3 (*HR3*), of *H. armigera*, is silenced resulting in downregulation of the target gene affecting the molting and larval growth cycle [173]. The gene arginine kinase is required for the cellular energy metabolism of larvae. It is prevented by the gene silencing process, resulting in defective larval growth and survival in *H. armigera* [173]. RNAi products such as bruising-resistant Arctic[®] apples [173] and bruising and black spot-resistant Innate[®] potatoes are deregulated and considered safe for consumption by the United States Food and Drug Administration [174].

Even though there is a considerable development in utilizing the techniques for crop improvement, still it has

its challenges in developing and commercializing products. In addition to the miRbase; which is the information gained on novel miRNAs of plants and deposited in a public repository, several bioinformatics tools are necessary for the identification of target sequences [175]. Even if the identification is properly done, desired gene manipulation using short RNA (sRNA) could lead to undesirable pleiotropic changes in the plant. Therefore, the technique should be improved to avoid unwanted effects on the ecosystem. However, most current RNA-based research work is carried out on specific or combined stress conditions faced by the plant.

Genome editing

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 [176]. It is a novel, highly used technique and, a fast crop improvement method [177]. Engineered homing endonucleases/meganucleases (EMTs), ZF nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) 9 [178, 179] are some highlighted gene-editing tools in plant research.

ZFN was initially used to edit the genome by acting as a binding domain for a specific DNA using the recognition of three base pairs at the target site [180]. Though these were first used in targeting protein reagents, later it was commonly used for several genome modifications of plants such as *Arabidopsis thaliana*, tobacco, and maize [180]. In *Brassica napus*, activation of transcriptional machinery of the *b-ketoacyl-ACP Synthase II* gene [181], edition of herbicide transmission-related *DCL* gene, and *PAT* gene of soybean and maize [182] 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 [183]. However, the complicated nature of designing ZFNs, challenges in technical processes, and the low efficacy are highlighted as its drawbacks [180].

Following the concept of ZFN, another site-driven mutagenesis genome-editing technique was discovered, called TALENs [183]. Instead of using three nucleotides like in ZFN, TALENs can target a single nucleotide in the target site, making a more precise edition in the genome [184]. Successful utilization of TALENs was performed in several crops including soybean, barley, wheat, tomato [185]. In soybean, *fad2-1a* and *fad2-1b* genes were generated in double mutants, improving economic value and oxidative stability. Similarly, in wheat mutant lines,

TALENs have been used to target three *TaMLO* homologous alleles in making the resistance to powdery mildew [185]. In addition, the nutrition profiles of several crops have been modified with the use of TALENs. Soybeans with a high oleic acid content, tubers containing reduced sugars are some examples [185]. Even though there is a vast potential of TALEN in crop improvement, its low efficacy and challenging nature of construction of TALE repeats required for the technique [176], evolved the need to have more reliable gene-editing tools.

CRISPR has been developed as a versatile genome-editing tool that can be applied for several applications [186], due to its ability to perform on multiple target sites, efficacy, simplicity, and low cost compared to ZFN and TALENs [187]. However, CRISPR is relying on simple Watson–Crick base pairing between the target DNA sequence and its guide RNA that edit the genome without any sophisticated protein engineering steps as in ZFN and TALEN [187]. Research reports from 2014 to 2017 suggest that CRISPR-mediated genome editing has been applied on several fruit and crop genomes, and rice (*Oryza sativa*) was the most studied and nearly 40% of them were successful with an improvement of yield [186]. 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 technique [188]. Modification of the gene *CsLOB1* has resulted in plants' resistance to the severe disease called "citrus canker". Mostly the CRISPR system has been utilized in mutating promoter sequences [189], the protein-coding sequences [190], insertion of alleles and replacement of alleles [191], complete deletion of genes or chromosomal fragments, and in protein fusion [192]. A large number of genomes including rice, maize, wheat, soybean have been modified using CRISPR in making plant genomes "climate-ready". The ability of this technique to avoid tedious screening and its less tricky nature highlights itself over other methods. However, compared with its use on animal cells, the success rate of plant cells is considered low [191, 192].

Climate-ready crops

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 [193]. Some efforts carried out on major cereal and non-cereal crops where advanced biotechnological approaches have been used in making them "climate-ready" are mentioned below and summarized in Table 3.

Cereals

Rice

Rice, being the staple food crop in several developing countries, is a major crop used in research work. The result of a study based on 227 properly irrigated farms has forecasted a considerable negative impact on rice yield due to expected warming conditions [194]. Therefore, efforts have been made to enrich rice crops with climate-ready characters leading to better performance under several stress conditions.

In terms of improving drought-resistant characteristics, the transgenic rice expressing *Capsicum annum* methionine sulfoxide reductase B2 (*CaMsrB2*) gene, which is known to have drought tolerance at the growth and reproductive stages of the plant [195], is considered as one of the major findings. Overexpression of the drought inducible gene promoter *OsHOX24* of rice has been showing a higher sensitivity to abiotic stresses in transgenic *Arabidopsis* while showing an increased susceptibility to water-deficit stress as well. Further *OsHOX24* rice transgenics were able to possess the impaired ability of stomatal closure, converting itself into a modulator of abiotic stress responses [196]. Similarly, several transcription factors are known to have a considerable effect on the abiotic stress responses in plants [197]. When plants are under stress such as chilling, drought, and salinity, several toxic aldehydes can accumulate in plants [198]. Methylglyoxal is known as a cytotoxic metabolite that accumulates in plants during stress conditions. It is found that the prevention of accumulation of methylglyoxal is a promising way to improve plant stress tolerance, such as the responses of rice especially against low temperature, salinity changes, heavy metals, drought, submerge conditions. Therefore, genetic manipulation of glyoxylate pathways has been successfully used in making rice, resistant to biotic and abiotic stresses [199]. Regulatory genes are mostly targeted in these instances. The *receptor for activated C-kinase 1* (*RACK1*) is one such target, a highly conserved protein scaffold proven to be important in plant growth. RNAi technique has been used to silence the *RACK1* gene expression in rice and the role of *RACK1* in drought responses has been identified [200]. The gene *DNA helicase-47* (*PDH47*), is known to have multiple roles during conditions such as cold and salt stress, but no relationship has been found on drought. Its relationship with drought has been revealed by the transformation of the *PDH47* gene from the *Pisum sativum* plant to the ASD Indica rice through *Agrobacterium tumefaciens* using immature embryos. The Cauliflower mosaic virus 35S-CaMV promoter, is known to be upregulated during drought conditions and known to regulate several stress-responsive genes related to drought stress in transgenic rice plants [201]. The *PDH47* transcripts

Table 3 Climate-ready crops improved using genetic engineering

| Crop | Gene and genetic engineering methods used | Target trait | Trait improvement | References |
|-----------------------------|---|--|---|------------|
| Rice | Transgenic rice expressing Capsicum annum methionine sulfoxide reductase B2 (<i>CaMsrb2</i>) gene | Drought resistance | Drought tolerance at reproductive stage | [195] |
| Rice | RNAi silencing of <i>RACK1</i> gene expression | Drought resistance | Higher growth even at water stress | [200] |
| ASD India Rice | Transgenic of gene DNA helicase-47 (<i>PDH47</i>) from <i>Pisum sativum</i> | Drought resistance | Regulate several stress response genes | [201] |
| <i>Arabidopsis thaliana</i> | Abscisic acid-responsive transcription | Drought resistance | Deeper root system | [203] |
| Rice | CRISPR editing on MADS-box transcription factors for gene <i>MADS78</i> and <i>MADS79</i> | Seed germination | Endosperm cellularization and early seed development | [231] |
| Rice | Knockdown of <i>gna1a</i> , <i>dep1</i> , and <i>gs3</i> gene | Abiotic stress resistance—Climate-ready crop | High yield, large grain size, grain number, improved grain weight | [232] |
| Rice | CRISPR edition of 3' end of OsLOGL5 coding sequence | Drought resistance | Increase in grain yield | [232] |
| Rice | Cytokinin homeostasis | Stress resistance | Increase in grain yield | [222] |
| Rice | CRISPR of <i>gs3</i> and <i>dep1</i> genes | Salinity tolerance | | [232] |
| Rice | Silencing the ERF transcription factor gene <i>OsERF922</i> y CRISPR editing | Disease resistance | Resistant to rice blast in both seedling and tillering stages | [229] |
| Maize | Transgenic maize preserving RNA stability and translation of Cold shock protein B | Drought resistance | Maintain the cellular functions under water stress conditions | [238] |
| Maize | Transgenic maize with homologous <i>ZmNF-YB2</i> | Drought resistance | 50% increase in grain yield | [241] |
| Maize | CRISPR/Cas9 system to edit <i>ARGOS8</i> | Drought resistance | Increase in plant yield | [243] |
| Maize | ZFN technique to knock out of TMS5, the <i>thermo-sensitive male sterile 5</i> gene | Heat resistance | Thermos-sensitive male-sterile maize crops | [244] |
| Maize | RNAi technology in putative V-ATPaseA coding region | Pest resistance | Resistant to Western corn rootworm | [249] |
| Soybean | Overexpression of Arabidopsis gene <i>Δ1-pyrroline-5-carboxylate synthase (P5CR)</i> | Drought resistance | Tolerate high-temperature condition | [254] |
| Soybean | Transformed with <i>AtDREB1A</i> gene under rd29A | Drought resistance | Increase in plant photosynthetic rate, plant chlorophyll content with a higher stomatal conductance | [256] |
| Soybean | Virus-induced gene silencing of <i>WRKY</i> transcription factors | Stress resistance | Resistant to biotic and abiotic stress | [257] |
| Soybean | Transgenic with <i>csr1-2</i> gene from Arabidopsis thaliana | herbicide- resistance | Resistant to imidazolinone chemical class | [261] |
| Soybean | Transgenic with <i>cry1Ab</i> gene from <i>Bt</i> | Pest resistance | Resistant to larval feeding and growth of <i>Anticarsia gemmatilis</i> | [261] |
| Wheat | Dehydration-responsive element binding (DREB) gene | Water stress | Tolerance of water stress conditions | [268] |
| Wheat | Transgenic with manipulation in transgene <i>Pinellia pedatisecta</i> agglutinin (<i>PPA</i>) | Pest resistance | Resistance to Aphid damage | [274] |
| Wheat | TALEN, and CRISPR-mediated genome editing to the target gene <i>TaMLO</i> | Pest resistance | Resistance to Powdery mildew disease | [274] |
| Wheat | CRISPR/Cas9-mediated gene editing in <i>EDR1</i> gene | Pest resistance | Resistance to Powdery mildew disease | [275] |
| Wheat | CRISPR-mediated editing of gene <i>TaGASR7</i> length and weight | Drought resistance | Improved grain length and weight | [277] |
| Barley | overexpression of <i>HvSNAC1</i> stress responsible transcription factor | Drought resistance | Tolerate drought without a reduction in crop yield | [283] |
| Barley | CRISPR/Cas9-mediated editing of <i>MORC-1</i> gene | Pest resistance | Resistance to <i>Blumeria graminis f. sp. Hordei</i> and <i>Fusarium graminearum</i> damage | [287] |
| Potato | Transgenic with tuber-specific gene <i>AmA1</i> | Enhance nutritional value | Increase in protein content | [298] |
| Potato | <i>bch</i> gene silencing using RNAi approach | Enhance nutritional value | Increase in beta-carotene and lutein content | [300] |

Table 3 (continued)

| Crop | Gene and genetic engineering methods used | Target trait | Trait improvement | References |
|---------|---|---------------------------|---|------------|
| Potato | Transgenic with cry1Ac9 and cry9Aa2 genes from <i>Bt</i> | Pest resistance | Resistant to larval feeding and growth of <i>Anticarsia gemmatilis</i> | [303] |
| Tomato | Antisense RNA technology | Increase in Shelf life | The slowdown in ripening and prevention from softening | [305] |
| Tomato | Transgenic and overexpression of banana MYB transcription factor <i>MaMYB3</i> | Increase in Shelf life | Inhibition of starch degradation and delay in fruit ripening | [307] |
| Tomato | Transgenic and overexpression of apple vacuolar H ⁺ -translocating inorganic pyrophosphatase (<i>MdVHP1</i>) | Abiotic stress resistance | Resistant to salinity and drought | [307] |
| Tomato | Deletion of QTLs, <i>Pto</i> gene | Pest resistance | Resistant to <i>Pseudomonas syringae</i> races and <i>Cladosopum fulvum</i> strains | [316] |
| Cassava | Knockdown of <i>eIF4E</i> genes, <i>ncbp1</i> and <i>ncbp2</i> , using CRISPR/Cas9 technique | Pest resistance | Resistance to Potyviridae viruses damage | [317] |
| Banana | Overexpression of <i>MaPIP1;1</i> gene | Drought resistance | Higher ABA content | [327] |
| Banana | Knockdown of gibberellins biosynthesis genes | Submergence tolerance | Well-developed root system | [329] |

were found to be upregulated in leaves and root tissues in drought conditions and it is found to be correlated with the accumulation of osmolytes in the plant. Therefore, heterologous expression of *PDH47* is considered to regulate several endogenous stress-responsive genes in rice during drought conditions [202]

Similarly, the effect of growth regulators in several stress conditions has been studied especially in, *Arabidopsis* and cotton plants to check the role of abscisic acid-responsive transcription factors in regulating drought tolerance. Transgenic lines developed with increased expression of abscisic acid-responsive transcription factors have shown a better stress tolerance than the wild types [203]. Having a deeper root system is an advantageous characteristic for a drought-tolerant plant. Among the identified genes controlling root system architecture (RSA), the QTL *DEEPER ROOTING 1 (DRO1)* is known for its usability for breeding climate-resilient rice, The *DRO 1* loci in chromosome 9 were proved to be involved in the regulation of deep rooting by affecting root growth angle and also found to enhance grain yields under drought conditions. Identification of *DRO1* homologs such as *qSOR1* and proper characterization of them [204] is considered to fasten the development of rice cultivars that are robust to environmental stress [205]. Among various stress conditions, heat stress is considered to damage rice plants by inhibiting metabolic activities and other biological processes such as seed setting, pollen fertility, and plant growth [206–208]. Genes such as zinc finger proteins (*ZFP*) and *OsWRKY11* are related to plant heat tolerance and used in genetic engineering rice cultivars. *ZFPs* are known to have a great diversity [209] and are reported to play a major role in plant growth and

development and are found to be highly involved in the regulation of environmental stress in plants. By using high-throughput techniques such as MeDIP-chip several expression levels of specially C₂H₂ type *ZFPs* in rice plants have been identified [210]. Even though the functional characterization of rice *ZFP* genes is still under investigation, *Oryza sativa drought-responsive zinc finger protein 1 (OsDRZ1)* was found to positively regulate stress conditions and has been utilized in developing drought-tolerant transgenic rice plants [211].

Even though the role of Novel stress-associated proteins (*SAP*) in stress conditions is analyzed in several studies [212, 213] its role in plant growth and development is still less covered. *WRKY* is one of the genes known to encode several transcription factors which participate in abiotic stress tolerance. Rice plants overexpressing *WRKY* were shown a higher survival rate and non-impaired growth [214, 215].

The frequency and intensity of flooding are increasing due to global warming and therefore the production of cereal crops with the ability to withstand the waterlogging and/or extended submergence conditions is important. In rice, three non-*SUB1* QTLs were identified from IR72, suggesting that alternative pathways may exist independent of the ethylene-dependent pathway of the *SUB1* gene [216]. The major QTL for salinity tolerance on rice chromosome 1, named *SalTol*, has been mapped, and several salt-tolerant rice varieties such as BR23, BRRI dhan40, BRRI dhan41, BRRI dhan53, and BRRI dhan54 have so far been released [217]. In terms of salt tolerance, such rice varieties have been improved by targeting the changes in mineral transportation in plants [218]. Salt-tolerant Pokkali and salt-sensitive IR64 have

been identified as two rice genotypes showing contrasting responses to salinity stress due to the presence and absence of salt-tolerant proteins [219]. Genes responsible for cell wall integrity, detoxifying ROS, and photosynthesis are expressed in higher amounts. And overexpression of the transcription factor *PeWRKY1* was found to enhance salinity tolerance while increasing the rate of root growth, photosynthesis, survival under stress, and ion fluxes. Salinity tolerant recombinant lines have derived from IR29/Pokkali in Bangladesh using conventional breeding approaches and markers assisted breeding methods. CRISPR technique has been used to create mutants of *gs3* and *dep1* genes, in enhancing rice tolerance, especially to salinity conditions along with various other abiotic stresses [220–222].

Insect-resistant crops are considered to have the second-largest cultivation area in the world, which is 23.3 million hectares in 2017 [80]. In terms of resistance to biotic stresses, genetically modified rice lines have been designed incorporating the *Bt* gene from *Bacillus thuringiensis* in several countries [223]. Results have indicated the ability of genetic improvement in minimizing losses due to lepidopteran pests in Asia [224], high tolerance against rice leaf folder in China field trials [225], insect resistance in Pakistan [226] and Mediterranean regions [227]. And the potential effect of *Bt* proteins against humans, other animals including nontargets are demonstrated to be negligible. Among the large array of isolated *Bt* genes, *CryIA(a)* and *CryIA(c)* proteins are most used in developing transgenic plants resistant to Lepidoptera. The chimeric expression of *cry2Aa* and *cry2Ac* were found to be effective against rice leaf folder in rice plants [228]. After successful results of the cotton plant, *cry* genes have been incorporated in several other crops such as rice, soybean, chickpea, tomato to provide resistance against lepidopteran insect pests [229]. 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. CRISPR has been used in conferring resistance to rice blast in both seedling and tillering stages by silencing the ERF transcription factor gene *OsERF922* [229]. Other than that, CRISPR has been successfully used to knockout the *Os8n3* or the *xa1* gene in rice which makes it resistant to *Xanthomonas oryzae* (xoo) infection [230]. According to Ni et al. 2021, engineering the promoters of susceptibility genes has resulted in rice lines successfully demonstrating Xoo and Xoc resistance while affecting no agronomic traits of the rice plant.

In terms of plant biological processes, CRISPR genome editing has been used on MADS-box transcription factors for gene MADS78 and MADS79 which are

important for the endosperm cellularization and early seed development of rice [231]. To date, there are several genes identified such as *OsPIN5b* responsible for panicle length and *GS3* for grain size to influence the rice yield. Normally cytokinin is degraded in rice inflorescence, and if that mechanism is disrupted, cytokinin levels can be elevated. Such conditions have produced more filled rice grains even the crops were in stress conditions [231]. CRISPR-mediated knockdown of *gna1a*, *dep1*, and *gs3* genes are found to be involved in the development of climate-ready rice [232]. 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 plant [233, 234]. Similarly dense and erect panicle gene (*dep1*) was found to result in a dense and erect panicle with increased 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 a consequent increase in grain weight. CRISPR edition of 3' end 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 [232]. The mechanism of cytokinin homeostasis of rice is used to increase its grain yield [222].

Maize

Maize, being the second most important cereal crop in nearly 125 developing countries, is calculated to have a global production of about 1040 million metric tons from 2016- to 2017 [235]. Consequences of climate change such as drought conditions result in a considerable reduction in its growth and yield [236]. From 1981 to 2016, the yield loss in the United States due to drought stress alone is reported to be around 37% [237].

The genetically modified “MON87460” transgenic maize is a popular creation 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 [238]. 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 [239]. The hybrid maize “DroughtGard™” was developed based on the transgenic maize, bred, and released for farming in the United States in 2013 and is considered to save water required for cultivation by reducing the growth of leaves especially during the critical flowering stages [240]. Maize homologs to the Arabidopsis NFB1 that confers resistance to drought *ZmNF-YB2* 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 [241]. Several drought-responsive transcription factors such as *AP2*, *bZIP*, *NAC*, *HD-Zip*, *MYB* have been identified to play an active role in maize drought tolerance [242]. Drought tolerance characteristics of maize have been improved using the CRISPR/Cas9 system where the *ARGOS8* gene was edited leading to an increased yield and no yield loss under water-stressed conditions [243]. Similarly, the ZFN technique has been used in targeted knock out of the thermo-sensitive male sterile 5 (*TMS5*) gene, in generating temperature male-sterile maize crops [244].

With the development of new techniques, RNA sequencing has been used in the identification of differentially expressed genes (DEG) at the transcriptome level providing an understanding of the molecular basis of abiotic stress responses of maize. This technique has been successfully used in obtaining the transcriptome profile of B73 seedling leaves in response to abiotic stresses such as salinity, heat, drought, etc., allowing them to be used in detecting genes and pathways responding to different abiotic stresses in maize [237]. Root transcriptome analysis at different growth stages of the plant has revealed the molecular mechanism of root growth and development of maize [245].

Potassium homeostasis in plants is regulated by *SKCI* in chromosome 1, providing it the salt-tolerant ability, while acting as a molecular marker suitable for selecting salt-tolerant cultivars [246].

Flooding or water-logged conditions are another possible consequence of climate change that most of the crops are highly susceptible to. When compared with other crops, maize is considered a crop relatively sensitive to flooding, and interestingly there are several maize relatives such as *Z. nicaraguensis*, and *Z. luxurians* with a higher flood resistance. Multiple QTL-related studies are carried out on these crops to locate the resistant genes [247]. Several miRNAs related to waterlogging conditions have been identified. For instance, *asmir159*, *miR164*, *miR167*, *miR393*, *miR408*, and *miR528* are considered to be upregulated in short-term water-logging conditions giving the plant a higher resistance [248].

RNAi technology has been used to design insect-resistant maize using the putative V-ATPaseA coding region from Western corn rootworm (war). The resulting F1 hybrid plants were equipped with resistance to or and it is considered a successful control of lepidopteran pests. Genome editing tools such as ZFN have been used to edit maize gene *ZMIPK1*, improving it with herbicide tolerance characteristics [249, 250].

Soybean

Soybean is an important crop that can be used as a food for human and animal consumption. It is also a source of oil for industrial uses and has made itself one of the main targets in crop improvement programs [251]. However, its productivity, seed quality, and plant growth-like characteristics are found to be considerably affected by a range of abiotic and biotic stresses [252]. Drought is considered the main abiotic stress that results in approximately a 40% reduction in the growth of the plant [253]. Therefore, efforts have been made to improve drought and heat tolerance in plants using overexpression of *Arabidopsis* gene Δ 1-pyrroline-5-carboxylate synthase (*P5CR*) [254]. Several transcription factors regulating stress-related genes have been identified and among them, the *ERF* family of transcription factors are highlighted [255]. The drought-sensitive soybean cultivar BR16 has been transformed using the *AtDREB1A* gene under *rd29A*, drought inducible promoter from *Arabidopsis*. Its overexpression is found to enhance drought tolerance in soybean while increasing plant photosynthetic rate, plant chlorophyll content, and a higher stomatal conductance [256].

Both abiotic and biotic stress-related plant responses are regulated by using *WRKY* transcription factors superfamily genes. Virus-induced gene silencing technique has been used to silence 64 soybean *WRKY* transcription factors. Silencing of *GmWRKY36*, *GmWRKY40*, and *GmWRKY45* was highly related to plant stress resistance [257].

Several soy varieties are currently commercialized and Roundup-ready (RR) crops, registered under trademarks of Monsanto Technology LLC [258]. RR crops are designed tolerant to the herbicide glyphosate through the expression of the 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*) gene from *Agrobacterium* spp. strain CP4. Roundup is known to block the plant *EPSPS*, blocking the synthesis of aromatic acids. In the presence of glyphosate catalyzing the reaction of shikimate-3-phosphate and phosphoenolpyruvate to form 5-enolpyruvyl-shikimate-3-phosphate is found to fulfill all plant's aromatic amino acid requirements [259, 260]. Several research efforts have been carried out for the development of a second-generation glyphosate-tolerant soybean using an identical CP4 EPSPS protein, with a higher yield compared to the first [260]. Similarly, Soybean CV127 was genetically modified tolerant to herbicides of the imidazolinone chemical class, expressing an altered *csr1-2* gene from *Arabidopsis thaliana* [261]. The soybean *Bt* lines are approved for commercialization in Brazil producing more than 114-million-ton production in 2019 (FAO). Expression of the *cry1Ab* gene is reported to be successful in the prevention of larval feeding and growth

of *Anticarsia gemmatalis*. The expression of the synthetic *cryIAc* gene is also found to complete larval mortality of *Anticarsia gemmatalis* while reducing the larval survival of *Pseudoplusia* includes and *Helicoverpa zea* in several studies [261, 262].

Wheat

Wheat, being one of the most important crops that affect global food security is known as the food source for more than 50% of the world population. It is included in the “very sensitive” category according to the response to water-deficit conditions [263], and stress conditions such as drought are considered to severely compromise the yield of wheat crops [263]. The loss due to the impact of climate change and associated water scarcity is calculated to decline by 2 million in India alone [264]. An increase in temperature has been found to affect seed germination, increasing the risk of crop failure. Molecular control mechanisms for abiotic stress tolerance are based on activation and regulation of specific stress-related genes. Therefore, revealing the temperature-dependent molecular mechanisms that could influence the seed germination is important and the metabolism and signaling of growth regulators such as abscisic acid and gibberellic acid can be considered as a turning point in terms of wheat seed germination [265]. The tissue-specific data are integrated with biochemical networks to generate a comprehensive map of molecular processes during grain filling and the developmental processes of wheat [266]. The findings could be used in improving wheat, its wild relatives as there are no considerable differences in the gene expression of wheat and its wild relatives [267], and also the underline principle could be used in other crop improvements as well. Further, the transcription factors are also investigated in improving the water stress of crops. The dehydration-responsive element binding (*DREB*) gene is one of them 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 [268]. Even though so far, a significant yield improvement was not observed in the transgenic plants, they are known to demonstrate a considerable adaptation to water stress conditions when compared to controls [269]. In terms of plants’ resistance to stress conditions [270], has 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 in the world [271]. The QTLs of 150 winter wheat cultivars have been evaluated and identified revealing SNPs in 37 quantitative trait loci associated with the salt tolerance traits. Those have been effectively used in wheat improvement and identified polymorphism is expected

to be used in future breeding programs as well [272]. 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 [273]. Manipulation of the transgene *Pinellia pedatisecta agglutinin (PPA)* for improvement of aphid resistance, TALEN, and CRISPR-mediated genome editing to improve wheat Powdery mildew resistance by manipulating the target gene *TaMLO* [274] are highlighted. Using CRISPR/Cas9, an additional effort was made to target *EDR1* as an alternate mechanism for achieving powdery mildew resistance: edited wheat plants, such as the *MLO* edited lines, were resistant to powdery mildew, as evidenced by reductions in fungal structures and microcolonies [275]. CRISPR-mediated editing of gene *TaGASR7* for the improvement of grain length and weight [276], *PDS* gene for the improvement of plant chlorophyll synthesis [277] are a few of the successful modifications in the world.

Barley

Barley is considered the fourth most important cereal crop in the world after rice, maize, and wheat [278]. However, among them, barley is highlighted as a crop that can be adapted to abiotic stresses in a relatively little time [279]. Because of barley’s natural stress tolerance, researchers are increasingly interested in identifying stress-sensitive genes using small/large-scale omics investigations, comparative genomics, and genetic transformation to overexpress some of these genes [280]. Abscisic acid is considered to be involved in several metabolic pathways that play a role in drought, salinity, and cold tolerance in the plant [281]. 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 [282]. Similarly, overexpression of *HvSNAC1*, a stress-responsive transcription factor, improves the drought tolerance with no reduction in the plant’s yield [283]. Several such mechanisms underlying stress-related gene expression have been evaluated and known to have a major impact on the improvement of barley productivity with the increasing changes in climatic conditions [284]. Sequencing and related mapping efforts have been performed on several barley species and among them, Tibetan hullless barley has been highlighted as a resource to further study barley genomics due to its high gene completeness and high collinearity of genome synteny with the previously reported barley genomes [285]. Comparison of genomes is performed to reveal the unidentified genes and the barley stem rust resistance gene has been identified from rice–barley comparisons [286]. 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 resistance have been identified in the genome of wild species of barley (AWCS276) [287] paving the pathway to several improvements in a barley crop. Identification of important QTLs is also a major concern in crop improvements. Exome-QTL sequencing has been used to successfully map the black lemma and pericarp (*Blp*) loci and the QTLs responsible for resistance to net blotch disease (caused by the fungus *Pyrenophora teres*) [288]. In terms of frost tolerance, frost-resistant loci (*Fr-H1* and *Fr-H2*) have also been mapped [289] and considered an interesting focus of research related to cold tolerance and flowering time of plants. High-quality malting, being the most preferred use, barley varieties have been developed using isogenic lines and are accepted by several brewers as varieties providing high yield and high malting quality [290]. QTLs also govern several abiotic stress tolerance characters such as drought tolerance, submergence tolerance (*SUB1*), and salinity tolerance [291]. Genome editing tools such as CRISPR/Cas9 induced mutation is found to reduce the callose deposition in sieve tubes, making the plants resistant to aphid infections. Molecular markers have been developed for major resistance genes and quantitative trait loci (QTL) against many pathogens in barley such as rust (*Puccinia* spp.) and powdery mildew (*Blumeria graminis* f. sp. *hordei*), *Rhynchosporium commune* [292]. The use of developed markers in marker-assisted selection on barley within recent years is still considered scarce. However high-throughput phenotyping techniques along with NGS are considered to increase the ability to identify loci related to several important traits.

Other crops

Potato

Potato, the world's fourth most important food crop after rice, wheat, and maize, has been evaluated for its performance. It is predicted to have a considerable reduction in the world potato yield from 2 to 6% by 2055. The rise in temperature along with CO₂ concentration is considered to be the reason for the decline in potato production [293].

Tuberization; the most important aspect of potatoes is directly linked with the flowering time of crops. The genes *GIGANTEA* (*gi*), *CONSTANS* (*CO*), and flowering locus T (*FT*) have been found to affect plant flowering in long days, and experiments are still on in this regard [294]. Plant growth hormones such as gibberellins and auxins are considered to play a major role in tuberization.

Genes such as *StGA2ox1* were found to be upregulated during the early stages of potato tuber development along with differential expression of two *PIN*-like genes, *adr11-2* and *acrA*- genes [295]. In terms of tuberization, *StSP6A* (FT-like; Arabidopsis ortholog), the proteins *StBEL5* and *POTH1* (transcription factors belonging to TALE super-class) are found to be positively regulated in potato. Several genes such as *POTM1*, *StPA2Ac*, *StTUB19*, and *StTUB7*, have been identified and suggested to incorporate in genetic engineering through their overexpression. Genes such as *StCO TE*, *StSP5G*, and *StSUT4* that are responsible for sucrose transportation, have been found to inhibit tuberization and their suppression is expected to promote tuberization in potato [296].

The salinity tolerance of plants has been improved using single gene transfer techniques. The sparse and shallow root system in potatoes has made them vulnerable mainly to drought and salinity. The *DREBIA* gene that has been identified as a gene under the control of a stress-inducible promoter (*rd29A*) from Arabidopsis, has been used in creating transformants exhibiting tolerance to salinity [297]. Several genetic manipulations have been practiced for the enhancement of the nutritional value of potatoes. The tuber-specific gene *AmAI* is considered to be associated with an increased protein content [298]. Transgenic potato tubers with this gene were showing more than 60% increase in their protein content compared to controls [299]. An increase in beta-carotene and lutein content was observed with silencing the *bch* gene using the RNAi approach [300]. In terms of commercial releases, GM potato "NewLeaf" produced by Monsanto was commercially available in the USA from 1996 to 2000. It was a modification containing the *Cry3A* gene against Colorado potato beetle (CPB). Even though it was considered a useful solution to prevent high chemical insecticidal use in the agricultural sector, "NewLeaf" was later considered not useful with the introduction of a novel insecticide that controls both beetles and aphids that damage the cultivation [301]. The protease inhibitor (*PI*) gene is also used in developing insect resistance in several plants as it is a natural component present in the seeds and other storage organs of the plants, which aids the plant defense function [302]. This concept has been used both in potato and tomato crops observing a rapid accumulation of protease inhibitors in leaves attacked by Colorado potato beetles. Similar 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 [303].

Tomato

Tomato is the first vegetable consumed in the world, and its yield is considered to be strongly dependent on cultivars and growth conditions [304]. FLAVR SAVR™ tomato, which was created using antisense RNA technology to increase the shelf life, can be considered as the first transgenic variety approved by FDA (USA) and commercialized in 1994 [305]. However, with the introduction of several other transgenic products, it was removed from the market in 1999 [306]. Lately, the tomato was used for heterologous expression of target genes of other species such as apple, peach, banana, etc. With genetic engineering, overexpression of banana *MYB* transcription factor *MaMYB3* has resulted in inhibition of starch degradation and delayed food ripening in tomatoes. Similarly, apple vacuolar H⁺-translocating inorganic pyrophosphatase (*MdVHP1*) was overexpressed in tomatoes, resulting in tolerance characteristics to drought conditions [307]. Transcriptome analysis has been used to reveal nearly 130 drought-responsive genes in Tomato [308] and transcription factors regulating stress-responsive genes have also been identified [309]. However, the identification of the most efficient gene has been challenging. Cytokinin is known to inhibit leaf senescence. Isopentenyl transferase (*IPT*) is an important enzyme for cytokinin biosynthesis and its expression has been used to create transgenic plants. In terms of resistance to salt stress, regulation of transcription factor C-repeat/dehydration responsive element-binding factor (*CBF*) and other ion transporters have been used. By using *A. thaliana* tonoplast membrane Na⁺/H⁺ antiporter, *AtNHX1* under the regulation of strong promoters, transgenic tomatoes have been designed with the ability to set fruit under 200 mM NaCl salt conditions [310]. Tomato's resistance to chilling conditions has been revealed using Brassinosteroids (BS) mediated regulation [311]. During chilling conditions, BS has shown a positive regulation through a signaling cascade involving *glutaredoxin* genes and some antioxidant enzymes. Due to the expensive nature of the external application of BS, its' biosynthesis in plants is manipulated [312]. Parasitic nematodes are known to cause more than \$150 billion losses annually in several crops including tomato, positive regulation of BS via RESPIRATORY BURST OXIDASE HOMOLOG-dependent increases in mitogen-activated protein kinases is considered a worth finding [313]. Revealing enhanced tolerance to water deficit, salt stress, and chilling conditions using the transcription factor *CBF* driven by *ABTCl* is considered a great finding as results were not affecting plant growth and yield under normal growing conditions [314]. In tomatoes, more than 100 loci responsible for the resistance of 30 major diseases have been mapped and molecular markers associated with those traits have been

reported [315]. Some of those diseases include Tomato yellow leaf curl virus (TYLCV), Potato Virus Y (PVY) and Tobacco etch virus (TEV), and corky root rot. Among the detected QTLs, the *Pto* gene, conferring resistance to *Pseudomonas syringae* races, a leucine-rich repeat protein conferring resistance to *Cladosopum fulvum* strains, conferring *Verticillium* wilt resistance, encoding surface-like receptors [316] have been cloned into tomato plants and are functionally validated.

Cassava

Cassava is an important staple food in developing countries, feeding almost half a billion people a basic diet. After rice and maize, cassava is considered the third-largest source of dietary carbohydrates in the tropics. It is one of the most drought-tolerant crops, able to thrive in even the aridest conditions. Gene editing has been used to combat the brown streak virus, which can cause yield losses of up to 70% in cassava. 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. The root disease severity and viral titer have been significantly reduced in edited cassava lines [317]. Efforts have also been made to develop resistance to the African cassava mosaic virus (ACMV). However, resistance to ACMV was not achieved in the glasshouse experiment, indicating that this virus is mutagenic and warrants further investigation [318].

Banana

Bananas (*Musa* spp.), native to Southeast Asia, are widely cultivated throughout the tropics and subtropics, where they form a staple diet and are an important source of nutrition for more than 500 million people [319]. Banana has the largest market share of all fruits in the world because of its widespread popularity [320]. The United Nations Food and Agriculture Organization considers bananas to be the fourth most important food crop after rice, wheat, and maize for alleviating human starvation in Africa because they are nutritious and starchy [321].

Water transport is an essential part of maintaining the ability to withstand drought and high salt stress [322–324]. Since banana plants have shallow roots, they can be adversely affected by drought, salt, and cold stresses that result in water shortage conditions, a condition that significantly reduces banana quality and yield [325, 326]

Jin, Xu, and Song, 2021 have investigated plants' ability to survive abiotic stressors by improving aquaporins. However, the proper mechanism is still unknown. Overexpression of *MaPIP1;1* in bananas has improved tolerance to numerous stressors. Ion leakage and

malondialdehyde levels were decreased in transgenic plants, whereas proline, chlorophyll, soluble sugar, and abscisic acid (ABA) levels were increased, the transgenic lines have shown higher levels of ABA production and response genes than the wild type. In a previous study it was shown that *MaMADS3* may bind to the promoter region of *MaPIP1;1*, by consequently controlling *MaPIP1;1* expression and impacting the drought tolerance of the banana plant. In addition, 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 and the produced proteins have shown the ability to bind to *MaPIP1;1*'s promoter area. The findings of this study have contributed to revealing the stress-resistant regulatory network of aquaporin genes and are important for the development of stress resistance in banana [327].

In addition, a semi-dwarf banana variety has been developed by Shao et al., 2020 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 [328]. In many parts of the world, the Banana streak virus (BSV) is considered a major hindrance to the breeding and distribution of banana cultivars (*Musa* spp.) [329]. It is considered to affect many important agronomic banana species, while also limiting the ability to use *Musa balbisiana* as source material due to the presence of the latent virus. In the study of Tripathi et al., 2019 the knockouts of the endogenous virus have resulted in lines in which 75% of edited plants remained asymptomatic after being subjected to water stress. The study has demonstrated the efficacy of attacking an integrated plant virus in a plant genome for the first time and has provided a promising pathway to address a major barrier in banana production and breeding [330].

Current challenges and prospects

There is an urgency to double the crop production by 2050 to feed the ever-rising human population which is projected to reach 10 billion. The current food production system would not be sufficient to reach the future food demand. Thus, a series of steps must be urgently planned and executed to allow the global food system from a sub-optimal state to fully cater to the demand. Proper identification of the remaining challenges in crop improvement could pave the way to successfully introduce biotechnological products to future generations.

In terms of agronomy, considerable attention should go to the underutilized crops. Horticultural crops including fruits and vegetables also need attention towards genomic assisted breeding to combat abiotic and biotic

stresses. Only 34% of fruits and vegetables of global demand have been produced so far. Despite this, the accomplishment of sustainable development goals (SDG) by 2030 needs immense production of them to ensure global food security.

Genomic assisted breeding techniques have been directed towards major cereal crops. *Colocasia esculenta* (Taro) is one underutilized crop that has been identified as a potential source of antioxidants and to mitigate chronic malnutrition and hunger. 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” [331, 332]. 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. Brazil is known to use them as “cash crops” to purchase requirements such as medicine and food. Despite their importance, they are less addressed in biotechnological efforts. That could be one of the reasons for less usage of the GM crops because the crop of the target in research is a non-target in multiple regions of the world. *Dioscorea* spp. (yams) can be considered as another good example of that. 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 [333]. 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. They could be modified to contain no toxins, so more germplasm becomes available for utilization. A similar condition could be considered for the crop *Lathyrus*, which is a protein-rich legume, known to use after soaking overnight to clear the toxins contained in split seeds. The maximum possible contribution from identified genes is still not taken into effective usage in crop improvement. In terms of SNPs, their distribution in genome maps is found to be unequal and composed of several gaps. Effective mapping strategies are required to overcome the issues as in plant genetic research and breeding, identification of the exact gene loci is fundamental to all the steps ahead. Therefore, the error-prone nature of some NGS approaches is considered to result in false-positive SNPs. To overcome these issues, machine learning approaches are recommended to use instead of the binary composition of nucleotides. Integrated SNP Mining and utilization (ISMU) is one of

the methods recently used to increase the pool of accurate data in research.

No “Silver bullet technology” would solve the impact of climate change. Therefore, an integrated strategic plan is needed to solve the issue. In terms of research practices, diversity is preferred over the regularly used protocols. For example, in making insect-resistant crops, *Bt* toxins are the most used proteinaceous molecule for most species. The spectrum of molecules could be increased such as by using peptides, or other compounds such as secondary metabolites from other species using novel genetic engineering approaches [334]. Since there are several plants containing a range of medicinal properties the gene pool can be broadened to increase the diversity as different techniques are used even the same crop is modified for the same purpose. 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. Meanwhile, all the transgenic watermelon plants harbored chicory phytoene desaturase gene (*CiPDS*) mutations and developed clear or mosaic albino phenotype, indicating that CRISPR/Cas9 system has 100% genome-editing efficiency in transgenic watermelon lines to introduce new functions [335].

Automated platforms could be considered as a background for several improvements in crops [336]. Even though several types of research and finding have been published in genetic engineering of crops, dissemination, and utilization of such modern technologies all around the world remain slow. The primary barrier of implementing climate change adaptation plans is insufficient to transfer technology. Especially most of the developing countries are facing lack in high technology facilities. Up to date, only Bangladesh is in the trial of producing transgenic crops in South Asia. Interestingly, Genetic Engineering Appraisal Committee (GEAC) has approved field trials of *Bt* Brinjal in eight states in 2020 in India [1, 3]. Generally, many countries’ climate change strategic plans include adaptation in farming practices, productive irrigation practices, reducing greenhouse gas in the farm, developing resilience crop varieties for harsh environmental conditions, and production of biofuels. It becomes important because even though transgenic crops, such as *Bt* cotton is known to result in a higher yield, it strongly depends on the available environmental conditions and the availability of water. In such cases, investments in transgenic crops can be considered a risk for farmers. Therefore, agronomic evaluations on improved crops should match the local conditions of the countries where the product will be used. Since in the real world, plants are undergoing several stresses in combination with

alterations in the periods, they should be properly studied and understood before commercialization. For example, the designed herbicide-tolerant crops were found to establish slowly in African and South Asian countries. The reason was the un-match between the crops with those countries’ climatic, environmental conditions, and farming practices. Similar situations could limit the applications of crop improvements while reducing the value of the effort that was taken in improving it.

In developing countries such as India, a huge amount of labor opportunities has become available by using them for weed removal in cultivation. Due to the development of herbicide-tolerant crops, the time spent on manual weed control seems to be reduced and that could give rise to a conflict between job security and innovations in the economy. Also, that could be a reason for the rejection of GM crops by a large number of people. In that case, several factors arise to be considered before applying the techniques, especially in developing countries.

Further, there should be a proper practice and a system to gradually replace the previous cultivars and agronomic practices using suitable mechanization. If the farmers have better access to the market, then only the processing food will be able to generate a considerable income for the farmers, while delivering better products to the consumers to contribute to food security. The image of “GM” food in the market is dynamic. Therefore, it’s another challenge to get approval for GM plants by certifying their safety of consumption. The factors which influence public decisions should be addressed using discussions or forums to identify the factors that could help locally adopted technologies. It’s important to conduct them on multiple levels to match the people’s literacy in different regions. A transparent discussion public–private partnership including farmers could increase the pace of GM acceptance by the general public. The local government and international authorities should pay attention to policies to appropriate utilization of climate-ready crops without environmental destruction [337, 338]. Meanwhile, there should be an international practice to reduce factors that support global warming, such as the invention of a universal protocol to calculate global carbon emission and other greenhouse gases. Considering all the issues, a proper determination on financial prioritization should be done by the national leaders of countries. Especially more consideration regarding the determination of research needs of the country becomes important. Similarly, investment support should be available for scientific requirements along with the most important resources.

Cultural and religious aspects are also an issue, especially in developing countries. For example, certain cultures consider cattle as gods, and transgenic crops

created using DNA taken from such organisms may not be accepted by those religions. The safety measures are of high concern regarding GM crops, it should be certified by an acceptable authority for the public to be confident about consuming the product. Since there are already formulated biosafety regulations and guidelines in this regard, the process of safety insurance should not prevail as a barrier in society for a future of “crop improvement”.

Conclusion

Adapting climate-smart agriculture is considered to ensure a sustainable increase in agricultural productivity to satisfy the global food demand. This review provides an overview of the development and application of plant biotechnology as a positive contribution to climate change adaptation in the field of agriculture. Genetic engineering approaches and new tools are used to improve plants adapted to an increasing number of challenges brought by the changing climate. Specific examples on crops are discussed on their improvement showing that these measures integrating modern biotechnology can be effectively used to improve the agricultural productivity of the world. Despite the efforts that are being taken to design climate-ready crops using modern biotechnology. Improved research efforts, filling the technical and knowledge gaps could substantially accelerate the process of creating crops adapted to the ever-changing climatic conditions.

Abbreviations

ABA: Abscisic acid; ABTC1: ABA-responsive complex; ACMV: African cassava mosaic virus; ACP: Acyl carrier protein; acrA: Auxin-regulated gene containing a GTP-binding site; adr: Auxin downregulated; Adr: Auxin downregulated; Agro-SS: *Agrobacterium*-Mediated site-specific integration; AmA: Apical membrane antigen; amA1: *Amaranthus hypochondriacus*1; AP2: APETALA 2; ARGOS: Auxin-regulated gene involved in Organ Size; AtDREB: *Agrobacterium tumefaciens* Dehydration-responsive binding protein; ATP: Adenosine tri-phosphate; AtNHX1: *Arabidopsis thaliana* Sodium proton exchanger 1; AWCS276: Long-kernel wild barley genotype; bch: β -Carotene hydroxylase; BEL: Bentazon sensitive lethal; BEL5: BEL 1 like protein; Blp: Black lemma and pericarp; BR: Bangladesh rice; BS: Brassinosteroids; BRRI: Bangladesh Rice Research Institute; BSV: Banana streak virus; Bt: *Bacillus thuringiensis*; bZIP: Basic leucine zipper; CaMsrb: *Capsicum annuum* Methionine sulfoxide reductase B; Cas9: CRISPR-associated protein 9; CBF: C-repeat/dehydration responsive element-binding factor; CIPDs: Chicory phytoene desaturase; CK: Cytokinin; CMV: Cauliflower mosaic virus; CO: CONSTANTS; CO₂: Carbon dioxide; CPB: Colorado potato beetle; CRISPR: Clustered regularly interspaced short palindromic repeats; Cry: Crystal proteins; CSA: Climate-smart agriculture; CsLOB: *Citrus sinensis* Osbeck lateral organ boundary; CspB: Cold shock protein from *B. subtilis*; csr: Chlor-sulfuron-resistant; DCL: Dicer-like; DEG: Differentially expressed genes; DEP: Dense and erect panicle; DNA: Deoxyribonucleic acid; DREB: Dehydration-responsive element binding; DRO: Deeper rooting; DRZ: Drought-responsive zinc finger protein; dsRNA: Double-stranded ribonucleic acid; DvSnf: UCROSE-NON-FERMENTING7 gene of western corn worm; EDR1: Early Development Regulator 1; eIF4E: Encoding translation initiation factor 4E; EMN: Engineered endonucleases/meganucleases; EMT: Engineered homing endonucleases/meganucleases; ENSO: El Niño-Southern Oscillation; EPSPS: 5-Enol-pyruvylshikimate-3-phosphate synthase; ERF: Ethylene response factor; FAD: Fatty acid desaturase; FAO: Food and Agriculture Organization; FDA: Food and Drug

Administration; Fr-H1 and Fr H2: Frost-resistant loci; FT: Flowering locus T; GA2ox: GA 2-oxidase gene; GAPIT: Genome Association and Prediction Integrated Tool; GBS: Genotyping by sequencing; GDP: Gross domestic product; GEAC: Genetic Engineering Appraisal Committee; GHG: Green-house gas; gi: GIGANTEA; GM: Genetically modified; GMO: Genetically modified organisms; Gn: Grain number; gs: Glutamine synthetase; GS: Grain size; GWAS: Genome-wide association studies; HD-ZIP: Homeodomain-leucine zipper; HR: Hormone receptor; HOX: Homeobox; HVA1: *H. vulgare* Abundant protein 1; HvSNAC: *Hordeum vulgare* Transcription factor NAC; InDels: Insertion-deletion; IPT: Isopentyl transferase; IR: Rice developed by International Rice Research Institute; ISMU: Integrated SNP mining and utilization; Kg: Kilograms; L: Liters; LOG: The LONELY GUY; MADS: Minichromosome maintenance 1 (MCM1) genes in yeast, Agamous (AG) in *Arabidopsis*, Deficiens (DEF) in antirrhinum and serum response factor (SRF) in humans; MaPIP: Banana plasma membrane intrinsic proteins; MAS: Marker-assisted selection; MdVHP1: H⁺-translocating inorganic pyrophosphatase; Mha: Million hectare; miRNA: MicroRNA; MLO: The powdery mildew locus O; MON: Monsanto; MORC1: Microorchidia 1; mRNA: Messenger RNA; MYB: Myeloblastosis; myb: β -Amylase gene isolated; NAC: No apical meristem (NAM), *Arabidopsis thaliana* transcription activation factor (ATAF1/2) and cup-shaped cotyledon (CUC2); NAPs: National action plans; NATP-2: National Agricultural Technology Program-Phase II Project; ncbp: Novel cap-binding protein; NFB: Neurofibromatosis; NGS: Next-generation sequencing; OS: *Oryza sativa*; PIN: Proteinase inhibitors isolated; P5CR: Δ 1-Pyrroline-5-carboxylate synthase; PacBio: Pacific biosciences; PAT: Paroxysmal atrial tachycardia; PAV: Presence and absence variations; PDH: DNA helicase; PDS: Phytoene desaturase; PI: Protease inhibitor; PIN: Plant-specific pin-formed; PLINK: Population linkage; POTH1: Potato homeobox1; POTM1: Potato MADS-box gene cDNA; PPA: *Pinellia pedatisecta* Agglutinin; Ppm: Parts per million; PTGS: Post transcriptional gene silencing; PTO: Gene resistance in tomato to *Pseudomonas syringae* pv tomato strains; PVY: Potato Virus Y; qSOR: QTL soil surface rooting; QTCAT: Quantitative trait cluster association test; QTL: Quantitative trait loci; RACK: Receptor for activated C-kinase; RCP: Representative concentration pathways; rd29A: Response-to-dehydration 29A; rDNA: Recombinant deoxyribonucleic acid; Rhg: Resistance to *Heterodera glycines*; RNA: Ribonucleic acid; RNAi: RNA interference; ROS: Reactive oxygen species; RR: Roundup-ready; RSA: Root system architecture; RT-PCR: Reverse transcription polymerase chain reaction; SAP: Stress-associated proteins; SCM2: STRONG CULM2; SDG: Sustainable development goals; 4SE: Standard error; siRNA: Short Interfering RNAs; SKC: Streptokinase C; SNP: Single nucleotide polymorphism; SP6A: Flowering locus T; Spp: Species; sRNA: Small RNA; SST: Somatostatin; St: *Solanum tuberosum*; SUB: Submergence; TaGASR: *Triticum aestivum* Gene encoding snakin/GASA; TALE: TAL effector; TALENs: Transcription activator-like effector nucleases; TaMLO: Mildew resistance locus O; T-DNA: Transfer-deoxyribonucleic acid; TEV: Tobacco etch virus; TMS: Thermos-sensitive male sterile; TUB: Beta-tubulin; TYLCV: Tomato yellow leaf curl virus; USA: United States of America; USD: United States dollars; WCR: Western corn worm; WGR: Whole-genome regression; WRKY: Transcription factors with conserved amino acid sequence motif WRKYGQK; Xoc: *Xanthomonas oryzae* Pv. oryzicola; Xoo: *Xanthomonas oryzae* Pv. oryzae; ZFN: Zinc-finger nucleases; ZMIPK: Maize inositol phosphatase kinase; ZmNF-Y: Maize nuclear factor Y.

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Author details

¹Department of Applied BioSciences, Graduate School of Bio Agricultural Sciences, Nagoya University, Nagoya, Japan. ²Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka 20400.

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