Channa S. Prakash · Sajid Fiaz ·
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Abdul Qayyum *Editors*

Sustainable Agriculture in the Era of the OMICs Revolution

Springer

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Chapter 1
The Utilization of Speed Breeding and Genome Editing to Achieve Zero Hunger

Khazin Hussain, Mahrukh, Raheeba Tun Nisa, Abbu Zaid, and Muntazir Mushtaq

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Introduction

According to the UN's Hunger Report, hunger is the term used to define periods when populationsare experiencing severe food insecurity, meaning that they go for entire days without eating due to lack of money, lack of access to food, or other resources. Around the world, more than enoughfood is produced to feed the global population—but as many as 811 million people still go hungry (SDG2). After steadily declining for a decade, world hunger is on the rise, affecting 9.9% of people globally. From 2019 to 2020, the number of undernourished people grew by as many as 161 million, a crisis driven largely by conflict, climate change, and the COVID-19 pandemic (Action Against Hunger International Nutrition Security Policy). In 2015, world leaders charted a new set of Sustainable Development Goals (SDGs) to "end hunger, achieve food security and improved nutrition and promote sustainable agriculture" by 2030. As the year 2030 draws closer, the achievement of the world's commitment to Zero Hunger is tragically distant. Current projections based on the Global Hunger Index (GHI) show that the world as a whole—and 47 countries in particular—will fail to achieve even low hunger by 2030. With the increasing global population, farmers around the world will have to dramatically increase food production to meet demand in the face of climate change, land and water scarcity, and the demand for more diverse, protein-rich diets among populations with increasing incomes. Yet since 1990, aggregate farm yield growth has stagnated and even reversed course in some areas (FAO, 2016). The pace of yield augmentation in most crop breeding efforts is insufficient to meet the growing food demand generated by the world's constantly expanding population. In plant breeding, the creation of superior crop varieties is hindered by the unusually prolonged crop duration. A new cultivar can take one or two decades to develop because of the multiple stages of crossing, selection, and testing needed in the generation of new plant types. The creation of high-yielding, nutritional crops for future conditions has several hurdles. One limiting element is generation time, which extends the time it takes to conduct research and produce plants.

Traditional agricultural practices, which have been used for a long period, have reduced crop evolutionary divergence. Several conventional and molecular approaches have been used to improve agronomic traits associated with yield, quality, and resistance to biotic and abiotic stresses in crop plants, including gene manipulation, mutational breeding, soma-clonal variabilities, whole-genome sequence-based strategies, physical maps, and functional genomic techniques. New plant breeding techniques that are developed due to advances in scientific research, enable more precise and faster changes in the plant's genome than conventional plant breeding techniques. Recent developments in genome editing technology, however, employing customizable nucleases, clustered regularly interspaced short palindromic repeats (CRISPR), and CRISPR-associated (Cas) proteins, have ushered in a new age of plant breeding. To improve crop breeding performance, plant breeders and researchers throughout the world are employing revolutionary tactics such as speed breeding (SB), genome editing technologies, and high-throughput phenotyping. These novel approaches involve a sequence of developments from genome editing techniques to SB and the incorporation of omics technology offering relevant, versatile, cost-effective, and less time-consuming ways of achieving fidelity in plant breeding.

The emergence of SB and genome editing techniques have led to a substantial leap in the ability to generate new genetically modified plant varieties (GMOs) expanding the potential for crop improvement. The main objective behind SB and genome editing is the development of high-yielding varieties, resistance to pests and diseases, change in maturity period, elimination of toxic substances, synchronous maturity, photo insensitivity, resistance to abiotic stress, and improved quality and ultimately leads to the achievement of zero hunger goals (Fig. 1.1). SB has revolutionized agriculture by shortening the crop cycle by producing 5–6 generations of a crop in a year. Unlike doubled haploid (DH) technique, which produces haploid embryos to provide totally homozygous lines, SB is acceptable for various germplasm and it does not require specialized *in vitro* culturing facilities (Slama-Ayed et al., 2019). The concept behind SB is by using optimal light intensity, temperature, and daytime length control (22-h light, 22 °C Day/17 °C Night, and high light intensity) to increase the frequency of photosynthesis, which primary user early flowering, in conjunction with annual seed harvesting to truncate the generation time. SB has been an emerging way to reduce the hunger crisis by reducing the generation

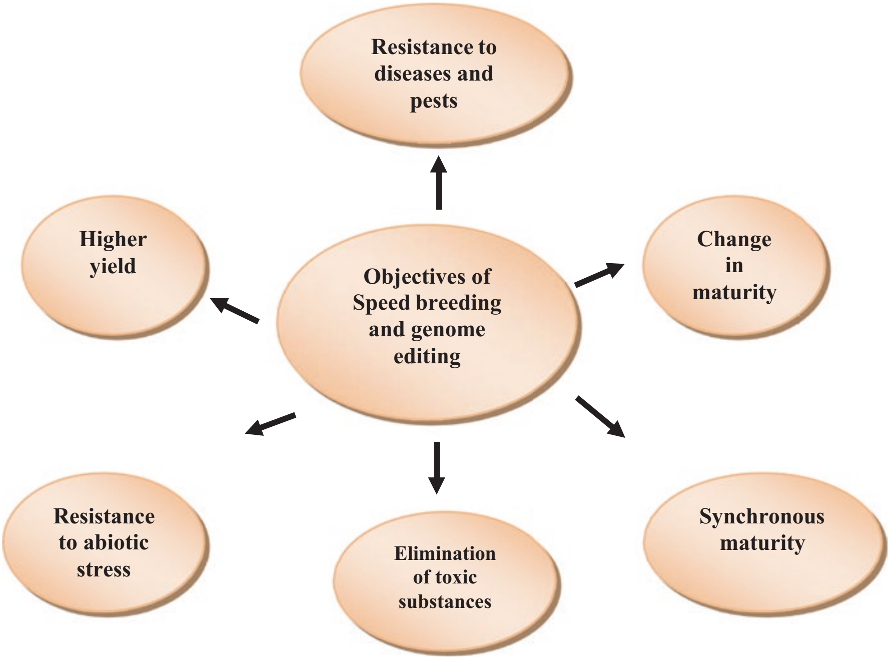

Fig. 1.1 Objectives of speed breeding and genome editing to achieve zero hunger

gaps. With SB, you can achieve up to six generations per year for spring wheat (*Triticum aestivum*), durum wheat (*Triticum durum*), barley (*Hordeum vulgare*), chickpea (*Cicer arietinum*), and pea (*Pisum sativum*), and four generations per year for canola (*Brassica napus*), instead of two to three under traditional methods (Watson et al., 2018). The advent of genome-editing technologies has transformed the field of plant sciences. To create a double-stranded cut in the host genome at a specific site, genome-editing technologies employ site-specific endonucleases such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR-Cas-based systems. The sequence is then repaired by the cell's endogenous DNA repair machinery *via* nonhomologous end joining (NHEJ) or homologous recombination (HR) (Chiurugwi et al., 2018). By producing minor deletions, NHEJ repair can wipe out gene function, whereas HR repair employing a template can make specific alterations to the DNA sequence at the desired area. Rather than producing double-stranded DNA breaks at the target spot, epigenetic alterations can be caused by changing the DNA sequence by base editing or affecting the chromatin structure. Moreover, there are a few genome editing tools (GE) that work independently without inducing the double-stranded DNA breaks (DSB), including prime editing, base editing, and dCas9-based epigenetic modification. These genome editing tools (GE) can be responsible for unwanted DNA mutations. A conventional breeding process takes almost 8–12 years to develop an improved variety. With the rapidly changing environmental conditions, the newly developed varieties are needed in a short period. Meganucleases were the first genome editing tools to improve the maize and wheat genomes. Meganucleases are naturally occurring molecular DNA scissors that can recognize up to 12–40 DNA bases. Zinc finger nucleases work on the same pattern as meganucleases and can recognize up to 9–18 base pairs. TALENs have an advantage over other site-specific nucleases (Meganucleases and Zinc Finger nucleases) due to its nature that it targets a single nucleotide as compared to three in meganucleases and zinc finger nucleases (ZFN). TALENs were successfully used in many cereal crops. CRISPR/Cas9 and CRISPR/Cpf1 were associated with off-target mutations, and this issue was resolved by using the modified genome editing tools such as prime editing, base editing, and dCas9-based epigenetic modification.

Evolution of SB and Genome Editing

In mid-1980, NASA collaborated with "Utah State University" to explore the rapid growth cycle of plants under the constant light of a space station on wheat. NASA developed SB, or rapid generation advancement, to safeguard the food supply and psychological well-being of astronauts during long-haul missions of the future. Dr. Lee Hickey and his co-workers were the first to adopt NASA's Plan for the production of wheat and peanut at the University of Queensland, John Innes Centre and the University of Sydney in Australia. Since the advent of recombinant DNA technology in Paul Berg's laboratory (Singer, 1979) in 1972, genetic engineering has come a long way and achieved enormous success. Many molecular and genetic mechanisms and phenomena have been discovered and studied in detail and the knowledge accumulated now permits researchers to reproduce experiments *in vitro*. Several decades-long investigations in molecular genetics and biochemistry of bacteria and viruses have allowed researchers to develop new methods of manipulating DNA through the creation of various vector systems and tools for their delivery into the cell. All of these developments allow the successful creation of not only transgenic microorganisms but also genetically modified higher organisms including various plant and crop species. The creation of novel tools for breeding and biotechnology, an application area of genetic engineering, has received significant focus resulting in the accelerated development of useful tools. In 1996, for the first time, it was shown that protein domains such as "zinc fingers" coupled with FokI endonuclease domains act as site-specific nucleases (zinc finger nucleases; ZFNs), which cleave the DNA *in vitro* in strictly defined regions. Such a chimeric protein has a modular structure because each of the "zinc finger" domains recognizes one triplet of nucleotides. This method became the basis for the editing of cultured cells, including model and non-model plants. Continued efforts and investigations led to the development of new genome editing tools such as TALENs (transcription activator-like effector nucleases) and CRISPR/Cas (clustered regularly interspaced short palindromic repeats). Designing TALENs requires the reengineering of a new protein for each of the targets. However, the design process has been streamlined recently by making the modules of repeat combinations available that essentially reduces the cloning required for the design. On the other hand, the designing and use of CRISPR are simple. Both TALEN and CRISPR systems have been shown to work in human cells, animals, and plants. Such editing systems when used for efficient manipulation of genomes could solve complex problems including the creation of mutant and transgenic plants.

Time-Saving Tools for Achieving Zero Hunger

Plant Breeding Acceleration Through SB

SB is a suite of techniques that involves the manipulation of environmental conditions under which crop genotypes are grown, aiming to accelerate flowering and seed set to advance to the next breeding generation as quickly as possible. Procedures of SB technology involve the following steps: preparing seed for sowing, monitoring key growth stages, parameters, and phenotyping, harvesting of seeds, and last is monitoring the use of energy (Fig. 1.2). The method saves breeding time and resources through rapid generation advancement. For most crop plants, the breeding of new, advanced cultivars takes several years. Following the crossing of selected parent lines, 4–6 generations of inbreeding are typically required to develop genetically stable lines for evaluation of agronomic traits and yield. This is particularly time consuming for field-grown crops that are often limited to only one to two

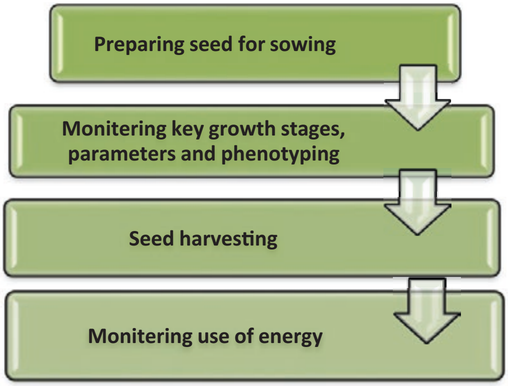

Fig. 1.2 Steps of SB

generations per year. SB techniques have been used on various crops to rapidly develop homozygous lines after initial crosses of selected parents with complementary traits. The technique depends on the manipulation of photoperiod, light intensity, temperature, soil moisture, soil nutrition, and high-density planting. The method allows for the production of 3–9 breeding generations per year. For numerous crops, SB procedures are now being developed. By lengthening photoperiods, SB has been shown to reduce generation time. Certain crop species, such as radish (*Raphanus sativus*), pepper (*Capsicum annum*), green vegetables like Amaranth (*Amaranthus* spp.), and sunflower (*Helianthus annuus*), responded favorably to longer days (Chiurugwi et al., 2018). Positive results in peanut SB (*Arachis hypogaea*) have been reported, also Amaranth (*Amaranthus* spp.) was able to produce more generations per year as the day length lengthened. SB can increase vegetative growth in staple food crops that require shorter photoperiods to enter the reproductive phases, such as rice (*Oryza sativa*) and maize (*Zea mays*). It is possible to generate repeated generations of enhanced crops for field testing *via* SSD using SB, which is less expensive than producing DHs. Gene insert (common haplotypes) of diverse phenotypes is also favored by SB, followed by MAS of superior hybrid populations (Achigan-Dako et al., 2014). SB can increase vegetative growth in staple food crops that require shorter photoperiods to enter the reproductive phases, such as rice (*Oryza sativa*) and maize (*Zea mays*) (Collard et al., 2017). It is possible to generate repeated generations of enhanced crops for field testing *via* SSD using SB, which is less expensive than producing DHs. Gene insertion (common haplotypes) of diverse phenotypes is also favored by SB, accompanied by MAS of superior hybrid lines (Collard et al., 2017). SB is routinely used for generation advancement without phenotypic selection. However, modern technologies (e.g., high-throughput genotyping methods, marker-assisted selection, etc.) can be successfully integrated for target trait selection. The combination of SB and effective selection methods should allow for the maintenance of a good breeding population and genetic diversity in the environments that restrict plant growth and for maximum yield production (Johnston et al., 2019). Conventional selection methods such as bulk, mass, recurrent, pedigree, and pure line selection require a genetically stable plant population for the selection of optimally yielding genotypes. These methods are not ideal for SB due to the long inbreeding and selection cycles that they require. The most appropriate selection methods amenable with SB are single seed descent (SSD), single pod descent (SPD), and single plant selection (SPS) methods.

Genome Editing Strategies: A Progressive Way Toward Zero Hunger Goal

Genome editing is a process where an organism's genetic code is changed. Scientists use enzymes to "cut" DNA creating a double-strand break (DSB). DSB repair occurs by non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ produces random mutations (gene knockout), while HDR uses additional DNA to create a desired sequence within the genome (gene knock-in). In recent years, the emergence of highly versatile genome editing technologies has provided investigators with the ability to rapidly and economically introduce sequence-specific modifications into the genomes of a broad spectrum of cell types and organisms. Genome editing technologies have been employed (Fig. 1.3) to increase resistance against plant pathogens, increase yield, tolerance to abiotic stress, herbicide tolerance, and in turn mitigate crop losses to provide enough food for the growing population and achieve the motive of zero hunger.

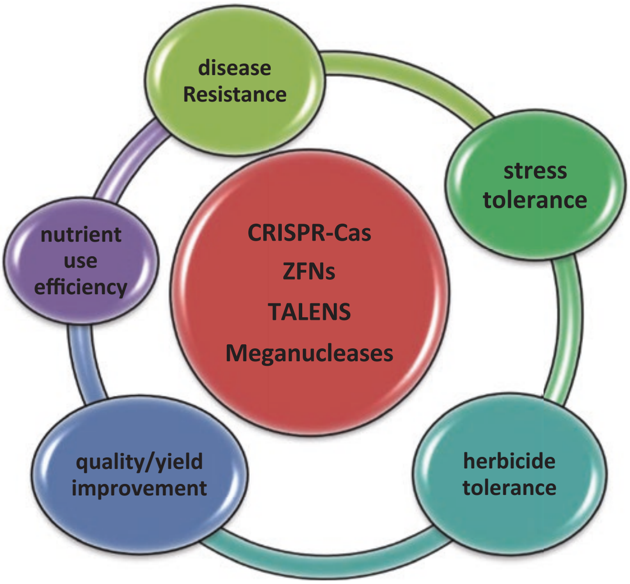

Fig. 1.3 Utilization of genome editing technologies for improvement of disease resistance, nutrient use efficiency, stress tolerance, herbicide tolerance, and increase yield

Enhancing Resistance via Genome Editing

Plant pathogens such as bacteria, fungi, and viruses cause a variety of plant diseases that significantly reduce yield; additionally, herbivores such as insects reduce yield through direct trauma and deal as disease vectors. The change in climate has a negative impact on plant tolerance to various biotic stresses. Direct crop losses due to biotic stresses are estimated to be between 20% and 40%. A crop modification has been done through genome editing technologies in order to achieve a motive of zero hunger. The core technologies now most commonly used to facilitate genome editing are (1) clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9), (2) transcription activator-like effector nucleases (TALENs), (3) zinc-finger nucleases (ZFNs), and (4) homing endonucleases or meganucleases. The CRISPR/Cas9, and related versions, are the most advanced genome editing techniques in plant biology. The Cas9 system requires a short guide sequence (sgRNA) to direct Cas9 nuclease to cleave the target site. Targeting susceptibility genes in a plant genome with CRISPR-Cas is the best alternative for improving disease resistance and thereby enhancing the crop yield. These susceptible genes encode factors used by the pathogen to aid in an infection. Researchers use targeted mutagenesis to knock out such genes and in turn reduce disease susceptibility. There are several crops in which susceptible genes have been edited with the help of genome editing technologies such as CRISPR-Cas, Zinc-finger nuclease, meganucleases, and TALENS. For example, bacterial blight is caused by the bacterium *Xanthomonas oryzae* pv *oryzae*, which results in considerable yield losses in rice under ideal conditions. The bacterial pathogen produces transcription activator-like effectors (TALEs) following infection, which stimulate the transcription of genes expressing sugar transporters (e.g., OsSWEET11, OsSWEET13, OsSWEET14, and so on). SWEET genes are well-known Su genes for bacterial blight disease since they help bacteria multiply in plant tissues. Researchers used the CRISPR-Cas system to break the connection between TALEs and SWEET genes, resulting in rice varieties that are resistant to bacterial blight across the range. They accomplished this by altering the TALE binding site in the promoters of OsSWEET11 (Xa13), OsSWEET13, and OsSWEET14. The plants that resulted were more resistant to the bacterial infection (Oliva et al., 2019). Blast disease, caused by the fungus *Magnaporthe oryzae*, has a negative impact on global rice output. By knocking off particular rice susceptible genes, such as OsERF92231 and Pi21, rice lines resistant to rice blast disease have been created (Wang et al., 2016). Similarly, wheat resistance to powdery mildew, a disease caused by the fungus *Podosphaera xanthii*, has been increased by using CRISPR-Cas to target the Mildew Locus O (MLO) gene (Wang et al., 2014). In polyploid crops like wheat, knocking off Su loci become more difficult; nevertheless, CRISPR-Cas can be used to concurrently target multiple homologs (gene copies present on several copies of the genome). Wheat resilience to many diseases has been boosted by utilizing CRISPR/Cas to target susceptibility genes. Wheat cultivars resistant to powdery mildew, for instance, were created by attacking all three homologs of MLO at the same time (Zeng et al., 2020). Surprisingly, the disease-resistant plants had better yield-related features, indicating that disease-resistant plants may be developed using CRISPR technology to help accomplish the zero hunger objectives.

Increasing Abiotic Tolerance via Genome Editing

In addition to biotic challenges, plants are subjected to a variety of abiotic stresses, such as temperature, salt, heavy metals, and drought, all of which halt crop growth and result in considerable, often disastrous yield losses across the world. Sensitivity genes are plant genes that exacerbate the negative impacts of abiotic stressors. Genome editing methods, notably CRISPR- Cas, have been utilized to increase abiotic stress tolerance in a variety of plant species, including grain, vegetable, and fruit crops, by altering these sensitive genes. CRISPR-Cas has been employed to disrupt OsMYB30 in rice in order to develop cold-tolerant rice lines (Zeng et al., 2020). Similarly, in maize, a gene–ARGOS8–has been edited via CRISPR-Cas9 to improve drought tolerance (Shi et al., 2017).

Improving Herbicide Tolerance Through Genome Editing

Herbicides are used by farmers to minimize weed competition for light, water, and nutrients, in addition, to directly attacking crops. Herbicide-tolerant crops have so shown to be quite valuable in modern agriculture. Transgenic technologies or significant mutagenesis were once necessary to create a new herbicide-tolerant cultivar. CRISPR-Cas9 base editing and prime editing methods were recently employed to increase herbicide tolerance in maize by altering Acetolactate Synthase 1 (ZmALS1) and ZmALS2. The fusion of Cas9 endonuclease and VirD2 enhances the cleavage of the OsALS gene in rice, and concomitant HDR-mediated repair of the OsALS generesulted in herbicide resistance (Ali et al., 2020).

Improvement of Crop Yield Through Genome Editing

Genome editing tools have been employed to increase yield in various crops. Certain genes in plants that proportionally regulated the grain yield function have been edited by such tools to increase the yield. For example, in comparison to wild type, multiplex gene editing of three genes (GS3, GW2, and Gn1a) boosted grain production in altered plants. Another study used CRISPR- Cas9 to knock out OsPIN5b (which controls panicle length), GS3, and OsMYB30 (which regulates drought tolerance in rice) all at the same time, resulting in high-yielding drought-resistant rice lines (Lacchini et al., 2020). Furthermore, CRISPR-based editing of the OsLOGL5 gene, which encodes a cytokinin-activation enzyme, inhibits root development, tiller number, and rice production.

Optimization of Nutrient Use Efficiency Through Genome Editing

Fertilizers use enhanced productivity during the Green Revolution, but it also increased expenses and polluted the environment owing to fertilizer runoff. The development of crops with higher nitrogen use efficiency (NUE) will minimize fertilizer use, boost farmer earnings, and reduce pollution. Greater nutrient absorption from the soil, more effective operation and transport of nutrients in plants, and improved plant growth and production might all help to improve Nutrient Use Efficiency. Some genes suppress nutrient absorption, which has a detrimental influence on Nutrient Use Efficiency in plants. Researchers developed crops that are having incredible nutrient use efficiency and in turn have better crop growth. For example, in rice, nitrate transporter gene NRT1.1B was modified with the help of the CRISPR-based cytosine base editing system (CBE), and the resulted plants showed improved NUE compared with non-edited plants (Lu & Zhu, 2017).

Integration of SB and Genome Editing Technologies for Achievement of Zero Hunger

SB, alongside different state-of-the-art technologies such as gene editing, is the best way to develop a pipeline of new crops to tackle hunger issues. SB and genome editing are important biotechnologies—essentially plant scientists now can edit any gene they want to, at will, using very refined and targeted systems in the lab. However, plant breeding technologies have to be scalable. There are a lot of bottlenecks in genome editing which prevent scaling developments up and delivering improved crop varieties to people globally. The lab holds us back when it comes to plant breeding. Anything that goes to the lab is a bottleneck—for example, the lengthy passages through tissue culture systems. It can take 9 months from particle bombardment to producing seed from those transformed plants. Often the whole process is limited to just one or two specific genotypes that are very favored for the tissue culture regeneration process.

Integrating genome editing and SB without tissue culture requires many technological breakthroughs, with the optimal outcomes being allelic modification without tissue culture or the application of exogenous DNA, as these could avoid the genetically modified organism label. It has been widely demonstrated that single or multiplex edits can be achieved (Zhang et al., 2016), and this could now be implemented using the following tissue-culture-free techniques. Immature embryos or protoplasts have been used as the target tissue, and ideally, this methodology would be optimized for mature seeds or germinating seedlings.

Shortcomings of SB and Genome Editing

The use of SB techniques is a prized approach to accelerate conventional breeding programs. However, the technology requires proficiency, effective and complementary plant phenomics facilities, appropriate infrastructure, and continuous financial support for research and development (Shimelis et al., 2019). Some other limitations of SB are that different plant species can have markedly different responses when exposed to extended photoperiod, phenotyping of some seed traits is interfered by an early harvest of immature seeds, there is a high initial investment and no universal protocol (Fig. 1.4). For these resources to be in place, it requires that SB approaches are recognized as essential for conventional plant breeding, marker-assisted selection, and genetic engineering. The integrated suite of tools requires proficiency in plant breeding and biotechnology, long-term funding, and government policy support. The use of modern breeding tools in the public sector is limited by technical, economic, and institutional challenges (Morris & Bellon, 2004). SB methods could accelerate the release of both conventional and genetically modified crop cultivars around the globe. Despite this, the most common challenges hampering the use of SB include (a) access to suitable facilities, (b) staff trained in the

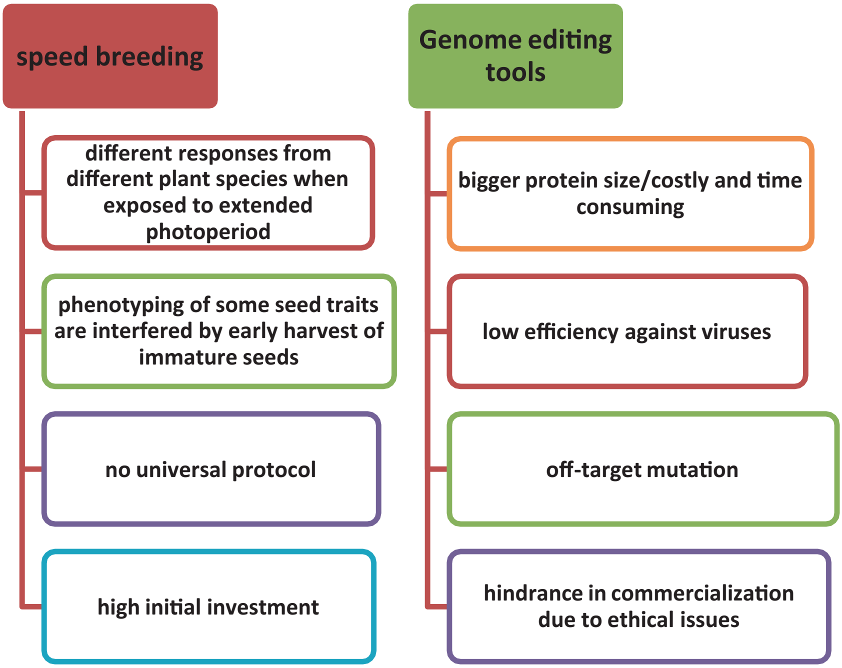

Fig. 1.4 Limitations of SB and genome editing tools

protocol, (c) adopting major changes to breeding program design and operations, and (d) the need for long-term funding.

Despite numerous agronomic, environmental, and economic assessments reaffirming the benefits that GM crops entail, there is still a polarized debate about whether countries should adopt them or not (Brookes & Barfoot, 2018; Kangmennaang et al., 2016). The safety of genome editing depends (Fig. 1.4), in part, on whether the changes are directed to predetermined sites (which would reduce or eliminate unintended changes, so-called off-target mutations) or to targets (which would eliminate unintended effects of the intended changes) (SAM, 2017; Agapito-Tenfen, 2016). The precision and efficiency of genome editing are expected to lower the frequency of some sources of unwanted downstream events, and therefore yield fewer potential hazards at the product level (SAM, 2017). Yet, for staple food crops with large and complex genomes, such as wheat, barley, or maize, off-target editing is more likely to occur (Agapito-Tenfen, 2016). Similar to varieties derived from chemical or radiation mutagenesis, unexpected risks and negative externalities (i.e., potential harm to human health and the environment) cannot be ruled out. The current knowledge about the safety of genome editing in plants is relatively limited (Xie et al., 2014; Zhang et al., 2018; Nekrasov et al., 2013; Zhao & Wolt, 2017).

In addition, current detection and identification strategies (e.g., bioinformatics) and emerging analytical tools (e.g., next-generation sequencing) face potential shortfalls. This is complicated because reliable and harmonized procedures for the detection of unwanted editing are still lacking (Agapito-Tenfen, 2016). Two main principle-based approaches are used to regulate new technologies: precautionary approach vs substantial equivalence. The contrasting biotechnology regulatory approaches exemplify two different paradigms. The EU approach, with an eclectic outlook as to the sources of risk, considers it best to wait for evidence of no risk whatsoever in order to proceed. The US approach considers impeding technology only when there is evidence of risk. The difference in approach can be partly explained by the way through which biotechnology is perceived in both jurisdictions. Despite their difference, both governance methods are limited to scientific risk assessment of human and environmental safety and are unable to resolve public and policy concerns (Hartley et al., 2016). Advocacy groups are actively engaged in debates about emerging technologies. The commercialization of GM crops has been especially controversial, with social development and environmental organizations questioning not only safety but also a spectrum of issues including ethics, monopoly ownership, corporate control of crop varieties and the food chain, consumers and farmers right to know, and the nuances around coexistence of various agricultural practices (Hartley et al., 2016; Wieczorek & Wright, 2012).

Conclusion

The challenge of food and nutritional security poses serious threats to human life and health, especially in developing countries. Over recent years, biotic (such as bacteria, insects, fungi, and viruses), abiotic (such as limited water supply, edaphic factors, and heavy metal toxicity), and climatic (such as low and high temperatures, flooding, and rainfall shifts) stresses have impacted negatively on crop production. Based on the prevailing circumstances, researchers agree that traditional plant breeding methods alone cannot achieve a sustainable caloric supply for the expanding human population. Consequently, there is a need to switch to alternative cost-effective technologies with more flexibility and reliability to boost agricultural productivity with little or no pressure on nonrenewable natural resources. Plant research, both basic and applied, has been transformed by gene-editing technology. Gene-editing methods have progressed in several ways, from mega-nucleases to CRISPR-based prime editors. Several crop lines with better agronomic features and nutritive value have been created using CRISPR-based precision gene-editing methods. We suggest that CRISPR-Cas technologies eventually achieve zero hunger goals by the end of 2030 because of their significant potential for crop enhancement and widespread implementation in agriculture. In addition to using the CRISPR-Cas system to improve crop resistance to biotic and abiotic stresses and improve grain quality, CRISPR-Cas technology has aided in the ideotyping of high-yielding varieties by improving photosynthetic performance and plant architectural features in a short period, enabling for zero hunger and malnutrition. Natural selection has constraints when it comes to reaching the aim of zero hunger. Obtaining acceptable unique features takes a long time. This can be addressed because of the recent invention of CRISPR-Cas-dependent targeted gene-directed evolution, which produces highly functional genes in considerably less time. Bioengineering based on CRISPR-Cas can also avoid natural selection. These methods could be used to develop a variety of important plant genes in order to raise yield and attain the zero-hunger mission. In a country like India, where resources are very limited, SB can be one of the most viable options for shortening the breeding cycle and accelerating the research program aiming to achieve high yields to tackle zero hunger.

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Chapter 2
Multiomics Approach for Crop Improvement Under Climate Change

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Introduction

Climate change is an outcome of the global rise in temperature due to anthropogenic emanations of greenhouse gases and extensive shifts in weather phenomena. Climate change affects the agricultural sector including crop yield, market price, and trading and ultimately impacts human health. Food security is at greater threat with variation in the frequency and severity of droughts and floods. In developing countries, adverse environmental conditions predominantly affect the agricultural yield; high temperature and superfluous CO_2 accumulation phenomenon enforced researchers to devise new strategies to address such challenges (Rosenzweig et al., 2014). Food security and human health safety are vulnerable to grave weather conditions. The development and yield of plants are significantly influenced by abiotic stresses such as waterlogging, drought, heat, cold, UV-B, light intensity, flood, gas emissions, and salinity (Ashraf et al., 2018; Benevenuto et al., 2017). Thus, new climate-smart crop cultivars are a prerequisite in the current scenario (Wheeler & Von Braun 2013). Technological approaches are vital to enable the crops to adapt to fluctuating environmental stress (Ali et al., 2019a, b). The amalgamation of biotechnologies into crop improvement is a very dynamic field. So, metabolomics, proteomics, transcriptomics, and genomics transformation may act as supportive ways for crop improvement. Metabolomics and proteomics are the study of cellular metabolites and protein expressions, respectively. Transcriptomics provides detailed information about RNA and genetic pathways under environmental stress. These techniques commonly include structure, function, evolution, mapping of genomes, and genetic manipulation (Fig. 2.1). Resistance to herbicides and insects is an extensively used genetically modified trait in crops (maize, soybean, cotton, and canola) with large markets. Although herbicide and insect resistance traits greatly decrease soil tillage and insecticide use, respectively, they require careful management to avoid the natural selection of weeds or pests resistance (Duke, 2015; Tabashnik et al., 2013; Ali et al., 2019a, b). This chapter emphasizes on impact of climate change on crop yield and improvement by multi-omics approaches and several recent applied molecular techniques to meet the standard of market demand and food security.

Climate Change Scenario and Its Impact on Crop Yield

A climate scenario refers to a probable future climate that has been constructed for categorical use in investigating the potential consequences of anthropogenic climate change. It involves an array of possible environmental as well as socioeconomic impacts (Fiaz et al., 2019a, b). More frequent and severe extreme weather events such as heat waves, acute rainfall, floods, storms, droughts, and forest fires are anticipated outcomes of climate change. Several nations heralded a new epoch of collaboration to preclude a climate disaster. Investments in renewable energy and biotechnology produced an immediate decrease in emissions of greenhouse gases

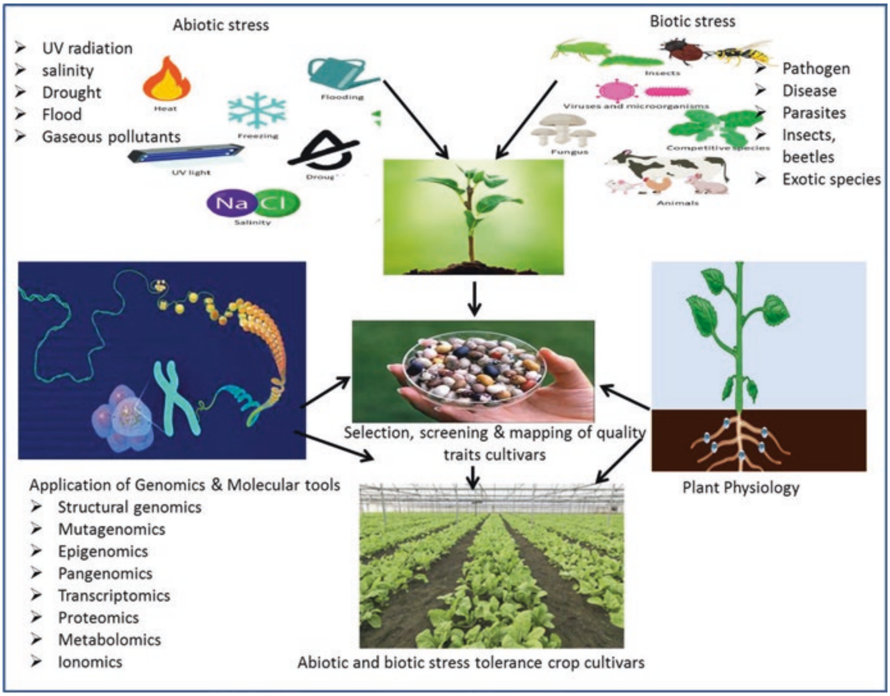

Fig. 2.1 Schematic representation genomic approaches could be employed to cultivate stress-tolerant crop cultivars

and limited global warming to around 1.5 °C above pre-industrial levels (O'Neill et al., 2017). Carbon emissions are rising, setting the stage for 5 °C of global warming by the end of the century. Here, climate scientists are required to explore diverse issues that may appear with varying levels of global warming and climatic catastrophe (Hausfather & Peters, 2020). So the development of scenarios to represent a range of upcoming future challenges would be faced by humanity. Although a momentary drip in greenhouse gases emissions from the 2020 outbreak, countries diverted to inexpensive fossil fuels to recuperate their economies postcrisis (IPCC, 2014). The major goal is to examine different environmental policies that may alter carbon and other harmful gaseous emissions, also the response of the planet to heat-trapping gas (Jeff, 2020; Hussain et al., 2018).

Prediction of climate change impact at a particular geographical location is not easy, however general adaptations are evident based on plant physiology. It includes the ability to yield and withstand: drought or flood, variations in temperature, pests, and pathogenic diseases. Net positive impact on crop growth is anticipated with the change in concentration of atmospheric CO_2 as it is an essential nutrient (Lawlor & Mitchell, 1991). Extreme weather disasters occurred during 1964–2007 reported national cereal production losses worldwide (Corey et al., 2016). Droughts and extreme heat owed a 9–10% reduction in national cereal production; however, the effect of floods and extreme cold could not be identified. Droughts are accountable for production losses due to constriction of harvested area and yields, whereas extreme heat primarily reduced cereal yields. Furthermore, the developed countries endured ~7% higher production damage from the latest droughts and 8–11% more than the developing ones. Without CO_2 fertilization, i.e., increased rate of photosynthesis, effective adaptation, genetic improvement, and surge in earth's mean temperature would decrease crop yields: wheat by 6.0%, rice by 3.2%, maize by 7.4%, and soybean by 3.1% world across. The outcomes are extremely heterogeneous across crops and geographical areas. Considering the impacts of climate on major crops on a worldwide scale, crop and region-specific adaptation strategies are suggested to ensure food security for an increasing world population (Chuang et al., 2017). A study at Maricopa, AZ, was conducted on wheat, wherein the crop was planted for a total of 12 planting dates commencing September to May over 2 years. Infrared heaters overhead the crop catered supplemental heating only for six planting dates, increasing canopy temperature by 1.3 °C and 2.7 °C during day and night, respectively. With every 1 °C rise in seasonal temperature above 16.3 °C, grain yield reduced to $42\,\mathrm{g\ m^{-2}}$ (6.9%). Supplemental heating affected the early fall plantings (Sept./Oct.) most, wherein the temperature was slightly below optimum (13.8 °C). Thus, a further rise in temperature may negatively impact the yield for late plantings and alter optimum planting windows to earlier dates in geographical regions of the world similar to the desert southwest of the USA (Ottman et al., 2012). C_3 plants (wheat and rice) are more affected by the increased CO_2 than C4 plants (maize) which have evolved mechanisms to optimize CO_2. Higher CO_2 concentrations improve water utilization tendency as fewer stomatal openings are needed for the exchange of gases. Due to climate change's impact, many regions are facing recurrent or grievous droughts and floods. Thus, improved water consumption and the ability to withstand yield under drought are vital to crop traits. Remarkable progress has been made in the regions of flooding tolerance in rice with the introduction of the Sub1 gene (Mackill et al., 2012; Xu et al., 2006; Shar et al., 2019). Escalated plant development and reduced transpiration linked to a high level of atmospheric CO_2 are anticipated to influence the nutritive content of food, with a decrease in nitrogen content in certain plant species (Cotrufo et al., 1998). A major impact may observe in developing nations wherein dietary nutrition is hitherto substandard. The assortment of quality characteristics, maintenance of yield, and increase in virulence of pests and diseases become more exigent underneath anticipated climate change scenarios (Garrett et al., 2006; Gregory et al., 2009; Batley & Edwards, 2016). These past research studies may assist to determine agricultural primacy in international disaster risk reduction and adaptation efforts.

Application of Advanced Omics Approaches

The current crop yield pattern is inadequate to fulfill global nutritional requirements by 2050. Superior and more stable crop production must be attained against a milieu of climatic stress that restricts yields, caused by alterations in pests and pathogens, extreme precipitation, and heat waves. Plant sciences may address post-Green Revolution agricultural challenges and search for emergent stratagems to enhance sustainable crop production and resilience in fluctuating climates (Bailey-Serres et al., 2019; Barman et al., 2019). Aggrandized crop improvement affects the naturally evolved characteristics and transformative engineering determined by mechanistic understanding, to confirm future yields. Notwithstanding engineered characters beneficial to farmers and end-users inclusive of virus-resistant papaya (Fitch et al., 1992), drought-tolerant corn (Castiglioni et al., 2008), rice (Potrykus, 2015) and pro-vitamin A fortified bananas (Paul et al., 2017), non-browning apples(Murata et al., 2001) and low-acrylamide potatoes (Rommens et al., 2008), the recognition of genetically modified characteristics is ambivalent in some nations, and the cultivation of such crops is majorly prohibited in the European Union (Bailey-Serres et al., 2019). The introduction of high-yielding crop varieties in regions with advanced agricultural practices has resulted in the loss of genetic variation which provides flexibility to suboptimal environments. Cultivation of crops with a greater number of resistance genes and/or planting a diversity of varieties is vital (Tabashnik et al., 2013). Evolutionary, genomic, and mechanistic findings indicate the requirement of comparatively fewer genetic components to confer nitrogen-fixation abilities. For instance, transference of nitrogenase to plants, the prerequisite constraint of genetic components was accomplished by a combination of distinguishing bacterial genetic units to build a minimal set of three genes compulsory for nitrogen fixation (Yang et al., 2018). Furthermore, specific components of nitrogenase can be firmly expressed in yeast and plants (Buren & Rubio, 2018). Legumes and arbuscular mycorrhizal fungi in association with the task of nitrogen fixation in legumes wherein many components are involved (Oldroyd, 2013) signifies that cereals have some essential fundamental units and the ability to rationalize engineering efforts to transfer the nitrogen-fixing association. This significant engineering challenge will require accurate transcriptional and posttranslational regulation of multiple heterologous genes in cereals (Julia et al., 2019).

Genomics deals with the study of genes and genomes and focuses on the structure, function, evolution, mapping, epigenomic, mutagenomic, and genome-editing aspects (Muthamilarasan et al., 2019; Gaballah et al., 2021). It has a crucial role and may explicate genetic variation, consequently improving crop breeding efficacy and genetic improvement of crop species (Rehman et al., 2021). Genomics can be classified into structural genomics, mutagenomics, epigenomics, and pangenomics as described below.

(a) **Structural Genomics**

Structural genomics includes sequence polymorphism and chromosomal organization and facilitates plant biologists to create physical and genetic maps to recognize characteristics of concern (Fiaz et al., 2019a, b). Molecular markers are relied extensively upon in structural genomics for concerned gene tagging and mapping and its ensuing utilization in crop breeding programs. The marker techniques are classified into different classes: (a) non-PCR-based techniques which involve restriction fragment length polymorphisms (RFLP), enabling detection of DNA polymorphism through hybridizing labeled DNA probe to a Southern blot of DNA digested by restriction enzymes and resulting in differential DNA fragment profile (Agarwal et al., 2008) (b) PCR-based techniques for markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), and single nucleotide polymorphisms (SNPs) (Vos et al., 1995; Li et al., 2021). Genome-wide association studies have also identified the (drought resistance) DR-related loci in rice crops (Guo et al., 2018). Moreover, frequent SNPs associated with drought-responsive TFs have been recognized using GWAS of maize crops (Shikha et al., 2017). Moreover, structural variants significantly contribute to the genetic control of agronomically essential traits in crops. The association of SVs with agronomical characteristics has been recorded in GWAS of *Bacillus napus* (Gabur et al., 2018), maize (Lu et al., 2015), and soybean (Zhou et al., 2015).

(b) **Mutagenomics**

Genetic modifications in mutant characteristics are categorized under mutagenomics. Erstwhile genome sequencing approaches, the identification of candidate genes involved strenuous techniques comprising suppression subtractive hybridization (SSH), expressed sequence tag (EST), and cDNA-amplified fragment length polymorphism (AFLP)-sequencing. Introduction of NGS eventually, has eased the tiresome approaches (Muthamilarasan et al., 2019). Reverse genetic approaches have enabled the investigation of gene function by silencing and interrupting the candidate genes. Among the reverse genetic techniques, RNA Interference (RNAi) is specifically used to screen/induce mutations in crops. Further, the approaches have been used in mutation screening in wheat, rice, maize, barley, tomato, sunflower, cotton, chickpea (*Cicer arietinum* L.), pea (*Pisum sativum* L.), and soybean crops (Dwivedi et al., 2008; Gupta et al., 2008; Tomlekova 2010). Mutagenomics is reportedly beneficial for improving crop growth, yield, and stress resistance.

(c) **Epigenomics**

Implementation of epigenomics may also prove crucial in crop improvement against abiotic/biotic stress. The unification of epigenetics and genomics known as epigenomics has gained recognition as an emerging omics technique to comprehend genetic regulation and its influence on cellular growth and stress responses (Callinan & Feinberg, 2006). Epigenetics is described as heritable changes apart from DNA sequence as a result of DNA methylation and posttranslational modification (PTM) of histones (Strahl & Allis, 2000; Novik et al., 2002). Lately, an epigenome study ascertained MANTLED locus is accountable for the mantled phenotype (hypomethylation) in the oil palm (*Elaeis guineensis*) (Ong-Abdullah et al., 2015). Whole-genome bisulfite sequencing (WGBS) identified ncRNAs in cotton crops under drought stress (Lu et al., 2017).

(d) **Pangenomics**

Mutagenomics and pangenomics are emerging omics approaches dedicated to crop sciences (Golicz et al., 2016; Goh, 2018; Muthamilarasan et al., 2019).

Pangenomics is the summation of a core genome, common to all entities, plus a dispensable genome partly shared or discrete (Tettelin et al., 2005). Various pangenomic studies on different crops including rice (Schatz et al., 2014; Wang et al., 2018; Zhao et al., 2018), soybean (Li et al., 2014), wheat (Montenegro et al., 2017), and *Brassica napus* (Hurgobin et al., 2018) have been done. The dispensable genes of a pangenome are decided by structural variation (Xu et al., 2012; Mace et al., 2013) and are enriched with genes associated with disease resistance in crops such as maize (Zuo et al., 2015) and rice (Fukuoka et al., 2009) and abiotic stress in barley (Francia et al., 2016) and sorghum (Magalhaes et al., 2007). The technique suggests the importance of dispensable genes in the maintenance of crop quality and diversity.

Transcriptomics

Transcriptomics involves the transcriptome, which refers to the complete set of RNA transcripts produced by the genome of an organism in a cell or tissue (Raza et al., 2021). Transcriptome analysis is dynamic and has become a promising technique for analyzing any stimulated gene expression within a specific time period (El-Metwally et al., 2014). Initially, traditional analyses like cDNAs-AFLP, differential display-PCR (DD-PCR), and SSH were used to analyze transcriptome dynamics, but these techniques provide low resolution (Nataraja et al., 2017).

Since then, the introduction of powerful technologies has made it possible to analyze RNA expression profiles using microarrays and digital gene expression profiles (Duque et al., 2013). Microarray analysis reveals the differential expression of soybean and barley genes at the developmental and reproductive stages under drought stress (Le et al., 2012). Similarly, the Affymetrix gene chip array was used to identify the differential expression of genes in soybeans under dehydration stress (Khan et al., 2017). The novel TFs, Cys-2/His-2-type zinc finger (C2H2-ZF) TF and drought and salt tolerance (DST) were found to control stomatal aperture in response to salt and drought stress in rice crops (Huang et al., 2009). Another study proved the function of WRKY TFs in response to wheat abiotic stress (Okay et al., 2014).

Proteomics

Proteomics is a technique involving the analysis of total expressed proteins in organisms. It is divided into four different parts, including sequence, structure, function, and phosphoproteomics (Aizat & Hassan, 2018).

(a) Structural proteomics deals with the structure of proteins to understand their assumed functions. Structural proteomics can be analyzed by a variety of methods, such as computer-based modeling and experimental methods, including nuclear magnetic resonance (NMR), crystallization, electron microscopy, and X-ray diffraction of protein crystals (Woolfson, 2018).

(b) Functional proteomics determines the functions of proteins. These functions are examined by various methods, such as yeast one or two hybrids and protein microarray analysis (Lueong et al., 2014).

(c) Phosphoproteomics aims to analyze protein phosphorylation by quantitatively or qualitatively detecting phosphoproteins and their phosphorylated amino acid residues (Mosa et al., 2017). In addition, proteomics and phosphoproteomics have been combined to study different functions of crops [e.g., wheat and grapevine (*Vitis vinifera* L.)] in response to phytoplasma and fungal pathogen (*Septoria tritici*) (Yang et al., 2013). Both drought-resistant and phytoplasma-resistant wheat varieties showed resistance (Zhang et al., 2014). Therefore, phosphorylated proteomics helps to identify crop varieties that are resistant and/or susceptible to various stresses.

Metabolomics

Metabolomics is defined as the comprehensive study of metabolites involved in different cellular events in biological systems. However, the metabolome refers to the complete set of metabolites synthesized through metabolic pathways in the plant system (Baharum & Azizan, 2018). Next-generation sequencing technology has become a promising tool to understand the regulation of gene expression and the molecular basis of cellular responses that occur in crops to deal with biotic and abiotic stresses (Abdelrahman et al., 2018). Metabolomics is particularly important in plant systems, because plants produce more metabolites than animals or microorganisms. The secondary metabolites produced by plants help cope with environmental pressures. Therefore, environmental metabolomics is a promising field in stress physiology during the response of plants to many abiotic stresses and changes in their metabolites (Brunetti et al., 2013; Viant & Sommer, 2013). In addition, many metabolite analyses related to drought, cold, and heat stress have been performed in wheat, corn, tomato, and soybean crops (Witt et al., 2012; Sun et al., 2016; Le et al., 2017; Paupiere et al., 2017).

Ionomics

Ionomics deals with the ion group, and the ion group refers to the composition of total mineral nutrients and trace elements, representing the cellular inorganic components of the plant system (Salt et al., 2008; Satismruti et al., 2013). Ionomics includes the quantitative measurement of the elemental composition of organisms and the determination of changes in mineral composition caused by various physiological stimuli, genetic modifications, or developmental conditions. Elemental analysis was also performed on tomato varieties to observe the concentration of trace and macronutrients under water stress (Sanchez-Rodríguez et al., 2010). Similarly, ionome analysis has been performed to analyze the nutritional balance of certain fruit species, including kiwi, orange, mango, apple, and blueberry (Parent et al., 2013). Therefore, these ionomics studies have shown the important role of crop improvement and response to various nonbiological and biological stimuli.

Phenomics

Phenomic is defined as the characterization of phenotype by obtaining high-dimensional phenotype data within the scope of the organism (Houle et al., 2010). However, phenotype refers to the phenotype as a whole, and plant phenotype can be determined by the interaction of genome, environment, and management (Gjuvsland et al., 2013; Großkinsky et al., 2018), and hence phenomenon is also referred to as genotype–phenotype–envirotype interactions (Zhao et al., 2019). Current research work also proposes a CropSight system based on the Internet of Things (IoT), which is used to expand and determine crop phenotypes and genotype–environment interactions. The system can perform high-quality crop phenotyping analysis and monitor the dynamics of microclimate conditions and has been applied to field wheat crop trials (Reynolds et al., 2019). In general, by combining phenomics with other omics tools, phenomics plays an important role in the development of crop-breeding strategies.

Emerging Molecular Techniques

Several developing molecular techniques are being listed here, and their applications with targeted crops are listed in Table 2.1.

Zinc Finger Nuclease (ZFN)

Generate a single mutation or short indel or introduce a new gene into a predetermined target site in the genome. Here we report a broadly applicable, versatile solution to this problem: the use of designed ZFNs that induce a double-stranded break at their target locus (Rebar et al., 2002).

Table 2.1 Emerging molecular techniques and its applications

Molecular techniques	Application	References
Zinc finger nuclease (ZFN)	Modify endogenous loci in plants of the crop species *Zea mays*	Shukla et al. (2009)
Oligonucleotide-directed mutagenesis	BFP transgene in *Arabidopsis thaliana* protoplasts resulted in up to 0.05% precisely edited GFP loci	Noel et al. (2015)
RNA-dependent DNA methylation	It is best characterized in angiosperms, particularly with *Arabidopsis thaliana*	Robert and Colette (2020)
Reverse breeding	Cucumber, onion, broccoli, cauliflower, sugar beet, maize, pea, sorghum, watermelon, rice, tomato, and eggplant	Wijnker et al. (2012)
Agroinfiltration	Increase agroinfiltration-based transient gene expression in *Nicotiana benthamiana* by improving all levels of transgenesis	Norkunas et al. (2018)
Synthetic genomics	Design and construction of a synthetic *Saccharomyces cerevisiae* genome, the Yeast 2.0 project	Sliva et al. (2015)

Oligonucleotide-Directed Mutagenesis (ODM)

Targeted mutations of one or several nucleotides are induced to occur in Cis-genesis and intra-genesis: genetically modified organisms (GMOs) are produced by inserting genetic material derived from the species itself or cross-compatible species and are contiguous and unchanged (cis-genesis) or the inserted DNA may be a new combination of DNA fragments, but it must still come from the species itself or from a cross-compatible species (Zhu et al., 2000).

RNA-Dependent DNA Methylation (RdDM)

Still improving, the modified gene expression is epigenetic, and the new phenotype has only been inherited for a few generations.

Reverse Breeding

The combination of recombinant DNA technology and cell biology procedures is used to quickly generate suitable homozygous parental lines without transgenes to reconstruct excellent heterozygous genotypes.

Agro-Infiltration

The agro-infiltration is mainly used in research environments, such as studying the interaction between plants and pathogens in living tissues, selecting parental lines, or evaluating the efficacy of transgenes. Liquid suspensions of Agrobacterium sp. contain the required genes for infiltrating plant tissues, mainly leaves so that the genes can be expressed locally and instantaneously at high levels (Norkunas et al., 2018).

Synthetic Genomics

Large functional DNA molecules synthesized without any natural templates are used to construct the smallest viable genome, which can be used as a platform for the biochemical production of chemicals such as biofuels and drugs pharmaceuticals (Chikelu et al., 2012).

Widely Studied Crop

Rice is one of the most important food crops in the world, and it is also a model system to study crop domestication (Islam et al., 2022). Though there are tons of literature discussing rice origin and domestication, the origin and history of rice domestication remain controversial. With the achievements of research on rice domestication, their applications in modern rice breeding are impressive. Genome-editing technology, which can efficiently modify target genomes predictably and precisely, is no doubt a revolutionary tool to perform molecular domestication to obtain desirable traits in the laboratory (Wang et al., 2019; Hua et al., 2019). Wild tomato has large genetic diversity and has been extensively studied to characterize certain traits favorable for breeding (Rick & Chetelat, 1995; Larry & Joanne, 2007). Potato cultivar varieties are autotetraploid and vegetatively propagated. Consequently, breeding efforts for tuber yield and quality improvement are very limited. Most potato germplasms bearing alleles controlling agronomically important traits are diploids (Spooner et al., 2014) (Table 2.2). The reinvention of inbred diploid varieties has been proposed to overcome this limitation and accelerate breeding (Jansky et al., 2016).

Table 2.2 Widely studied crops by molecular tools for better outcome

Crops	Molecular technique	Outcome	References
Rice (*Oryza sativa*)	Genome-editing technology, by editing qSH1 gene, broke down seed dormancy by knockout OsVP1, and developed superior alleles of yield genes by editing Gn1a and DEP1 genes	Reduced seed shattering	Sheng et al. (2020), Jung et al. (2019) and Huang et al. (2018)
Tomato (*Solanum pimpinellifolium*)	Edited six loci in wild tomato by genome editing	Generated highly productive progeny that could serve to breed improved cultivars	Zsögön et al. (2018)
Potato (*Solanum tuberosum*)	Edit S-RNase genes	Achieve self-compatible diploid potato varieties	Enciso-Rodriguez et al. (2019) and Ye et al. (2018)
Maize (*Zea mays*)	The endogenous maize gene *ZmIPK1* was disrupted by insertion of *PAT* gene cassettes	Resulted in herbicide tolerance and alteration of the inositol phosphate profile of developing maize seeds	Schreiber et al. (2018)

Conclusion and Future Prospects

There is a great challenge to deal with food security and climate change which may lead to food scarcity, a rise in inequality of wealth, and well-being across the world. Advances in multilevel genomics approaches have the potential to influence the production of stress-tolerant crops, improving world food security in the climate change scenario. Multiomics analysis could play an integral role in the identification of genetic processes, growth, and stress tolerance in numerous crops. Furthermore, transcriptomics and proteomics have proved to be potential tools to explicate biochemical processes and abiotic stress tolerance in some model crops. A combination of above-highlighted omics approaches might be beneficial for identifying potential candidate genes. With advances in molecular technologies, the amalgamation of such omics approaches has been promising in the agriculture field. Thus, it can be concluded that multiomic approaches with systematic biology by phenotype to genotype and by genotype to phenotype model can be helpful in crop improvement. It may improve the quality of agronomic traits for crop cultivars under environmental abiotic or biotic stress conditions.

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Chapter 3
The Intervention of Multi-Omics Approaches for Developing Abiotic Stress Resistance in Cotton Crop Under Climate Change

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Introduction

Cotton has the utmost importance in the textile industry. It provides a range of quality fibers for basic needs, from 2017 to 2018, the gross production/utilization of cotton was projected to be 25.1 million tons globally. It has been estimated that by 2026 the production could reach 26.1 million tons in totality. The genus *Gossypium* resides in species that number above 50. The ploidy of this genus has been classified into two groups—diploid ($2n = 2x = 26$) and tetraploid ($2n = 4x = 52$). Of the 50 species, 45 are diploid and five are tetraploid. Only four species, two diploids such as *Gossypium herbaceum* and *Gossypium arboreum* and two tetraploids such as *Gossypium hirsutum* and *Gossypium barbadense* are cultivated domestically. *G. hirsutum* produces high lint, but fiber size is medium and contributes approximately 90% of total cotton production due to its wide range of adaptability. However, *G. barbadense* is famous for producing superior-high-quality extra-long fiber in demand by the textile industries (Rahman et al., 2021). Thus, the *Gossypium* species are diverse and suitable model plants for evolutionary studies, polyploidy, and domestication of species. Ploidal impacts on crucial agronomical traits could also be studied such as fiber quality and gene inheritance and expression pattern. Cotton is grown in more than 90 countries worldwide. The top six countries which produce cotton are China, India, USA, Brazil, Australia, and Pakistan (Rahman et al., 2021). Cotton is a cash crop for Pakistan, and it constitutes 55% of foreign exchange, 1.5% of GDP, and 7.1% of the agriculture sector (Saeed et al., 2021).

Cotton yields natural fiber that is used in textiles. The edible oil is extracted from its seed. Cotton seed cake is a raw product obtained during the extraction of oil from cotton seed and is used as animals feed (Shuli et al., 2018). Heat stress, water depletion, and salt stress are the principal abiotic stresses which decrease cotton production, and approximately 50% decrease in cotton production is because of abiotic stress globally (Hassan et al., 2020).

Cotton production is significantly influenced by abiotic stresses, and these stresses bring a broad range of morphological, biochemical, and physiological alterations (Khan et al., 2017). Overall, abiotic stress greatly limits cotton growth, for example, it adversely decreases the number of nodes, plant height, leaf area index, fresh/dry weights (root/shoots), photosynthesis, transpiration rate, low yield, stomatal conductance, canopy, quality of fiber, and root development (Abdelraheem et al., 2019). Globally, 50–70% decrease in crop productivity is due to environmental or abiotic stresses, which is a major global problem. Consequently, climate change is further worsening the situation, so it has become challenging and problematic to feed the rising population. Several environmental factors have a negative influence on a crop's survival, developmental, reproductive, and growth processes. Environmental stresses including drought, nutrient starvation, salinity, cold, heat, heavy metals, flooding, light intensity, photoperiod, waterlogging, sub-zero temperature, gale-force winds, and elevated greenhouse gasses would have a destructive influence on crucial agricultural crops such as cotton (Saeed et al., 2021).

During the last 20 years, studies have shown that a number of genes are involved when a plant is exposed to abiotic stresses. These genes are downregulated and upregulated by fluctuating dynamic activity. As a result, numerous scientists have employed several "omics" techniques to achieve an internal image of adverse environmental conditions (abiotic stresses) and plant responses (Xiaxiang Zhang et al., 2019; Xiaodan Zhang et al., 2018). Different omics techniques are emerging as central tools for addressing and studying plant genomics and their activities, uncovering biological networks, and advancing translational research (Mehta et al., 2019). Omics is a set of studies that examines the roles and interconnections of biological data in various life clusters. It entails the investigation of phenotype (phenomics), transcripts (transcriptomics), genes (genomics and epigenomics), metabolites (metabolomics), lipids (lipidomics), interactions (interactomics), and proteins (proteomics) (Fahimirad & Ghorbanpour, 2019; Li et al., 2021). Several innovative omics technologies have been developed in recent decades. Applications of these omics approaches in different plant studies have proven and shown their efficiency in studying genetic variation, DNA methylation, protein's function, and other molecular mechanisms under stressed conditions (Muthamilarasan et al., 2019). Several studies have been conducted yet, to prove the efficiency and relation of omics approaches (genomics, mutagenomics, proteomics, transcriptomics, metabolomics, phenomics, and ionomics) in studying plant response to abiotic stress (Yang et al., 2021). The relationship (signaling) between chloroplast and nucleolus under heat stress has been proven. However, this communication network between these organelles is still not fully understood. Multi-omics approaches (especially genomics, transcriptomics and proteomics) will play a vital role in studying and further understanding this communication network for the improvement of crops under heat stress (Lamelas et al., 2021).

Next-generation sequencing (NGS) has enabled scientists to generate significant data for panomics techniques (Großkinsky et al., 2018). Several omics techniques together might unravel gene functions and networks under abiotic stresses (U. M. Singh et al., 2013). Ample knowledge of multifarious omics techniques with resilient approaches has been adopted to pinpoint and unravel quintessential factors against responses to various stress conditions, yields, and senescence in numerous significant crop plants such as soybean, millet, and wheat (Yang et al., 2021; Iqbal et al., 2022).

This book chapter gives a deep insight into multi-omics approaches and their possible implementations to enhance cotton production and increased tolerance against abiotic stresses such as salinity, heat, and drought. With the advent of integration of multi-omics approaches, it will provide a core idea to augment production, breeding science, and resistance to abiotic stresses in cotton crops.

Impact of Abiotic Stresses on Cotton

The agriculture sector is highly prone to abrupt changes in climatic conditions because it is open to the natural environment (Rosenzweig et al., 2014), such as elevation in temperature, shifts in rainfall, and the enhanced likelihood of severe weather circumstances in different regions of the world (Change, 2014), particularly in Pakistan (Nasim et al., 2016). These anticipated shifts in the weather pattern will have negative consequences on crops in Pakistan, specifically in arid and semi-arid areas (Abbas et al., 2017; Ahmad et al., 2015). Escalated temperature and shifts in precipitation could have harmful effects on cotton growth and productivity (Iqbal et al., 2016). The ultimate idea of any cotton improvement program is to achieve sustainable production of cotton. Fiber quality coupled with low yield is negatively influenced by different abiotic stress conditions (Hassan et al., 2020).

Elevated temperature coupled with persistent drought phase and saline soil are prime environmental stress conditions that bring together an approximate 50% reduction in cotton productivity (Bita & Gerats, 2013). The shift in weather is an alarming situation for the world as it will exacerbate eventually resulting in yield loss. The upland cotton (*G. hirsutum*) becomes enervated to various stresses at different developmental stages (Azeem et al., 2022). For instance, a decline in normal temperature combined with salt stress could hamper cotton growth at the germination and seedling stage (Bolek et al., 2016). The ideal growing conditions are prerequisites for maximum cotton production just like other crops. For instance, the ideal temperature for boll formation is 27–32 °C. In cotton, temperature over 36 °C is a primary limiting factor for carbon fixation while the ideal temperature for maximum photosynthesis is approximately 33 °C (Bibi et al., 2010). The membrane thermostability and photosynthesis are impeded by elevated temperature which greatly impacts plant metabolism. Protein gets denatured at high temperatures as a result the enzymatic processes of plants disrupt (Leakey et al., 2009). Abiotic stresses including salinity and drought impact more than 20% of cultivable land and around 40% of irrigated land around the world. Furthermore, salinity and extreme temperatures are thought to harm approximately one-third of the cultivable land of the globe (Hee-Dong et al., 2019).

The development and cell growth of cotton crops are influenced by drought stress because of disturbed turgor pressure. The drought stress also negatively affects photosynthesis activity and carbohydrate metabolism explicitly and implicitly. Any alteration in carbon intake as a consequence of drought stress has an explicit impact on the photosynthetic process, leading to a reduction in leaf area and boll maintenance in cotton crops. Various environmental stresses negatively impact growth, yield, and quality (Leakey et al., 2009). In comparison to this, the heat rise is a dire challenge for a few cotton-producing countries; but in areas where climate change is a problem, irrigation systems are inefficient, and fertilizer is overused, their impact will be severe (Rahimian & Poormohammadi, 2012). Cotton is a plant that grows in the tropics as a result, it is sensitive to cold temperatures. Low temperatures have been shown to lower germination percentages as well as leaf growth, resulting in necrosis and withering (Zafar & Zhang, 2021).

Physiological Responses of Cotton Toward Abiotic Stresses

Drought and elevated temperature together alter plant physiological responses such as photosynthesis rate, transcriptional expression, accumulation of lipid, and hormonal profile (Rizhsky et al., 2004). During abiotic stresses, the plant turns on its different physiological mechanisms for its survival; as result, the photosensitive activity of sensitive crops is shuttered due to stomatal closure, and also the activity of protein associated with chlorophyll and photosystem is reduced (Pirzad et al., 2011). The lower intake of CO_2 leads to a lower amount of photosynthates (David W Lawlor & Tezara, 2009), and as a result, the plant becomes vulnerable to photodamage (D W Lawlor & Cornic, 2002).

Hormonal signaling/networking pathways inclusive of phytohormones finely dictate/tune several plant responses against escalated temperatures and water-depleted conditions (Llanes et al., 2016; Peleg & Blumwald, 2011). Hormonal crosstalk and plant hormones play an important role in the molecular mechanics which optimize and regulate plant responses that occur simultaneously in the environment. The prime phytohormone that contributes to many growth stages is Abscisic acid (ABA); it is crucial in many developmental stages including but not limited to leaf senescence, movement of stomata, shoot growth inhibition, and it also interacts as a regulator in response to abiotic stress, differing signaling molecules, for example, reactive oxygen species (ROS), calcium ions, and many kinases (protein) are necessary and play a crucial role in the signal network of ABA with regards to the response of plants toward stressful conditions (drought and heat). Calcium ions and ROS also have key importance in the signal transmission of ABA during abiotic stress (Ding et al., 2013b). Enormous genes are repressed or induced under abiotic stresses (Lindemose et al., 2013). A large number of research projects have clearly shown transcription factors (TFs) like *bZIP*, *AP2/ERF*, *HD-ZIP*, *NAC*, *MYB,* and *MYC* play an important role in plant responses to stressful circumstances. As an example, researchers pinpointed and studied two drought-induced 19 (*Di19*) proteins in cotton, and they deduced that *Di19* proteins could possibly be 3′ prime end targets of ABA signaling pathways in plants suffering from abiotic stress conditions (Qin et al., 2016).

As a result, signaling from active *Di19* proteins is transmitted toward stress-related genes and ABA, boosting plant performance in stress conditions. On the contrary, different hormones, for example, auxins, ethylene & gibberellins might also collaborate to develop tolerance against stress in cotton crops. Consequently, Lu et al., 2016 ascertained that the cotton plant showed elevated expression of long noncoding RNAs (lncRNAs) during different pathways of hormone signal transduction under drought stress. Thus, ethylene and gibberellins amounts were high in these plants. This plant response might be linked to enhancing protein and DNA biosynthesis for promoting the utilization of water under adverse circumstances. Growth hormones such as auxins and cytokinins contents decrease to some extent as plant minimize their growth hormone contents and energy so they survive and go into the tolerance phase (X. Lu et al., 2016; D. Pandey et al., 2003). But a profound understanding of the phytohormones that regulate heat and drought stress responses would be a significant move for the betterment of cotton plant development under these adverse environments.

Consequently, plants that are exposed to different stresses like heat and drought along with their combined impact alter their fluorescence and photosynthesis activity. Plant development is impeded by high temperature, which reduces shoot net absorption rates; eventually, the plant's total dry weight also reduces (Wahid et al., 2007). Under heat stress, several injuries i.e., the searing of leaves and stems, leaf abscission, hindering of the shoot and root development, and damage to fruit occur causing low yield (Vollenweider & Günthardt-Goerg, 2005).

Conversely, to improve electrolyte leakage and permeability, modification occurs in membrane structure thus, due to increased electrolyte leakage and permeability cell death occurs under elevated heat stress (Abro et al., 2015). Heat stress has a direct impact on fluid leakage from the cell, which was shown to be greater in the leaves of heat-susceptible cultivars (Cottee et al., 2010). But the drought stress accelerates electrolyte leakage in the leaves of the plants (Sibel & Birol, 2007).

Usually, in response to heat and drought stress, plant modify their morphological structures such as petioles and hypocotyls. Heat and drought prolong in cotton in response to stress, just like plants respond morphologically to avoid shade for survival in heat stress conditions (Hua, 2009). These morphological changes are alterations in the cell, organelles, cytoskeleton structure, and function of membranes (Weis & Berry, 1988), thus, cells minimize the rate of normal protein synthesis, leading to an increase in the synthesis of heat shock proteins (Bray, 2000).

Just like the above changes, modifications in hormone concentrations/signaling pathways rely on hormone, synthesis of antioxidants, and defensive molecule occurs under elevated temperature stress. Additionally, chlorophyll contents (chlorophyll a and chlorophyll b) are substantial contributors to the photosynthesis process subsequently enhancing crop growth and yield. Chlorophyll contents (a, b, and total chlorophyll) and a/b ratio is dramatically affected via drought (Sabagh et al., 2020).

Salinity stress reduces the photosynthesis rate as it interferes with the photosynthetic apparatus. Osmotic stress is triggered by elevated NaCl concentration in soil, which restricts cell expansion rate and lowers stomata aperture size, as a result, the activity of photosynthesis is reduced. The initial observable symptom of salt stress is reduced leaf area. The decline in the surface of the leaf is critical because of limited cell expansion at the early stage as compared with a reduced amount of photosynthetic rate per unit area (PUA) (Shabala & Lew, 2002). Additionally, reduced photosynthetic activity is linked with reduced ribulose bisphosphate (RuBP) carboxylase efficacy due to a restricted supply of RuBP thus PSII becomes sensitive and fails to regenerate RuBP (Seemann & Sharkey, 1986). Salt stress is thought to intervene with photosynthetic biochemistry by distorting the chloroplast lamellar system, resulting in the integrity of the chloroplast being lost. Consequently, photosystem activity is reduced. The effect of salinity on enzymes of photosynthesis is subsidiary as a lower amount of carbon dioxides enters in leaves because stomata are closed. Photosynthetic enzymes are indirectly affected by salt stress because of a lower amount of CO_2 entry via closed stomata. Modification in chlorophyll structures under abiotic stresses results in low chlorophyll contents which ultimately leads to the reduction of the photosynthetic product (Meng et al., 2011).

Different varieties of cotton have shown a major reduction in chlorophyll a & b contents if salt levels increased (L. Zhang et al., 2014a). It might be due to the inhibition of certain enzymes involved in chlorophyll production (M. H. Lee et al., 2013). As a result, chlorophyll concentration might be considered a good signal for choosing salt-tolerant cultivars among physiological criteria. Carotenoids are a kind of pigment found in photosynthetic machinery that contributes to capturing light energy during photosynthesis. Under salinity, genes of carotenoids biosynthesis are not expressed properly, resulting reduction in photosynthetic rate, which reduces yield (Shah et al., 2017). Elevated salt stress significantly reduced carotenoid contents in cotton genotypes according to an early stated study (L. Zhang et al., 2014a).

However, Rafique et al. (2003) found that under salt stress, carotenoids are degraded gradually in comparison with chlorophyll contents. Moreover, carotenoids, flavonoids, and anthocyanins levels were increased whereas chlorophyll a & b were decreased simultaneously under different concentrations of salt stress. According to many researchers, a change in inorganic ions concentration has been recorded due to salt stress. The plant responds differently due to elevated levels of Na^+ and Cl^- ions as a result concentration of cations such as K^+, Ca^{2+}, and Mg^{2+} are reduced (Sharif et al., 2019). Plant physiological activities are adversely affected by elevated levels of Na^+. The availability of water is also reduced due to the excessive concentration of Na^+. In cotton leaves, higher amounts of Na^+ and Cl^- ions are found with a low ratio of K^+/Na^+ (Pervaiz et al., 2007). In salt-tolerant plants, Na^+ ions are excluded through root and susceptible plants are unable to sustain Na^+ homeostasis. Plant responses during saline conditions are governed by keeping the appropriate ratio of K^+/Na^+ ions instead of Na^+ elimination according to various studies (Dai et al., 2014).

Under salt stress, along with Na^+ and Cl^-, Mn, Zn, and N levels also elevate but P, Ca^{2+}, and S levels stay constant. However, Mg^{2+}, Fe, K^+, and Cu ions get reduced. According to Leidi and Saiz (1997), salt-tolerant plant leaves had a high amount of dry matter, water potential, and water contents instead of high Na^+ ions accumulation as compared to susceptible genotypes. These results proposed that greater Na^+ ion uptake and water content are associated with the tolerance mechanism (Sharif et al., 2019). Na^+ and Cl^- ions are rivals of nutrients such as K^+, NO^3, and Ca^{2+} thus ionic imbalance hinders nutrient availability and movement within plants, resulting in lower concentrations of N, Mg^{2+}, K^+, P, and Ca^{2+} in leaves and roots (L. Zhang et al., 2014a).

Molecular Changes Due to Abiotic Stresses

Abiotic stress has a multitude of detrimental impacts at cellular and molecular levels, as well as at the DNA and protein levels. When a cell is exposed to abiotic stress, it begins to operate abnormally, resulting in substantial molecular reprogramming at transcriptional and posttranscriptional stages (Zameer et al., 2021). Transcriptional factors are gene regulators that may control several genes at once and respond to adversity in stressful circumstances (Yamaguchi-Shinozaki & Shinozaki, 2006). The bulk of regulation takes place during transcription, posttranscription, and posttranslation; however, the transcriptional level gets the most attention since it includes modification of chromatin *architecture*, and coding regions of genes are upregulated and downregulated (M. Singh et al., 2021).

Regulation of miRNAs During Abiotic Stresses

Drought, escalated temperature, osmotic, and salinity stress have been discovered to activate various expressions of a particular array of miRNAs in different plants, like maize, *Arabidopsis,* tobacco, rice, and populous (G. Sun, 2012). However, the exact mechanism of miRNAs in cotton crops is not fully understood (Z. Yin et al., 2012). During water deficit conditions, different miRNA expressions (such as miR156, miR158, miR159, miR165, miR167, miR168, miR169, miR171, miR319, miR393, miR394, and miR396) have been reported in *Arabidopsis* (Q. Liu et al., 2009). In cotton, different miRNAs such as miR828, miR2118, miR869, miR159, miR1030, miR165, miR170, miR1884, miR319, and miR529 have been found which are activated during elevated temperature stress. The discovery of miRNAs that are responsible for heat stress has paved the road for future molecular breeding. In cotton, high-temperature responsive miRNAs can be discovered for various attributes using innovative techniques. If sets of miRNAs are overexpressed in the cotton plants via the help of modern techniques can help scientists to produce tolerant varieties against heat stress (Zahid et al., 2016).

Hormonal Regulation During Abiotic Stress

Abscisic acid (ABA) is crucial for growth and plant adjustment to adverse circumstances. Under abiotic stress, ABA controls various growth stages via production, signal transduction, and regulation (Cutler et al., 2010).

It is important to highlight a few crucial characteristics of ABA to have a better understanding of how it operates under stress. This will aid in defending yield losses and gaining a better understanding of ABA involvement in signal transmission during drought. ABA is a potent growth inhibitor that impedes cell propagation by preventing the cell from loosening before cell elongation. Moreover, it is an important contributor to signaling pathways under drought conditions. During water deficit conditions, ABA is enhanced in roots that is subsequently transferred toward shoots, where it is augmented by 50% in the leaves of the plant. Due to the elevated amount of ABA in leaves, potassium and different related ions exit the guard cells. As a result, stomata shut and thus limit the rate of evaporation (Zahid et al., 2016). The hydraulic conductivity of the roots is improved by ABA and elevates the shoot/root ratio under water-depleting conditions (Taiz & Zeiger, 2002). The comprehensive knowledge about the role and molecular characteristics of ABA may reveal the complex phenomenon of stress in cotton as well as for other crops. The understanding of this complex mechanism could help breeders to exploit germplasm for developing stress-tolerant varieties (Ali & Yan, 2012).

Calcium Signaling During Abiotic Stress

Apart from ABA, the signal transduction system in crop plants is quite complicated and various additional factors contribute too. The most significant is calcium, which has a strong feedback link with important genes that control ABA production (Murata et al., 2001). The introduction of [Ca^{2+}] cyt in guard cells is indirectly connected to the action of ABA on stomatal opening, implying that [Ca^{2+}] cyt may govern the decrease in an aperture of stomata. Furthermore, Ca^{2+} in guard cells induced by ABA has an important contribution to the transduction of signals aiming to control the functions of stomata. Former research has shown that the application of exogenous Ca^{2+} can enhance drought tolerance, maintain cell membrane integrity, decrease ROS formation, control photosynthetic activity, and biosynthesize plant hormones (Zahid et al., 2016).

Furthermore, the variety of signal transduction pathways activated by cellular Ca^{2+} under drought stress not only simply controls the physiological responses of plants but also turns on key genes accountable for tolerating the stressful environment (Tuberosa et al., 2007). As Ca^{2+} is part of signal transduction, it also protects and provides tolerance to plants against a variety of abiotic stresses. Ca^{2+} signals decoding proteins control the entire system of signaling/transportation of cellular Ca^{2+}. The modulation of stomata dependent on Ca^{2+} has been shown to increase [Ca^2+] cyt levels, which causes the adjacent guard cells to lose turgor (Zahid et al., 2016).

Type of Abiotic Stress-Induced Genes

Genes responsible for abiotic stress are split into two classes depending on the products of proteins they produce. The first class consists of genes that code for products that permit cells to fight against environmental stresses, like late embryogenesis abundant (LEA) protein, osmotic regulatory protein, enzymes producing proline, betamine, malondialdehyde (MDA), anti-freezing proteins, and osmotic regulators (Ciarmiello et al., 2011). The second class comprises regulatory proteins that have vital functions in the signaling pathways, like functional proteins, molecular chaperones, and TFs or kinases (Kimotho et al., 2019).

Role of Heat Shock Proteins During Abiotic Stress

Specific proteins aggregate in cells under elevated temperatures that protect the plants from heat shock. These proteins are called heat shock proteins (HSPs). These rescuer proteins are enormously important as it helps the crop plant to withstand adverse circumstances and to provide substantial yield. Under high stress, the pace of HSPs production was enhanced in the natural environment (Abrol & Ingram, 1996). Every time plant is exposed to high-temperature stress, it turns on its defensive system and sends signals to produce HSPs in a larger amount for defense. Cotton plants were subjected to a high temperature approximately 38–41 °C in a laboratory and a high rate of synthesis and accumulation of HSPs was recorded (Burke et al., 1985) (Fig. 3.1).

It was found from the genomic studies that HSPs expression is associated with temperature. HSPs had previously been divided into three groups based on their molecular weights: HSP90, HSP70, and low molecular weight (LMW) proteins with a MW of 15–30 kDa. When crops were exposed to elevated temperatures, they synthesized and deposited extremely high levels of LMW (200×), HSP70 (10×), and HSP90 (10×) as compared to usual. Along with their large numbers, LMWs mostly of 15–30, 70, and 90 kDa have the remarkable potential to pile up in plants at all phases of growth during high temperatures. HSPs function in achieving heat tolerance, avoiding protein degradation, maintaining cell integrity, and protecting PSII was revealed a few years ago (Vierling, 1991).

To date, overexpressing the HSPs in order to develop tolerance in plants against high temperatures has shown a low rate of success. Lee et al. (1995) effectively incorporated HSP-reporter fusion genes into *Arabidopsis;* as a result, it acquired tolerance to high temperatures. Different molecular techniques may be used to identify the genes accountable for the synthesis of these proteins, and having this information will help scientists (J. H. Lee et al., 1995).

Recently, several markers have been investigated in various kinds of cereal in order to find the flanking markers for various adaptive characteristics. Specifically, single nucleotide polymorphisms (SNPs) are present in abundance in rice and maize; both are now in common use as model plants for research studies. Different loci have been discovered via genome-wide association studies. So, breeders can manipulate traits according to breeding programs (Kump et al., 2011).

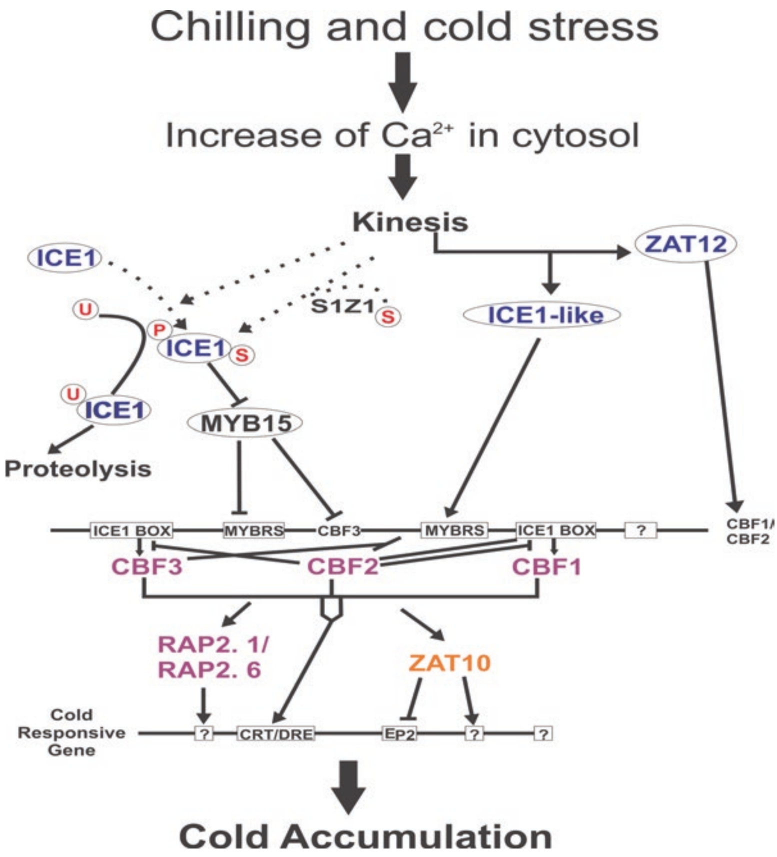

Fig. 3.1 Plant response of transcriptional network under cold stress. Cold stress regulates plant cell membrane mechanisms and other cellular changes which results in kinase protein activation which is necessary for plant tolerance to cold stress. Phosphorylation and sumoylation activate ICE1 expression at K393, which is necessary for *CBFs* transcription and *MYB15* repression. *CBFs* regulate the cold-responsive gene (COR) expression for developing resistance to freezing stress. *ZAT12* and *MYB15* negatively regulate *CBFs* expression. *ZAT10* expression is being regulated by *CBFs*, which may downregulate COR gene expression

Role of Transcription Factors Under Abiotic Stress

Transcription factors regulate normal cellular activities and also respond to different stresses. Transcription factors have a chief function in the signaling network during stress. Drought and stress-related genes are expressed via working with *cis*-acting elements found in the promoter region of stress-related genes that involves in signaling pathways. In such a manner, the signaling cascade for the whole network of drought stress-related genes is activated by transcription factors. These drought stress-related genes function together to induce tolerance in response to various abiotic stresses (M. Guo et al., 2016). Transcript factors are good choices for researchers to develop resistance against drought stress in cotton. *WRKY*, *MYB*, *ERF*, *bZIP,* and *NAC* are *TFs* engaged in both normal growth and stress response. Tolerance pathways against drought in cotton might be triggered and enhanced by the genetic engineering of genes related to TFs. Currently, a bZIP TFs gene such as *GhABF2* is identified in *Arabidopsis* and cotton, which develops tolerance against salt and drought stress. *GhABF2* regulating genes linked to ABA have been identified by transcriptomics. In domesticated cotton, CAT and SOD activities are enhanced by overexpression of *GhABF2* than in wild cotton. Furthermore, overexpressed plants performed better in the field, and their yield was greater in comparison with wild-type plants (Liang et al., 2016). GbMYB5, an R2R3-type MYB, TFs gene, was found to be favorably implicated against drought stress in cotton plants. Additionally, antioxidant enzymes and proline concentrations were improved and malondialdehyde (MDA) contents were decreased in transgenic lines as compared to wild species (Ullah et al., 2017).

Genomic and transcriptomic techniques have identified various transcription factors along with genes responsible for salt tolerance. The Son of Sevenless (SOS) gene family takes a lead to maintain ion homeostasis against salt stress amid the gene families (Sairam & Tyagi, 2004). The master regulators known as transcription factors control gene expression. There are many dehydrations responsive element binding (*DREB*), transcriptional factors families such as *NAC*, *WRKY*, *C2H2*, *bZIP* (basic leucine zipper protein) and *APETALA2* (*AP2*) that have a greater number of genes associated with stress. Scientists have examined gene expression of *bZIP* by showing that overexpression of genes in wheat that were susceptible to salt but in varieties with salt tolerance these genes downregulated (Arabbeigi et al., 2019). The overexpressed NAC family of transcription factors has been observed in rice and wheat. As a result, crops developed tolerance which aided in reducing the impact of salinity. Various kinases control certain TFs, which is very important for plant responses against salt stress. *OsRMC* (*Oryza sativa root meander curling*) has been reported in rice crops which codes for receptor-like kinases and pessimistic regulators for response to salinity stress. It has also been shown that the transcriptional level of gene *OsERBP1* is not considerably changed by salt, or ABA and extreme cold but is only weakly influenced by moderate drought and cold (M. Singh et al., 2021).

Mitogen-Activated Protein Kinase (MAPK) Signaling Pathway During Abiotic Stress

Researches on *Arabidopsis* and rice revealed that MAPKs play an important function in the signal transmission of plants against abiotic stresses like salt, drought, and oxidative and cold stress (Ullah et al., 2017). Recently, various genes engaged in the MAPK pathway have been discovered against abiotic stresses in cotton. Transcriptomics unveiled that different environmental stresses (ABA, cold, pH

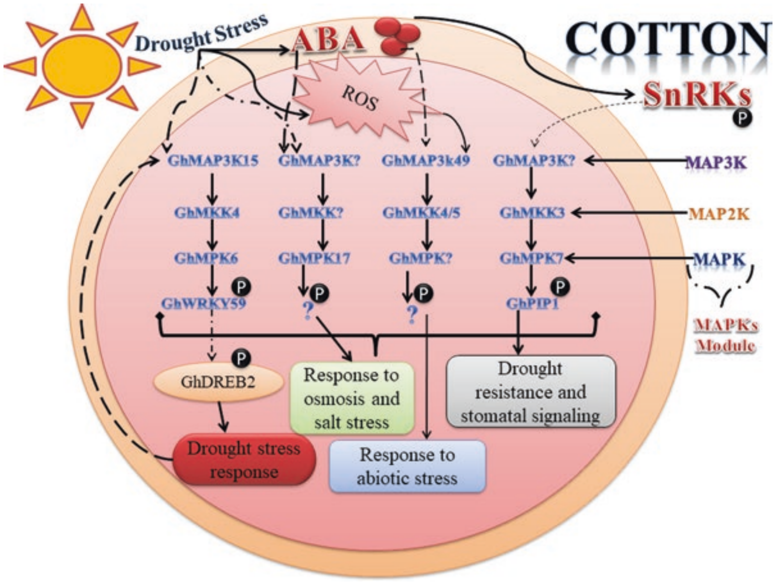

Fig. 3.2 Drought stress induces interaction among ROS, ABA, and MAPK signaling. ABA starts the regulation of different MAPK in cotton in water-deficit conditions. ABA activates and boosts the signaling pathways immediately after sensing drought stress

treatments, and drought) activate MAPK components (Y.-N. Zhu et al., 2013). In the genome of *G. raimondii* by using bioinformatics methods, 28 hypothetical MAPK genes scattered on chromosome number 11 were discovered. MAPK genes are divided into four groups: A, B, C, and D, and they serve a variety of roles (Xueying Zhang et al., 2014c). In cotton, MAPKs used in signaling pathways are engaged against a variety of environmental stressors (Ullah et al., 2017) (Fig. 3.2).

Role of Multi-Omics Approaches in Developing Tolerance to Abiotic Stresses

Genomics

Genomics is the study of the complex biological processes of a cell's genetic material, the genome, using high-throughput technologies. Whole-genome sequencing of plants became possible with the advancement in next-generation sequencing (NGS) techniques such as SOLiD (Applied biosystems), Roche, and Illumina platforms (Mochida & Shinozaki, 2011; Unamba et al., 2015). The cost of sequencing has decreased with the use of high-throughput technology, and bioinformatics analysis has become more effective for genome assembly from thousands of contigs has increased. To date, a total of 50 plant species have been sequenced yet (Unamba et al., 2015).

Advances in bioinformatics tools have made it easy for the gene identification of their regulatory elements within the genome due to the available sequenced genome. Functional genomics is used for the determination of gene functions with the help of genomic data. Comparative genomics is used in comparing the genome structure of different organisms. With the help of structural genomics, genetic loci can be determined on the genome using physical and genetic mapping. In cellular metabolism, functional genomics explores the functions of genes found in the genome. In this regard, functional genomics is the principal strategy to figure out the function of plant genomes (Rhee & Mutwil, 2014).

Genomic analysis is commonly utilized in agriculture to detect single-nucleotide polymorphisms (SNPs) via comparative genomics. The basic idea in the genomics analysis is the identification of SNPs or QTLs (quantitative trait loci) that are linked to a particular or agronomically important trait or traits. Nevertheless, due to the severe environmental impact on plants, SNPs are relatively widespread in the whole genome and generally are not able to cause an observable change in phenotype. This is of key importance to studying abiotic stress that is involving the observation of the desirable trait in plants. In this scenario, various tolerance mechanisms of abiotic stresses have already been tested under different environmental circumstances in order to limit the variations in desirable characters that are caused by genotype × environment (G × E) interaction. Therefore, GBS (genotyping by sequencing) and GWAS (genome-wide association studies) methods are usually applied in crops to find SNPs linked with abiotic stress tolerance. When there is a strong linkage between SNPs and tolerance, marker-assisted selection (MAS) helps in developing useful DNA markers for validation (He et al., 2014). In various crops, GBS and GWAS approaches have been applied to find abiotic stress-related SNPs or QTLs (Table 3.1).

The majority of studies have reported multiple (usually around a hundred) SNPs and QTLs linked to abiotic stress tolerance. However, only a few studies have confirmed SNPs and QTLs in separate genotypes. Usually, the GWAS method is used for the identification of SNPs linked with abiotic stress tolerance. Findings of this method reveal that plants can become tolerant under adverse environmental conditions due to some positive mutations in the sequence of genes. In breeding methods, QTLs and SNPs are used as primary markers for the improvement of tolerance in response to abiotic stress. The major problem with these markers is a change in observed phenotype under harsh environmental conditions. In plants, this environmental change restricts the functional validation of similar QTLs and SNPs in the selection of tolerant genotypes. Implications of climate change are the main reasons for the happening of multiple abiotic stresses at once. Such as the water level of plants can be decreased due to accelerated evaporation under heat stress, and this decrease in the availability of water ultimately leads to high salinity in crops. In other words, even if you have a salt-tolerant crop, yield loss will be an issue if it is heat sensitive. Thus, the identification process of QTLs and SNPs should concentrate on the combination of abiotic stresses, as well as possible scenarios of climate change implications at different cultivated regions (Gokce et al., 2020).

Table 3.1 Examples of GBS- and GWAS-based studies related to major abiotic stresses in crops

Crops	Stress studied	References
Gossypium hirsutum	Salinity	Cai et al. (2017)
	Drought	Baytar et al. (2018)
	Drought	X. Lu et al. (2016)
Hordeum vulgare	Salinity	Hazzouri et al. (2018)
	Drought	Merchuk-Ovnat et al. (2018)
Medicago truncatula	Salinity	Yaish et al. (2018)
Oryza sativa	Drought	V. Deshmukh et al. (2018) and Z. Guo et al. (2018)
	Salinity	Naveed et al. (2018)
	Chilling	Pandit et al. (2017)
Sorghum bicolor	Drought, chilling, salinity, heat, ABA, ROS	Woldesemayat et al. (2018)
Sesamum indicum	Drought, salinity	D. Li et al. (2018)
Triticum durum	Drought, heat	Sukumaran et al. (2018)
Brassica napus	Drought, salinity	Tan et al. (2017)
Triticum aestivum	Drought	Mwadzingeni et al. (2017)
Triticum aestivum	Drought, heat, drought and heat	ElBasyoni et al. (2017)
Zea mays	Drought	Shikha et al. (2017)
	Chilling	Shikha et al. (2017)
Glycine max	Drought	Kaler et al. (2017)
	Salinity	Zeng et al. (2017)
Medicago sativa	Salinity	X.-P. Liu and Yu (2017)
	Drought	Yu (2017)
Triticum turgidum	Salinity	Feng et al. (2017)

Millet et al. (2016) and Mangin et al. (2017) contribute with two considerations in the view of multiple abiotic stresses at one time on the effects of both stresses (drought and heat) in maize and oil content of sunflower, respectively. GWAS for the first time was practically applied as a genomics method for the development of abiotic stress tolerance in plants (Mangin et al., 2017; Millet et al., 2016). For the establishment of agricultural sustainability in the future, more research on different crops are needed.

Mutagenomics

Mutagenomics is a new omics technique for studying mutational events that comprise genetic alterations in mutant traits. High-throughput genomics tools such as TILLING (Targeted Induced Local Lesions IN Genomes), HRM (high-resolution melt), SAGE (serial analysis of gene expression), and microarray analysis can be used to analyze such mutational events (Prasanna & Jain, 2017). TILLING is generally used as a mutational technique in plants. In functional genomics, TILLING (McCallum et al., 2000) is used to induce and characterize mutations in crop plants (Mba, 2013). TILLING has quickly become a practical crop enhancement method and a viable alternative to biotech technology (Kurowska et al., 2011). The viability of this method has been demonstrated in a variety of significant crops, including wheat, rice, sunflower, rapeseed, tomato, and soybean (Kurowska et al., 2011; Witzel et al., 2015). As a result, this strategy has become very convenient for breeding programs as well as a viable approach for functional genetics (L. Chen et al., 2014).

The reverse genetic TILLING approach is used in a study to identify the mutants that control the composition of seed oil (Kumar et al., 2013). Variants abundant in oils and oleic acids have been detected in the soybean (Pathan & Sleper, 2008). TILLING method is also implemented on different crops like maize (Till et al., 2004), wheat (Dong et al., 2009), rice (Suzuki et al., 2008), tomato (Minoia et al., 2010) and barley (Caldwell et al., 2004) for the detection of mutations. Plant mutagenesis in comparison with transgene insertion caused more transcriptome alterations via multiple microarray analyses (Varshney et al., 2010).

Mutagenesis is a beneficial tool for finding genes and producing a wide variety of desirable agronomic traits (Varshney et al., 2010). All around the globe, over 3,000 different mutant varieties (776 mutants ensure the nutritional quality) of crops have been developed (Mohan Jain & Suprasanna, 2011). Mutagenomics has ensured induced mutagenesis in crop plants due to advancements in biological, functional, and breeding tools. Nevertheless, from a global perspective, several mutant traits in crops have been observed in the context of food development and quality improvement (Ahloowalia et al., 2004).

Mutagenomics has made it possible to use reverse genetic methods to mute and alter candidate genes to examine their role. Virus-Induced Gene Silencing (VIGS) and RNA Interference (RNAi) are two specialized reverse genetic methods used to screen/induce mutations in crop plants. The function of genes can also be studied with reverse genetic methods in the absence of mutant alleles (Talukdar & Sinjushin, 2015). Moreover, gene silencing and RNAi (reverse genetic methods) are applied to various crops like cotton, wheat, rice, chickpea, tomato, barley, and sunflower for the identification of mutations (Dwivedi et al., 2008; Gupta et al., 2008; Tomlekova, 2010). Thus, mutagenomics becomes a very promising technique for the improvement of stress tolerance, growth, and production of crops.

Epigenomics

The heritable changes apart from those in the DNA sequence are called epigenetics. These changes occur via posttranslational modification (PTM) in histones and DNA methylation (Novik et al., 2002; Strahl & Allis, 2000). Epigenomics is a new omics technology that came into being with the amalgamation of epigenetics and genomics. This technique aimed to figure out the regulation of genes and their effects in response to abiotic stress conditions (Callinan & Feinberg, 2006). In contrast with genomics, epigenomics is prone to environmental factors such as biotic and abiotic stress. However, genome-wide investigations might be carried out to identify anomalies caused by biotic stress (disease) environment (Muthamilarasan et al., 2019).

With relevance to PTM, the bisulfite sequencing method helps in the detection of DNA methylation in the genome. This technique has already been successfully applied to maize (Gent et al., 2013), soybean (Schmitz et al., 2013), and tomato (González et al., 2013). MSAP (methylation-sensitive amplified polymorphism) is a very commonly used technique for the quantification of DNA methylation in the genome. It has also been applied to foxtail millet and wheat under salinity stress (G. Pandey et al., 2017; L. Zhong et al., 2009). Furthermore, ChIPSeq (chromatin immunoprecipitation sequencing) is a novel method for analyzing DNA methylation and histone proteins (Van Dijk et al., 2010). It is also successfully implemented in rice in response to drought stress (Zong et al., 2013). In tomato (S. Zhong et al., 2013) and rice (Stroud et al., 2013) crops, DNA methylation research has also been conducted. WGBS (whole-genome bisulfite sequencing) revealed noncoding RNAs during drought stress conditions in cotton crops (X. Lu et al., 2017). On the whole, these findings suggest that epigenomics is very important for the improvement of crops under environmental stress conditions.

Phylogenomics

To find out the function of a gene for genome-scale data a term was coined phylogenomics by Eisen in 1998 (Eisen, 1998). Later on, this term was used in the context of phylogenetics (O'Brien & Stanyon, 1999). Scientists are working on phylogenomics to understand the functions of genomics and proteomics data on the genome level (Sjölander, 2004). Over the past two decades, phylogenomics had played an important role in DNA sequencing techniques (Metzker, 2010). Phylogenomics aims to construct the evolutionary history of an organism's genome or part of the genome (Patané et al., 2018).

LOX (plant lipoxygenase) is a nonheme iron-containing dioxygenase enzyme family, by which the oxidation of polyunsaturated fatty acid is catalyzed. LOX has been studied for biotic and abiotic stresses, but their knowledge is very limited in the case of cotton. Sixty-four LOX genes were identified from four Gossypium species after a genome-wide study. Duplication of the LOX gene was observed in the A and D genomes. Cis-acting elements were found regarding growth and stress in the promoter region of GhLOX. In response to abiotic stress such as heat stress and salinity stress, GhLOX genes were upregulated as revealed by gene expression analysis. Some genes showed particular expression in the response to exogenous phytohormones which suggests the role of these genes to cope with stress. Characterization of two genes (GhLOS12 and GhLOX13) by VIGS (virus-induced gene silencing) supported their function in salinity tolerance by regulating reactive oxygen species (ROS) (Shaban et al., 2018).

Genome-wide studies of AITR cotton genes (GhAITRs) were done with the evolutionary history of their origin in tetraploid cotton and its diploid parents. Results revealed that there is the possibility that the GhAITR gene helps in regulating plant response to drought and salt stress in cotton (T. Wang et al., 2021).

Pangenomics

Pangenome is a notion related to a species' entire genetic makeup, divided into core and accessory genes. Individuals share sets of core genes, whereas groups of accessory genes (called dispensable genes) only exist in a few plants (Tettelin et al., 2005). Dispensable genes play a vital role in crop diversification and quality improvement. Many pan genomic studies including wheat, rice, maize, soybean, wild turnip, and rapeseed have demonstrated the role of dispensable genes (Hirsch et al., 2014; Hurgobin et al., 2018; Y.-h. Li et al., 2014b; K. Lin et al., 2014; Montenegro et al., 2017; Zhao et al., 2018). Recently, pangenomics has been used to assess the genetic diversity in crop plants. Dispensable genes have more tendency of variability in comparison with core genes. Such as the study of soybean (Y.-h. Li et al., 2014b), *Brachypodium distachyon* (Gordon et al., 2017) and rice (W. Wang et al., 2018) crops indicate that higher concentration of SNPs is detected in accessory genes in comparison with core genes. In pangenome, dispensable genes are identified via structural variations (Mace et al., 2013; Xu et al., 2012). Dispensable genomes of sorghum and barley are enriched with abiotic stress-related genes (Francia et al., 2016; Magalhaes et al., 2007).

Moreover, pangenomics is also useful for the improvement of crop production. Most of the crop wild relatives (CWRs) can contribute genes for the improvement of traits, such as biotic/abiotic stress tolerance (Cao et al., 2011; D. Huang et al., 2013; Ram et al., 2007; Xia Zhang et al., 2006) and quality of grains (Campbell et al., 2016) in crops. So, pangenomic studies were probably used for the identification of novel genes in crop wild relatives for the elevation of crops (Fig. 3.3).

Transcriptomics

The term "transcriptome" is the collection of RNA copies created via an organism's genome in a cell or tissue, the study of the transcriptome is called transcriptomics (Raza et al., 2021). The expression of the response of the genes to various stimuli is analyzed with the help of a dynamic method known as transcriptome profiling (Duque et al., 2013; El-Metwally et al., 2014). This method is used for differential gene expression and provides basic information about the function of a gene. In the beginning, traditional profiling SSH, cDNAs-AFLP, and display-PCR were employed to explore transcriptome dynamics, but these methods provide a limited precision (Nataraja et al., 2017). RNAseq, NGS, SAGE, digital gene expression

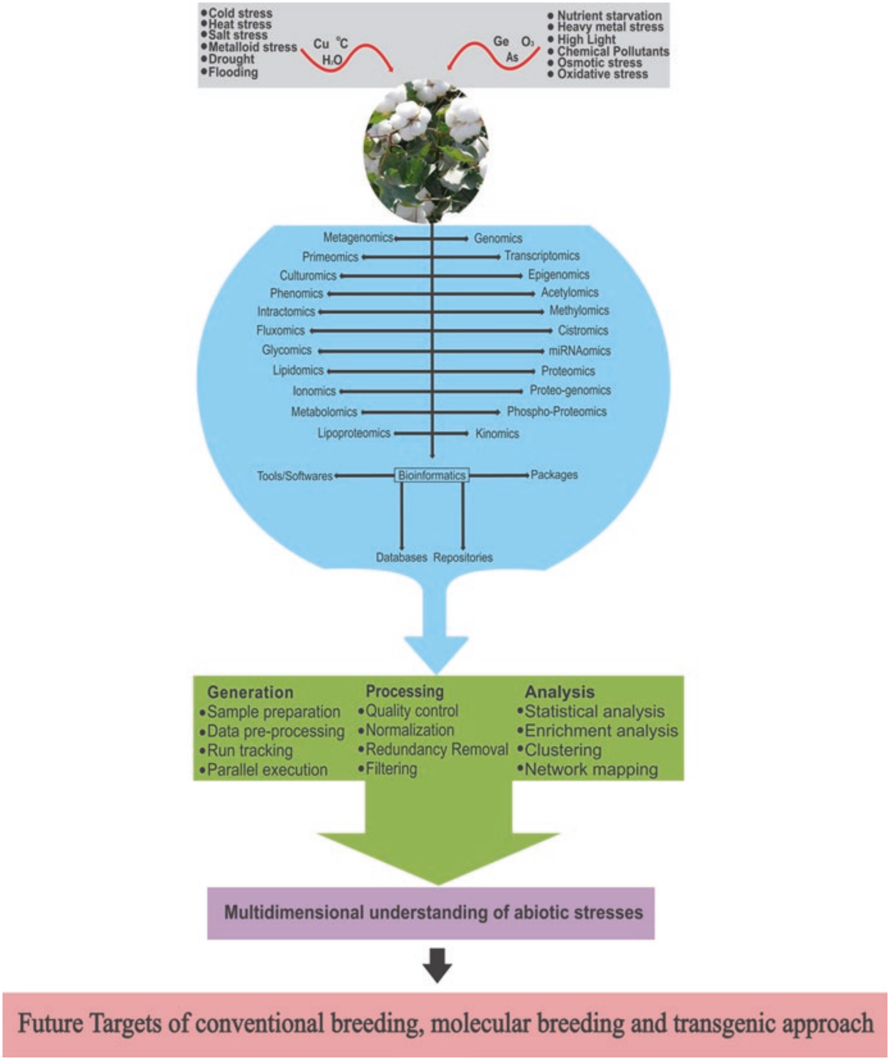

Fig. 3.3 Multi-omics approaches for the development of abiotic stress in plants

profiling, and microarray-based RNA expression profiling all were possible after the development of robust approaches (De Cremer et al., 2013; Duque et al., 2013; Kawahara et al., 2012).

Affymetrix Gene Chip array was used to identify differential gene expression in soybeans during water stress (Le et al., 2012). Similarly, microarray analysis revealed differential gene expression in soybean (Le et al., 2012) and barley (P. Guo et al., 2009) during embryonic and reproductive stages during drought stress. In rice, soybean, and Arabidopsis crop plants, the genetic expression alters several TFs under abiotic stress conditions (Wohlbach et al., 2008; Xiong et al., 2002).

Transcriptome studies in rice (Jin et al., 2013) and sorghum (Johnson et al., 2014) revealed differential expression (DE) of genes under heat, drought, and osmotic stress conditions. In the wheat crop, the role of WRKY transcription factors under abiotic stress is also demonstrated (Okay et al., 2014). These transcriptomic studies may be beneficial for the functional analysis of genes in plant sciences.

Despite the fact that noncoding RNAs, small peptides, and phytohormones are very significant in the functional regulation of genes during abiotic stress conditions in plants such as Arabidopsis (Matsui et al., 2008), tomato, wheat, and rice (Bashir et al., 2019; Chekanova, 2015). Many transcription factors (DREB2 and DRE/CRT) govern several phytohormone-independent abiotic stress responses in rice crops (Todaka et al., 2015). As a result, these studies show the role of transcriptomics in the development of crops.

RNA-seq analysis is an emerging technique in transcriptomics. This technique revealed tissue-specific expression related to biotic and abiotic stress conditions in sweet potato (Y. Lin et al., 2017), finger millet (Hittalmani et al., 2017), foxtail millet (Qi et al., 2013), and bambara groundnut (Bonthala et al., 2016). Researchers have also identified drought tolerance genes in maize (Kakumanu et al., 2012), rice (L. Huang et al., 2014), and rapeseed (Bhardwaj et al., 2015) using the RNA-seq approach.

Another tool for examining differential expression during stress conditions in various crops is comparative transcriptomics. In response to heat stress, comparative transcriptome analysis discovered sixteen combined genes in three crops (maize, wheat, rice) in comparison with the genes present in switchgrass (Ding et al., 2013a; Y.-F. Li et al., 2013). Multiple regulatory pathways in cotton (Y.-N. Zhu et al., 2013) and potato (Massa et al., 2013) have been discovered through microarray and comparative transcriptome analysis of abiotic stress. These stress-related pathways can be used as a beneficial tool for crop improvement. Alternative splicing (AS) is another method of transcriptomics for the production of various transcripts during abiotic stress conditions (Laloum et al., 2018). This approach is used in sorghum, maize, and rice under drought and heat-related environmental conditions (Chengjun Zhang et al., 2015). Thus, this technique explains the role of splicing factors that control the responses in crops during abiotic stress. Table 3.2 also describes some important applications to know the insight of abiotic stress tolerance mechanisms. As a whole, these transcriptomics approaches regulate the expression of genes that is very significant for the betterment of crop plants.

Proteomics

The process that involves the profiling of an organism's total expressed protein is termed proteomics. It is classified into sequence, structural, functional, and expression proteomics (Aizat & Hassan, 2018; Mosa et al., 2017). The arrangement of amino acids is determined in sequence proteomics. This arrangement is determined with HPLC (high-performance liquid chromatography) technique (George, 2013).

Table 3.2 Applications of transcriptomics related to abiotic stress

Crops	Technique	Results	References
Gossypium hirsutum	Comparative microarray analysis	Pathways related to abiotic stress identified	Y.-N. Zhu et al. (2013)
Triticum aestivum		Drought stress studied	Okay et al. (2014)
Panicum virgatum	Affymetrix gene chips	Heat stress studied	Y.-F. Li et al. (2013)
Chenopodium quinoa	RNA-Seq analysis	Genes identified related to drought tolerance	Raney (2012)
Eleusine coracana		Drought stress studied	Hittalmani et al. (2017)
Ipomoea batatas		Putative genes were identified	Y. Lin et al. (2017)
Cicer arietinum	NGS sequencing	Transcriptome assembly developed	Kudapa et al. (2014)
	Combined NGS and transcript profiling for GWAS	Salt stress studied	Molina et al. (2011)
	SAGE with NGS	Drought stress studied	Molina et al. (2008)
Glycine max	RNA-Seq analysis	Expression atlas of soybean genes	Severin et al. (2010)
	HiCEP (high-coverage expression profiling)	Flood stress studied	Komatsu et al. (2009)
	A custom array containing 9728 cDNAs	Differential expression of genes was identified	O'Rourke et al. (2007)
		Water stress	Le et al. (2012)
Oryza sativa	Rice oligo array	Salt stress studied	Ueda et al. (2006)
	SAGE	Differentially expressed genes studied	Matsumura et al. (1999)
	RNA sequencing mediated expression profiling	ER stress studied	Wakasa et al. (2014)

The structure of proteins is determined in structural proteomics for a better understanding of their possible functions. Experimental methods (NMR, X-ray diffraction, electron microscopy, crystallization) and computer-based modeling are techniques used for analysis in structural proteomics (Sali et al., 2003; Woolfson, 2018). The function of proteins is identified and analyzed using several techniques (including protein microarray profiling and yeast-one or two hybrids) in functional proteomics (Lueong et al., 2013). Plant proteomics can be developed with advancements in protein extraction and separation techniques (Nakagami et al., 2012). Affinity chromatography, ion exchange chromatography (IEC), and size exclusion chromatography (SEC) are examples of traditional proteomics techniques. While selective proteins analysis is done with the help of western blotting and ELISA (enzyme-linked immunosorbent assay), subsequently, the separation of proteins is done with the help of gel-based methods (SDS-PAGE, 2-DE, and 2D-DIGE). Protein microarrays are developed to detect not only protein activities but also their interactions even from a minute sample. Two-dimensional gel electrophoresis (2-DE) is used for the identification of proteins. Quantitative parameters of proteins are determined with the help of SDS-PAGE (Eldakak et al., 2013). Chromatography and spectrometry techniques are employed for the analysis of the molecular mass of the identified proteins (Fournier et al., 2007). In addition, MALDI-TOF, collision-induced dissociation (CID), and electrospray ionisation (ESI) have also been used to determine the molecular weights of proteins (Baggerman et al., 2005; McLuckey & Stephenson, 1998; Tanaka et al., 1988).

Furthermore, quantitative analysis of proteins is done with some modern methodologies including an isobaric tag for relative and absolute quantitation (iTRAQ), stable isotope labeling with amino acids in cell culture (SILAC), and isotopecoded affinity tag (ICAT) labeling. In recent times, the three-dimensional structure of proteins is identified with NMR spectroscopy and X-ray crystallography. These two modern approaches may be able to determine the function of proteins in different crop varieties (Aslam et al, 2017).

MALDI-TOF, SDS-PAGE, MS, 2-DE, and PMF are proteomics techniques used to identify the stress-responsive pathways in various crops including cotton (Deeba et al., 2012), wheat (Demirevska et al., 2008), rapeseed (Mohammadi et al., 2012), soybean (Nouri & Komatsu, 2010; Toorchi et al., 2009), and sugarcane (Jangpromma et al., 2010). Plants related to drought stress are also treated with these techniques (Ghosh & Xu, 2014).

The iTRAQ method of quantitative proteomics identifies protein's differential expression in potatoes during stress conditions (B. Liu et al., 2015). Furthermore, this method has revealed novel intuitions about somatic embryogenesis in cotton (H.-G. Zhu et al., 2018). In comparative proteomics, iTRAQ analysis determined several stress-responsive DEPs in varieties of coconut plants (Yang et al., 2020). So the abovementioned analysis plays a significant role in crop plants against various abiotic stress conditions (Basha et al., 2010).

Phosphoproteomics may be helpful in the detection of pathways related to cell function because it is linked with the functions of protein (Mosa et al., 2017). In two wheat crop varieties, phosphoproteomics with proteins discovered a substantial number of drought stress-related proteins (M. Zhang et al., 2014b). As a result, phosphoproteomics can be useful in the identification of susceptible or resistant cultivars in response to different abiotic stresses. Above all, Ghatak et al. (2017) thoroughly studied various techniques (including 2D-GE, SDS-PAGE, MALDI-TOF, ESI-IT- LC-MS/MS, and iTRAQ) in different crop plants like wheat, maize, rice, barley, sorghum, and pearl millet in response to drought stress (Ghatak et al., 2017). As a result, proteomics plays a significant role in crops against different abiotic stress conditions. Table 3.3 also illustrates some examples of proteomics studies.

Table 3.3 Applications of proteomics associated with abiotic stress in different crop varieties

Crops	Stress studied	References
Gossypium hirsutum	Salinity	W. Li et al. (2015) and Peng et al. (2018)
Oryza sativa	Drought	Y. Wang et al. (2017)
	Salinity	Chintakovid et al. (2017)
	Chilling	L. Ji et al. (2017)
Brassica napus	Salinity	Y. Yin et al. (2018)
Lolium multiflorum	Drought	Pan et al. (2018)
Lens culinaris	Salinity, drought	Muscolo et al. (2015) and Skliros et al. (2018)
Brassica juncea	Salinity	Yousuf et al. (2017)
Triticum aestivum	Drought	H. Ding et al. (2018)
	Salinity	Fercha et al. (2014)
	Heat	X. Wang et al. (2015)
	Chilling	Kosová et al. (2013)
Medicago sativa	Drought	Cuimei Zhang and Shi (2018)
	Salinity	Ma et al. (2017)
Zea mays	Drought	C. Sun et al. (2016)
	Salinity	R. Guo et al. (2017)
Allium cepa	Chilling	K. Chen et al. (2013)
Glycine max	Salinity	W. Ji et al. (2016)
	Drought	Silvente et al. (2012)
	Heat	Ahsan et al. (2010)
	Flooding	Oskuei et al. (2017)
Hordeum vulgare	Salinity	Gao et al. (2013)
	Chilling	Longo et al. (2017)
Pennisetum glaucum	Drought	Ghatak et al. (2016)
Phaseolus vulgaris	Drought	Zadražnik et al. (2013)
Pisum sativum	Drought	W.-Q. Wang et al. (2012)
Solanum lycopersicum	Salinity	Manaa et al. (2011)
	Chilling	Sanchez-Bel et al. (2012)
Cicer arietinum	Salinity	Dias et al. (2015)
	Heat	Chebrolu et al. (2016)
	Salinity	Y. Lu et al. (2013)
	Flooding	Komatsu et al. (2011)
Lotus japonicas	Drought	Sanchez et al. (2012)
Solanum tuberosum	Salinity, chilling	Evers et al. (2012)

Metabolomics

The study of small molecules (metabolites) within cells on a larger scale is referred to as metabolomics. Metabolomics in plants aims to identify the biological processes carried out at the cellular level, which provides a better understanding of different biochemical pathways and other underlying molecular mechanisms. To understand biological processes, primary and secondary metabolites are also necessary just like genes because transcriptomes and proteomes do not provide the complete information required for the practical application of omics (R. Deshmukh et al., 2014). Evaluating the functions of metabolites in plants, especially under abiotic stresses is the top objective of biotechnologists nowadays. Gas Chromatography–Mass Spectrometry (GC–MS) is being used to study the response of metabolites (Kaspar et al., 2011), CE–MS (capillary electrophoresis–Mass spectrometry (Monton & Soga, 2007) (Lee et al., 2012), and NMR (nuclear magnetic resonance) (Schripsema, 2010).

Accumulation of amino acids in leaves has been observed during the early stages of drought stress. In the late stage of drought stress, the leaves show significant enrichment of flavonoids, whereas, in the case of roots, the nucleotides and their derivatives are high (X. Zhang et al., 2021b). The tolerance of cotton to drought stress is linked with the enrichment of chitinase, callose, phenols, and flavonoids (Ibrahim et al., 2019). Proline and galactitol in cotton leaves have a positive association with yield under stress (Bozorov et al., 2018). In cotton, the polyphenol contents (a reducing agent) in leaves decrease under drought stress. In tolerant genotypes, the polyphenol levels are significantly lower as compared to sensitive ones (Yildiz-Aktas et al., 2009) thus low polyphenol content plays a crucial role in drought tolerance.

Chlorophyll and carotenoid levels were decreased during drought stress (Parida et al., 2007). According to the studies, polyphenols are associated with the maintenance of osmotic potential in cells in drought conditions. Higher salinity sensitivity is observed after the silencing of *GhERF12* (an ethylene-responsive gene) resulting in the increase of reactive oxygen species (ROS).

After silencing *GhERF12,* the peroxidase and superoxide dismutase activity declined and according to the research, the *GhERF12* gene had clear involvement in salinity tolerance (J. Zhang et al., 2021a). Salinity induces water stress and ion stress, decreases nutrient supply, and it also increases the level of reactive oxygen species (ROS). These abnormalities can cause membrane damage and DNA lesions which leads to irreversible metabolic failure or even leads to cell death (Miller et al., 2010; Noctor et al., 2014).

Ionomics

Ionomics is an approach used to detect and measure inorganic molecules having a low molecular weight and their possible function in plant metabolism at a specific growth stage and physiological state. Ionomics study is performed with the help of diverse techniques such as X-ray fluorescence (XRF), inductively coupled plasma mass spectrometer (ICP-MS), X-ray absorption spectroscopy (XAP), inductively coupled plasma-optical emission spectrometry, neutron activation analysis (NAA), and laser ablation inductively coupled plasma mass spectroscopy (LA-ICP-MS) (Satismruti et al., 2013; U. M. Singh et al., 2013). Inorganic compounds are vital as they control enzyme activity for cellular metabolic balance as well as inter/intracellular signal transmission. In this context, ionomics is critical from an agricultural aspect, particularly in smart agriculture techniques, to determine if plants can optimally utilize soil minerals and alter fertilization regimes appropriately. Ionomics provides knowledge about fluctuations in the physiological state of the plant against abiotic stress. Plant ionomics has only a few reports in the literature demonstrating its effectiveness in discovering novel gene functions, changing the nutritional profile of crops via smart agriculture approaches, and figuring out variations in the plant's ionome profile in response to various environmental conditions (Baxter, 2015; X.-Y. Huang & Salt, 2016; Watanabe et al., 2016).

However, efficient utilization of data acquired from ionomics studies, data integration on individual inorganic molecules, taking into account their interactions with each other and other subcellular molecules, and how plant change their phenotype in response to fluctuated environmental conditions is yet mandatory. It is obvious that the ionomics method has not been successful in determining the critical factors that allow field crops to withstand abiotic stress conditions on their own; nonetheless, additional research is needed to unravel the function of trace elements against abiotic stress.

Lipidomics

Lipidomics intends to study lipids within the cells using mass spectroscopy-based techniques to detect lipid compounds, concentrations, and changes to uncover their roles in membrane structure and production (Gross, 2017; Tenenboim et al., 2016). Lipids are used in membrane formation, signal transduction, and subcellular transportation. From an agriculture point of view, lipidomics helps to alter lipid composition to enhance abiotic stress tolerance if the functions and properties of all the lipid molecules that change during environmental stress conditions in the plants are well understood.

However, most lipidomics research has been done on *Arabidopsis thaliana*. So, there is still a long road ahead in terms of resolving issues such as lipidome isolation standardization, advanced data processing system, availability of suitable internal standards, and extensive use of high-throughput methods (Tenenboim et al., 2016; Welti et al., 2007).

Cytogenomics

A cell is the structural and functional unit of all living organisms; the branch of biology that deals with a cell is called cytology. The beginning of cytology as science started in 1839 after the discovery of cell theory (Buja, 2021). Cytogenetics refers to the study of the structure, function, and location of chromosomes in the cell. It also involves the study of chromosome appearance, number, and location of genes on the chromosome (locus) and behavior during cell division. The discovery of the chromosome made it possible to study and answer various questions regarding heredity. Today different techniques are available to study chromosomes, their function, and behavior within the cell and next generations, but the applications of omics in studying chromosomes opened an efficient and effective field known as cytogenomics. The term cytogenomics covers conventional and molecular cytogenetics (FISH, microarrays) as well as other molecular techniques (Silva et al., 2019). Cytogenomics microarray offers a basic strategy for evaluating chromosomal changes at a higher level. A reliable, efficient, and simplest way to determine chromosomal aberrations at high resolution is the application of cytogenomic microarrays. The microarray can detect chromosomal aberrations and provide accurate data. Today, cytogenomics is being used widely to study chromosomes and therefore replacing all the previous techniques used in chromosomal studies (Iourov, 2019).

Cotton yield has been drastically decreased by drought stress worldwide. Drought stress not only reduces the cotton yield but also reduces fiber quality. It is the requirement of time, to protect the cotton crop from abiotic stresses through the introduction of novel varieties that possess resilience and perform better under harsh environmental conditions. Association mapping gives a brief understanding regarding gene controlling traits related to drought and is the most applied example of cytogenomics in the recent era. An experiment was conducted on association mapping of traits related to drought in 99 cotton genotypes along with 177 simple sequence repeats (SSRs) markers. A total of 30 SSR markers loci were identified as associated with drought-related traits (Baytar et al., 2018). Due to continuous selection in the upland cotton genotypes, the genetic material of cotton has been greatly narrowed. Wild progenitors of *Gossypium* species have known to possess unique traits which could be exploited and inherited by elite varieties through hybridization and domestication to develop tolerance to abiotic stresses such as drought (Magwanga et al., 2018). A genetic map composed of 10,888 SNPs was constructed through genotyping by sequencing (GBS) for a population of *Gossypium Tomemtosum* (male parent with drought tolerance) and *Gossypium Hirsutum* (female parent sensitive to drought). As resultant information can be exploited in future projects (Magwanga et al., 2018), cytogenomics now has become an integral part of genomics. With the ease of PCR-based markers and association mapping technology today, it is possible to generate more reliable data based on which we can direct crop improvement projects more precisely and efficiently.

Phenomics

Phenomics was first introduced by Schork in 1997 and was characterized in resemblance to genomics. Phenomics deals with the phenotypic analysis of an organism by assessing all the qualitative and quantitative traits of the organism and its association with genetic and environmental factors (R. Deshmukh et al., 2014). Phenomics is a new field that has drawn extraordinary attention. But still, a lot of work is required to catch up with other disciplines such as genomics (Houle et al., 2010). Variation in the phenotype of an organism is the result of the dynamic interaction of the organism's genotype with the environment. Phenotypes often help us to figure out the potential alleles controlling phenotypic and pleiotropic factors (Houle et al., 2010), which can be exploited to improve desired traits, like stress tolerance, increased yield, and wider adaptability. In response to climatic conditions, highly efficient programs have been developed to study crop improvement. One of the efficient applications of phenomics i.e. modern high throughput phenotyping (HTP) techniques used to find out the cause of variation, plant developmental behavior, and association among traits (Brown et al., 2014).

Conventional phenotyping has many setbacks as it is labor intensive, has low throughput, and is time consuming. Getting a precise phenotype is one of the major hurdles in modern breeding (Furbank & Tester, 2011), but high-throughput phenomics technology has the power to overcome the drawbacks of conventional phenotyping. Automated imaging methodologies can be used to assess quantitative parameters of plant phenotype such as growth, stress tolerance, and yield (L. Li et al., 2014a). Phenomics provides enormous knowledge regarding biochemical and biophysical processes of a cell being combined with other omics approaches. The phenotype of the plant is the interaction of the plant's genotype with its environment (Bilder et al., 2009). HTP allows us a rapid and accurate phenotypic evaluation of large germplasm in a very short time. Bridging a gap between genomic and phenotypic data is of great concern for plant breeders and biotechnologists nowadays.

Panomics

Recently, the idea of panomics was given by Weckwerth et al. (2020). Panomics is the amalgamation of omics techniques, including genomics, transcriptomics, proteomics, metabolomics, and phenomics (Weckwerth et al., 2020). The basic idea behind panomics is to construct models that can directly predict complex traits through the combination of various omics techniques (Weckwerth, 2011, 2019).

In functional omics, multiple data integration along with panomics and environmental information can be applied for the detection of genes, markers, and QTLs. This data integration is also used in the construction of mathematical models that are further applicable to increase biotic and abiotic stress tolerance and for the improvement of cultivars (Weckwerth et al., 2020). The integrated data is further analyzed with the help of multi-omics tools, including KaPPA-view (Tokimatsu et al., 2005), COVAIN (X. Sun & Weckwerth, 2012), and PAINTOMICS (García-Alcalde et al., 2011). These tools are used to merge multi-omics datasets before any interpretation (Kuo et al., 2013).

Phenotypic variance in crop plants can be identified with the combination of panomics and GWAS. Significantly, the integration of omics datasets with GWAS can result in the detection of functional pathways related to complex traits and novel genes (Weckwerth et al., 2020). In maize crop, an integrated study "metabolome-based genome-wide association study" was performed for the detection of metabolite features related to kernel weight (Wen et al., 2014).

Moreover, precision breeding could also be improved through the combination of genome editing techniques including CRISPR/Cas9 and TALENs and panomics (Weckwerth et al., 2020). Currently, agronomically significant genes are determined through genomic selection techniques that interpret only ~40% of the phenotypic variance. But, the abovementioned method (combination of genome editing tools and panomics) could lead to the detection of the remaining ~60% of the phenotypic variance. This methodology can also become effective for the identification of agronomically significant genes. As a result, this methodology is very vital in the improvement of various cultivars (Weckwerth et al., 2020).

Role of Bioinformatics in Multi-omics Strategies

Bioinformatics is an amalgamation of various fields including biology, mathematics with statistics, and computer science. It is a practical approach to computational biology. Bioinformatics facilitates researchers in handling and analysis of biological data with the help of computer-based software (Raza et al., 2021; Dormatey et al., 2021). Especially in plants, the insights of molecular processes related to abiotic stress are determined with the assistance of panomics (integration of omics approaches) (Cramer et al., 2011; Jogaiah et al., 2013). When panomics combine with bioinformatics, it becomes vital for understanding the mechanisms of abiotic stress in crop plants (Ambrosino et al., 2020). Hence bioinformatics along with panomics plays an important role in data analysis.

Moreover, bioinformatics is also involved in the functional interpretation of genes. The integration of multi-omics approaches along with bioinformatics also provides a platform for computational modeling and simulation. Improvements in multi-omics platforms and recent bioinformatics tools (software packages and databases) are applied for multidimensional research in crop plants. These tools are not only intangible but also applied for the storage and analysis of data. Various multi-omics databases including KEGG, KomicMarket KNApSAcK, KOMICS, PlantTFDB, and PMND deliver information related to panomics for various crop plants. Furthermore, specialized or personalized databases are also present for significant crop plants including TAIR (arabidopsis), CottonFGD (cotton), MaizeGDB (maize), RiceXPro (rice), CerealsDB (wheat), TFGD (tomato), and SoyKB (soybean). The abovementioned databases give detailed information about plants that lead to multidisciplinary research. Recently more than 50 databases of plants have been available (Yang et al., 2021).

Several software packages are also available for multi-omics analysis, such as BioLeaf, EasyPCC, STRING, and GSDS. For individual omics analysis, various software packages are also available. This software includes LemnaLauncher (phenomics), SPPS (functional proteomics), VISTA (structural and comparative genomics), and AMDIS (ionome-integrated metabolic component analysis) (Yang et al., 2021). Thus, the abovementioned discussion demonstrates that bioinformatics plays an important role in the improvement of crop plants in response to various stress conditions.

Conclusion

The shift in climate is the root of all environmental stresses. It greatly impacts cotton growth and production worldwide. Cotton is a cash crop of Pakistan, but due to climate change, the yield of the cotton crop is adversely reduced thus affecting the economy of the country. Furthermore, abiotic stress impair the developmental mechanisms leading to reduction in quality of crop. Consequently, it is very important to investigate the stress-related mechanisms to increase cotton yield and quality under adverse environmental conditions. Omics tools have emerged as marvelous applications to induce tolerance against abiotic stresses.

Omics strategies have been widely applied to clarify the complex mechanisms underlying in response to abiotic stress tolerance in cotton plants. Different omics studies have provided large data about panomics (genomics, proteomics, transcriptomics, and metabolomics) to reveal different mechanisms (molecular, physiological, and metabolic) against abiotic stress tolerance in cotton. It has opened new avenues for the future cotton breeding programme. However, scientists need to work on stress-related genes responsible for developing tolerance in cotton plants. So, there is a need to develop cotton varieties that can survive with changing climate by integrating speed breeding, genome editing techniques, bioinformatics approaches, and panomics.

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Chapter 4
Big Data Revolution and Machine Learning to Solve Genetic Mysteries in Crop Breeding

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Introduction

Intelligence is a crucial aspect of life and is the reason *Homo sapiens* are termed as a superior race in comparison to every other life form. Over the passage of time, humans have developed a large number of machines that can be used for performing certain tasks ultimately aimed at making life more comfortable and more accessible in all sorts of matters. In this regard, modern-day computers have been a revolutionizing technology that paced up the evolution of mankind, and with the advent of modern artificial intelligence (AI) technology these machines are becoming more and more integrated into our daily life system. In general, AI is defined as certain computer systems which can augment human intelligence in order to perceive and analyze certain data packets and deduce conclusions from them for taking actions in response to them (Bini, 2018). This simply depicts that AI consists of a network of computer systems that can think much like the human brain, enabling machines to take actions for maximum possible success under various conditions (Poole et al., 1998).

From the early days of evolution, mankind has made consistent efforts to improve and facilitate their everyday life, from the discovery of the secret for growing their crops and food, to the discovery of the first steam engine. These monumental achievements were a huge turning point in human history as it leads to the initiation of the first industrial revolution in the mid-eighteenth century followed by transition into the second industrial revolution in the early twentieth century and later on the initiation of the third revolution in the mid- twentieth century. This revolution is often referred to as the digital revolution involving massive engagement in computer and internet-based tech industries in which machines and, in particular, computer systems have played an important role in shaping human life. Now, the world has entered the early phase of the fourth industrial revolution in which digital, physical, and biological fields are integrating for a better and more sustainable future (Xu et al., 2018). In this combination, modern AI and machine learning (ML) approaches are crucial elements for better understanding the secret of life and cosmos and how to unravel and utilize this knowledge for further development.

The concept of AI has been around for a while; British Mathematician and computer scientist Alan Turing presented the concept of basic computing sciences in 1930s in which he explained that when supplied with a set of instructions (program), a universal machine or a computing machine is capable of computing, analyzing, and performing certain types of function to great extent without an error in it (Turing, 1937). Furthermore, in 1950, he presented the hypothesis of machines having the ability to think which very much lead to the foundation of the concept and field of AI. In 1976, Jerrold Maxmen predicted development of certain intelligent machines in the coming century which will be able to perform many of such tasks commonly dependent on human thinking and perception. These machines will not just be able to carry out such functions but will also be intelligent enough to outsmart humans in performing many of these tasks (Maxmen, 1976; Naylor, 2018).

Although the concept of AI has been around for nearly a century, it is only until recently that it has become a major topic of conversation. A key reason behind this is the convergence of three main elements together; this includes the massive amount of large data sets (the Big Data), powerful advanced computer systems, and modern algorithms developed over decades of time to make AI a success story of today. Initially, the AI pipeline begins with a large amount of structured or unstructured data packets collected from various sources ranging from sensors to software systems, these large packets of data are then subjected to data refining, screening, and assembling, making information more accessible and easy to interpret. This processed information is further subjected to a series of algorithms that focuses on converting this information into the system's knowledge and utilizing it for generating response closely related to human intelligence and using for target missions or operations (Fig. 4.1 AI operational pipeline).

Machine learning (ML) is a key subset of the field of AI which mainly carries out the role of experimental learning by using a complex mixture of algorithms and big data sets. ML algorithms utilize a significantly large proportion of complex data sets to identify input and output patterns for effective learning strategy development (Haeberle et al., 2019). There are two main categories of ML: supervised and unsupervised learning; in the former kind, system algorithms are supplied with a certain set of data packages and are trained to perform the entire operation in a designated pattern to reach a specified conclusion, whereas in unsupervised learning, algorithms are not assigned with any previously mentioned standards instead the unsupervised algorithm randomly goes through all data sets and derive a certain naturally occurring conclusion from it. These AI- and ML-based approaches are now heavily embedded into our daily life system, continuously recording data related to personal preferences and choices, leading to assistance in daily life chores, from Apple's Siri

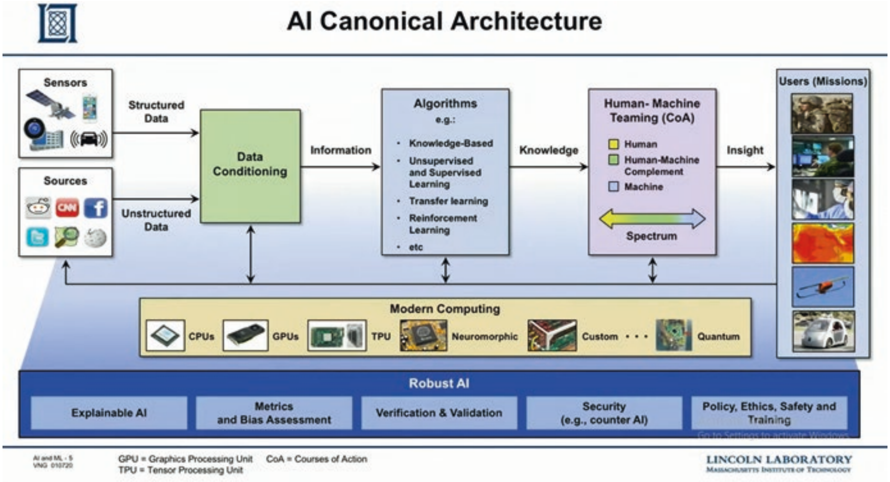

Fig. 4.1 AI operational pipeline. (*CPU* central processing units, *GPU* graphic processing units, *TPU* Tensor processing units, *CoA* Course of Action)

to Amazon's Alexa system and video ranking algorithms in online streaming and e-commerce websites, AI has now ingrained in every aspect of society and continues to operate in the background all time (Bini, 2018).

Just like in many other walks of life, the ML approaches are now also entering a critical phase within the field of agriculture and utilization of ML approaches for better processing of data and effective decision making.

ML and Big Data in Agriculture

Conventional breeding practices have been utilized for thousands of years in order to develop new crop varieties. Conventional plant breeding was greatly focused on ancient practices involving intense labor activities where plants with certain high-class physical traits were selected and subjected to crossing for preparing new and more sustainable crop types (Wang et al., 2018). Starting from around 10,000 BC, human efforts were mainly focused on randomly selecting various crops subjecting them to domestication and later on relying on certain natural mutations for trait enhancement and improvement of nutrition profile, this was often termed as the first phase of the crop breeding revolution (Sun et al., 2021). Fast forward to the nineteenth century, the discovery of Mendelian laws and chemical radiation-related technologies gave rise to cross-breeding and mutation breeding technologies, aimed at introducing desired traits at a faster pace, this entire period is commonly defined as the second breeding phase (Wallace et al., 2018). From the mid-twentieth century onward, the field of biology turned over a new page of scientific success with the discovery of the double-helical structure of DNA (Akther et al., 2021), this led to the foundation of molecular and genomic techniques and initiated the molecular breeding era. Marker-assisted breeding techniques were helpful in understanding genetic makeup and estimating the location of genes, and these techniques were of great success as the probe markers were highly specific to target genes and were instrumental in assimilation of multiple high-value traits in a single cultivar of the crop (Valenzuela et al., 2021). However, a major challenge faced in such approaches was the large demand for specific probe markers to cover the entire genome as they were highly specific in their attachments (Collard & Mackill, 2008). Genetic and molecular breeding also gave rise to the possibility of the creation of genetic combinations from multiple species, the result of such efforts was Bt cotton and other transgenic crops (James, 2004; Azeem et al., 2022). Reduction in the cost of sequencing technologies in integration with ever-improving statistical modeling is now equipping us with possibilities of massive genome-wide association studies (GWAS) (Rehman et al., 2021). These approaches are highly effective in the estimation of causal genes for numerous traits in complex crops in addition to this GWAS studies also enable the detection of single nucleotide mutations (Zhang et al., 2016), this is termed to be the third breeding revolution.

Now, we are in the fourth breeding revolution phase where an overlapping approach of data collection, analysis, and detection is being utilized in combination with the latest genetic engineering tools for precision breeding practices. Technologies like CRISPR are now used for efficient targeted editing of traits in crops. These efforts are supported by massive sequencing data powered by NGS technologies, enabling on-spot modification of traits and enhancement of crops in a short time (Eriksson, 2019; Chen et al., 2019).

Historic plant breeding efforts were of great success and provided updated versions of many crops including maize, rice, banana, and wheat, and these efforts were instrumental during the green revolution era and securing sustainable food supplies for the world population over a large period (Evenson & Gollin, 2003; Gaballah et al., 2021). Now the modern set of challenges is making old or a single kind of technologies difficult to keep up with ever-increasing demands for food and feed worldwide and thus requires innovations to resolve the issues (Fiaz & Wang, 2021). Challenges like climate change are of major concern for world food security and receding into compromised food supplies and increasing pressure on global food chains. This infers both biotic and abiotic challenges for plants making it ever more difficult to obtain effective yield outputs from desired crops (Younas et al., 2021). Multiple climate factors like rising temperatures, increasing intensity and frequency of extreme weather events, heat waves, and drought stress are some of the major climate-oriented challenges, and their influence can vary greatly from time to time owing to different spatial patterns, topography, and geographical attributes of a location (Ackerly et al., 2010; Gornall et al., 2010). Lower availability of water can lead to reduced supply of both water and minerals to plants imparting a negative impact on plant metabolic pathways, this ultimately leads to lesser biochemical activity in cells and slows the overall growth rate of the plant ultimately leading to lesser flowers, pods, and grains or fruit on plants. In a related manner, higher climate temperatures and heat waves tend to harm the productivity of the plant, estimate shows 1 °C rising in seasonal temperature can reduce the production of crops like wheat, maize, and soybean ranging anywhere between 3% and 7.4% (Rivero et al., 2007; Greven et al., 2009). Another major factor having a negative impact on growth and yield outputs is frequent extreme weather which tends to implicate physical harm to crops, although excessive water can be useful for some crop types, alongside this increase in levels of relative humidity can cause certain disease outburst rendering crop fields disease-ridden and making all product unfit for human consumptions (Rosenzweig et al., 2001).

In addition to abiotic stresses, biotic stress is also on the rise in damaging crop plants under rapidly changing climatic scenarios. The rising level of carbon dioxide is another major climate change concern and has been associated with enhanced susceptibility of crops to pathogens under high CO_2 concentrations in air (Trebicki et al., 2015). In addition to this, high humidity and temperature cause pathogens to complete a higher number of growth cycles in a quick fashion enabling crop infection at massive scales, estimation shows that pathogens like Fusarium and Septoria will spread to a large proportion of Asia, Europe, and Africa owing to rapidly changing climatic pattern; furthermore, weed incidence and insect attacks all will be more prevalent by the mid of twenty-first century (Shabani et al., 2014; Garrett et al., 2016).

Developing new crops which can grow in ever-changing environment is an uphill task and will require a combination of many existing modern tools to resolve the issue. Over the last two decades, the amount of data floating within the field of life sciences (including plant and agriculture sciences) has increased exponentially, mainly due to improved sequencing technologies, reduced costs, and high-throughput phenotyping (HTP) data in combination with modern sensors and drone technologies has ultimately provided with a swarm of data. A key challenge now lies in finding useful proportions in data, its interpretation, and using it for achieving targeted goals. Integrating all these genomic, phenomic, and environmental data sets, processing them, and deducing a conclusive result is a task far beyond normal human capacity. In this regard, modern computer systems, data management, and ML algorithms are keys to resolving these mysteries (Cobb et al., 2019).

Now, ML is the science of making computers able to learn from large sets of data and is now being used greatly in the field of plant sciences for interpreting large genomic and phenotypic information. The most commonly used ML algorithms are supervised, unsupervised and reinforcement learning algorithms; among these, the supervised learning algorithm has been reported to be more suitable for life sciences research and is used for both the predicted numeric and nonnumeric problem resolutions (Zheng et al., 2018; Wang et al., 2020). These ML approaches can analyze different sort of data ranging from imagery data to genomic sequence data and interpret it for detecting disease patterns, transposable genetic patterns or growth patterns in plants, etc. These algorithms are now being utilized by the research community around the globe for better assessment of data and precise decision-making for the development of sustainable crops (Hu et al., 2018; Orozco-Arias et al., 2019) (Fig. 4.2).

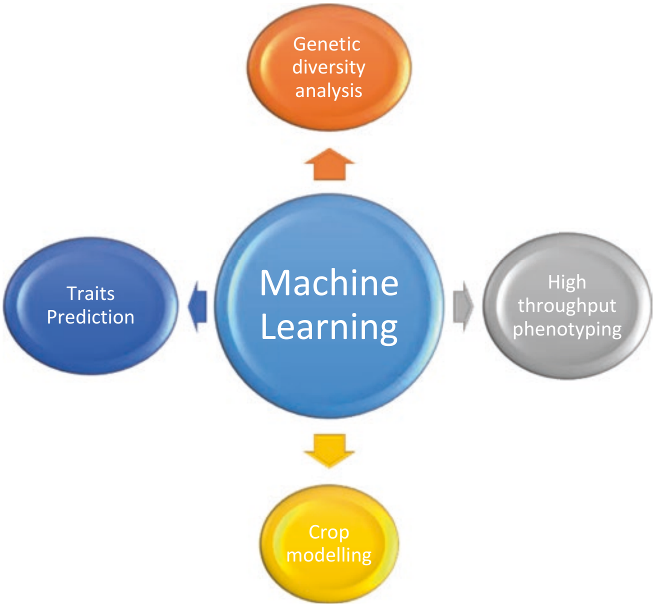

Fig. 4.2 Application of machine learning in plant sciences

ML and Its Impact in the Field of Genetics

Genes are center to every kind of study in life sciences as these are tiny bits of code present inside cells responsible for governing all tasks that are crucial for sustaining life. The genome is simply defined as the entire sum of the genetic material present inside the cell (Alberts et al., 2002). The study of the genome (genomics) is a rather broad field as it includes larger sets of genes, along with studies involved in understanding the function of these genes, how various genes interact with each other, what kind of genes act in a close cluster to each other sharing a common biochemical path in a cell, what type of regulatory sequences are present in the whole genome and how each of the genes is influenced by them, and profiling of corresponding proteome and metablome for functional studies of gene (Hieter & Boguski, 1997). These studies are also crucial in establishing the necessary connection between genotypic and phenotypic responses, discovering novel biomarkers, and screening various transcriptional enhancers (Ozak et al., 2002; Golub et al., 1999).

A genomic data set is generally too large and complex to be assessed visually for finding a relationship and thus requires assistance in form of analytical tools to carry out predictive analysis and draw productive conclusions (Ahmar et al., 2021). ML algorithms are an important tool in this regard as these algorithms can be designed and trained for the detection of trait patterns in genomic data (Libbrecht & Noble, 2015). A major issue with genomic data occurs with its preprocessing stage which is an error-prone and time-consuming activity. ML approaches offer a solution to this by embedding several layers of data processing into a single one and defining features of the model to attain desired results. Similarly, it also plays an important role in foretelling gene function and allowing the prediction of protein structures (Al Quraishi, 2019). In addition to this, ML model also enables real-time analysis of heterogeneous genomic data which includes single-cell RNA sequences and imaging data assessment simultaneously, and this enables real-time spatial recognition of a particular functioning cell and its location within the entire tissue system (Al Quraishi et al., 2019). Sequence analysis also requires certain mathematical and statistical formulations for data assessment, such technicalities are often laborious and time-consuming; however, ML can perform this reconfiguration for different type of data sets in real-time enabling rapid configuration of genomic data and extraction of necessary sequence related information. The immense amount of data being generated on a consistent basis from ever-improving sequence technology along with evolving ML approaches will soon be a part of everyday scientific analysis for predictive effects of genomic elements (Eraslan et al., 2019).

ML Approaches in Plant Breeding

Selection of high-yielding desirable plant cultivars is a major issue for the modern-day food security: on one side, there is a continuously rising challenge of climate change, and on the other end, a massive amount of data is to be assessed for trait screening and selections. Over the last half-century time period, increasing technological prowess has resulted in generation of a large swarm of data, including massive omics data (genomic, transcriptomic, metablomic, etc.), high-throughput phenotypic data, environmental data, and most recently pan-genomic data (Li et al., 2021) To deal with these rising challenges, ML approaches provide a potential solution for assessment of data and resolving breeding related solutions.

ML for Genetic Selections

Global population is increasing at a great pace with populations set to reach about 9.5 billion by 2050, indicating an inevitable higher food demand. For the satisfaction of these demands and to avoid catastrophic events, there is a dire need for increasing global food supplies (Foley et al., 2011; Dubey et al., 2019). In the meantime, climate change is another major challenge; fluctuating climatic responses are making it difficult for plants to assess their environment causing difficulties in attaining optimum production outputs. Yield and qualitative enhancement for major food crops is a key target for all research groups; however, the process of recording and accessing yield responses for specific cultivars is challenging as each of these targets is influenced by a series of quantitative and environmental factors (Cai et al., 2016; Collins et al., 2008). A combination of new tools and modern technologies focused on assessing the phenotypic and genotypic properties of plants are the keys to answering these questions. Modern high-throughput genotyping approaches are of great significance as they allow the development of trait-specific genetic markers used for the detection of mutation variations, ranging from frameshift mutation to single nucleotide polymorphism (SNP) and allow early stage detection of transposable elements (Patel et al., 2015; Din et al., 2022). Next-generation sequencing or the NGS advances are now resulting in fast detection of mutations and polymorphism with the SNPs being the most specific kind of mutations, utilizing such markers creates a chunk of mutation-related data and processing, analysis, and interpretation of such data is prerequisite for precision breeding of crops. Such as a recent publication focused on the genomes study of more than 3000 chickpea cultivars shows over a million SNPs present among all cultivars and the genome sequence of maize also showed a high degree of SNP mutations being detected in the genome (Gore et al., 2009; Varshney et al., 2021). Now analysis of such a large number of SNPs often results in such a large set of complex data that is highly complex and heterogeneous and is immensely difficult to be assessed by the human mind, at the meantime analysis of such data lies the foundation for precision breeding strategies.

Assessment of such complex data is now assisted by the use of modern AI algorithms which enable the thorough study of such traits for precision breeding purposes. These algorithms enable the use of not only the genetic information but also massive imaging data collected for evaluating traits of the plant at each stage which can then further be used for the detection of strong candidate lines for further breeding and development purposes. Some of the most commonly used ML algorithms for the purpose include convolution neural networks (CNNs), multilayer perception (MLP), and support vector machine (SVM). CNNs are widely utilized for making genetic studies of crop plants and in the estimation of polymorphic effects inside the genome. In addition to this, an assessment of various phenotypic traits is made using genomic data for breeding purposes (Ramstein et al., 2019). DeepGS ML algorithm is utilized for such phenotypic to genotypic interaction studies, which makes accurate trait-related predictions by utilizing the provided training data set. Similarly DeepSort CNN packages are utilized for ploidy differentiation among cultivars by targeting specific conserved regions (Ma et al., 2018; Veeramani et al., 2018). G2PDeep is another ML model used for genomic studies and is an open-source web-based program that makes use of genomic data sets available to make accurate predictions regarding phenotypic expressions, SNPs, and genetic biomarkers in various plant species (Zeng et al., 2021).

Studies have been made to assess the efficiency of various kinds of ML algorithms; a comparison between multilayer-perceptron (MLP), support vector machine (SVM), and the Bayesian threshold genomic best linear unbiased prediction model (TGBLUP) has shown that for performance predictions of targets based on genomic information the TGBLUP is the most accurate kind of algorithms, whereas the SVM was most time-efficient kind of ML algorithm (Montesinos-Lopez et al., 2019). ML programs including SVM and random forest (RF) models are being used for the assessment of toxic mutations in rice and pea genome based on complex training data sets of toxic and neutral mutations from *Arabidopsis* spp. (Sheekofa et al., 2014) (Table 4.1).

In addition to basic genomic studies, ML approaches are now also being utilized for assessing complex omic data sets (metablomic, phenomic, proteomic, etc.), as the concept of the central dogma of life presented by Francis Crick (1970) states the genetic information of a trait present on DNA is transcribed in form of an encrypted code to RNA which is then further translated in form of information into protein and these proteins interact with each other to influence the metabolism of living species, so assessment of these biochemical molecules sheds further light on to trait evaluation and selections of plant crops. Analysis of such datasets is of great significance for the exploration of a large chunk of information related to plant growth patterns and is further needed for proper evaluation and final assessment of mutagenesis or CRISPR-assisted modifications of the genome (Araus et al., 2018). Phenotypic analysis of plant crops is a complex task as it involves methods and inputs from the field of biology, statistics, computers, and artificial intelligence. Phenotypic data collection is carried out at various stages from organ level to HTP; organ level phenotyping is mainly focused on the extraction of plant organ data collected using high-resolution 2D image cameras or a multiview stereo approach that captures multiple images and collects motion data for plant organs, whereas HTP mainly focuses on a combination of ground-based and aerial high spectral reflectance imaging in combination with a network of sensors to record all phenotypic data and assess it using powerful computing tools (Zhao et al. 2019; Furbank et al., 2019). Another kind of phenotypic data collection technique is automated phenotyping which is normally carried out in certain controlled environments with sensors and

Table 4.1 ML approaches in plant sciences

Type of machine learning	Plant species	Target trait	REF
SVM and Gaussian Process Classifier (GPC)	Spinach (*Spinacia oleracea L.*)	Drought/abiotic stress	Raza et al. (2014)
Artificial Neural Networks (ANN)	Ajowan (*Trachyspermum ammi L.*)	Predictive models for seed yields	Niazin et al. (2018)
Simple Volume Maximization (SiVM)	Tomato (*Lycopersicum esculentum L.*)	Water stress	Romer et al. (2012)
ANN	Ajowan (*Trachyspermum ammi L.*)	Estimation of essential oil content	Niazin et al. (2018b)
Support Vector Machine (SVM)	Rice (*Oryza sativa L.*)	Nutrient stress evaluation	Chen et al. (2014)
SVM	*Arabidopsis thaliana*	Prediction of miRNA response to stress	Vakilian (2020)
ANN	Safflower (*Carthamus tinctorius L.*)	Seed yield modeling	Abdipour et al. (2019)
ANN	Sesame (*Sesamum indicum L.*)	Oil content estimation	Abdipour et al. (2018)
CNN	Soybean (*Glycine max*)	Estimating seed per pods	Uzal et al. (2018)
Multi layer percepton (MLP)	Chili pepper (*Capsicum annuum L.*)	Aflatoxins detection	Atas et al. (2012)
Deep neural network (DNN)	Soybean (*Glycine max*)	Evaluating density diversity of stomata	Sakoda et al. (2019)
ANN	Tomato (*Lycopersicum esculentum L.*)	Yield prediction on base of meteorological and fertilizer application data	Ravari et al. (2016)
ANN	Wheat (*Triticum aestivum L.*)	Yield prediction	Niedbała and Kozlowski (2019)
DNN	Wheat (*Triticum aestivum L.*)	Production forecast	Haider et al. (2019)
ANN	Grapevine root-stock	Assessment of genetic diversity	Costa et al. (2019)
CNN	Wheat (*Triticum aestivum L.*)	Genomic selection	Ma et al. (2018)
Random Forest (RF)	Carrot (*Daucus carota*)	Yield mapping for precision agriculture	Wei et al. (2020)
ANN	Rapeseed (*Brassica napus*)	Yield modeling of seeds	Niedbała et al. (2019)

(continued)

Table 4.1 (continued)

Type of machine learning	Plant species	Target trait	REF
CNN	Maize (*Zea mays L.*)	Identification of seed ploidy	Altuntaş et al. (2019)
ANN	Rapeseed (*Brassica napus*)	Yield estimation based on weather environmental forecast	Niedbała (2019)
ANN	Wheat (*Triticum aestivum L.)*	Modeling of in-vitro shoot regeneration	Hesami et al. (2020)
ANN	Tomato (*Lycopersicum esculentum L.*	Salinity tolerance estimation	Ravari et al. (2016)
Adaptive-neuro fuzzy interference system (ANFIS)	Chrysanthemum	Somatic Embryogenesis modeling	Hesami et al. (2019)

high-resolution cameras attached for data collection in addition to equipment placed for maintaining the internal environment, such phenotyping methods are often hampered by high-cost issues (Araus et al., 2018).

Phenotypic assessment is a complex task as it requires the collection of data sets of multiple domains and analysis of such large metadata requires high computing power. Another major challenge with such approaches is the reproducibility as environmental conditions continue to change, with various kinds of sensors used for data collection and fluctuations in plant responses to environmental influence all this make an impact in the final assessment of data. In this regard MIAPPE (Minimum Information about a Plant Phenotyping Experiment) guidelines for phenotyping experiments is of significance which allows incorporation of that phenomic data in cross with other systems thus making reusability of it possible to a great extent. MIAPPE also tends to resolve issues with data standardizations within experiment factors and allows a structured description for collected metadata sets (Bolger et al., 2019). Powerful statistical strategies are crucial to making use of the growing amount of complex phenotyping data and complex trait sets that describe crop cultivars (Zhao et al., 2016). ML-based approaches are used in identification, assessment, quantification, and creating predictive responses for the decision-making process of plant phenotyping activities (Singh et al., 2016). For instance, unsupervised learning method was used for processing of visual data in order to assess stress severity in soybean plants; similarly, SVM-based models were used in assessing drought in spinach plants.

Table 4.1 summarizes several ML approaches used in plant studies

The potential for ML approaches in plant sciences is immense, especially for unbiased trait selection of crops based on phenotypic and genetic data sets (Zhao et al., 2016). The use of complex data sets is of great significance for the development of future crops as a combination of knowledge is essential in developing better and sustainable crops for the future.

ML in Plant Disease Studies

Plant diseases and pests are of great significance in the field of agriculture and plant sciences for yield output perspectives. Detection of these pests and pathogens is rapidly becoming a hot topic of research in the field of AI and ML. Currently, a few ML-based programs have been developed which tend to detect the plant disease intensity based on visual responses from various cameras and vision devices. A series of image processing algorithms and case-specific classifiers are used in such vision-based programs for the detection of diseased plants, separating them from healthier ones, and estimating the overall disease rate in a plant population (Lee et al., 2017). Under actual field conditions, pest and pathogen detection is often met with a variety of challenges that includes: issue with detection of lesion area on the leaf surface, disturbance with natural light intensity, and variations in lesions shape and size. In an open field under natural conditions, it is often difficult for algorithms to eliminate all the difficulties acquainted with imaging by sunlight and other natural conditions. These algorithmic schemes for imaging are carefully designed by taking into account the properties of diseased plants along with the selection of opposite imaging angles and sources of illuminations (Tsaftaris et al., 2016).

Recently ML algorithms have evolved greatly in performing various complex tasks which were previously presumed impossible. Several general examples include the development of facial recognition systems, voice recognition, and language processing system (like Amazon’s Alexa, Apple’s Siri, and IBM’s Watson), predictive analytics for consumer markets (i.e. social media adverts), and in biological sciences in the form of personalized medicines, genomic analysis, and detection of diseased tissues (Sommer & Gerlich, 2013; Guyon et al., 2002; Singh et al., 2016). Advances in the field have resulted in the development of such software tools which enable the robust and efficient application of ML practices into a wide range of fields including the field of agriculture. ML approaches are now being widely used for disease detection in plants owing to technological advancements and lowering costs of it.

For detection of disease using ML the image data is collected and pre-trained initially to attain all possible characteristics of the image; this is followed by classification and ultimately determining the experimental efficacy of the model. Several ML models have achieved more than 90% accuracy in such disease analysis (Hasan et al., 2019). Table 4.2 summarizes several ML approaches used in plant disease and pest studies.

Conclusion and Future Outlook

Over the last many centuries, mankind has made consistent efforts to enhance global food outputs to ensure food security for all, the most prominent among these is the Green Revolution of 1960s and 1970s which provided the world with a perfect

Table 4.2 ML approaches in plant disease studies

Type of Machine Learning	Plant Species	Target Disease/Pest	References
Support Vector Machine (SVM)	Tomato (*Solanum lycopersicum L.*)	Powdery mildew disease	Raza et al. (2015)
Bayes Factor	Barley (*Hordeum vulgare L.*)	Rust disease	Wahabzada et al. (2015)
SVM	Citrus	Greening disease	Wetterich et al. (2013)
Convolution neural network (CNN)	Cucumber (*Cucumis sativus L.*)	Powdery mildew disease quantification	Lin et al. (2019)
None Preprocessing via segmentation	Cotton (*Gossypium hirsutum L.*)	Southern green stink bug	Camargo and Smith (2009)
SVM	Tomato (*Solanum lycopersicum L.*)	Yellow leaf curl virus infection	Mokhtar et al. (2015)
Deep neural networks (DNN)	Pearl millet (*Pennisetum glaucum L.*)	Mildew disease identification	Coulibaly et al. (2019)
ANN	*Phalaenopsis* spp.	Bacterial soft rot and brown rot	Huang (2007)
Random Forest (RF)	Potato (*Solanum tuberosum L.*)	Classifying cultivars infected with *Phytophthora infestans*	Gold et al. (2020)
Deep CNN	Cassava (*Manihot esculenta L.*)	Brown streak disease of cassava	Ramcharan et al. (2017)
SVM	Sugar beet	*Cercospora* leaf spot and rust disease of sugar beet	Rumpf et al. (2010)
CNN	Tomato (*Solanum lycopersicum L.*)	Grey mold disease and powdery mildew disease	Fuentes et al. (2017)
Neural Networks (NN)	Cotton (*Gossypium hirsutum L.*)	Bacterial blight	Revathi and Hemalatha (2014)
Neural Networks (NN) and image processing	Grape	Black rot	Kakade and Ahire (2015)

solution to its food problems. During this period, productivity and yield outputs increased massively across the globe, mainly in response to massive amounts of fertilizers, pesticides, and the use of high-yield cultivars. Now the twenty-first century comes with its own new sets of challenges, as the global demand for food is further projected to increase along with the negative impact of climate change and the reducing availability of land and other agricultural resources. The United Nations Food and Agriculture Organization presented a white paper in 2009, with a key emphasis on climate-smart agriculture in order to mitigate the impact of climate change and ensure sustainable food outputs for the world (Lipper et al., 2014).

During the past few decades, technological advancements have resulted in producing petabytes of "Big Data" related to genetic information and HTP of crop plants. The application of ML algorithms can result in screening out useful information sets and creating sustainable eco-friendly agriculture models with targeted yield outputs as part of optimized breeding and digital agriculture approaches. This modern optimized breeding approach with a combination of digital prowess and genotypic-phenotypic data sets of the plant will lead to enhancements of skill sets among the modern scientific community for advanced research leading to reduced developmental cycle durations for crop plants and ultimately resulting in the creation of eco-friendly agricultural products, industrial development, and value addition to the economic setup.

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Chapter 5
Applications of Multi-omics Approaches for Food and Nutritional Security

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Introduction

Access to safe, nutritious, and sufficient food is crucial for the growth and development of people. To date, it is estimated that up to 821 million people go hungry across the world, although one of the main targets of the 17 Sustainable Development Goals is to strive for Zero Hunger (FAO, IFAD, UNICEF, WFP, and WHO 2020). Numerous challenges to achieving Zero Hunger exist, and some of the most recent difficulties include the COVID-19 pandemic, social and political unrest, and pathogen attacks, among others (Béné et al., 2021; Brück et al., 2019). Food security was introduced to help reduce and eliminate hunger and poverty by ensuring that everyone has constant access to sufficient food and calories. While food security addresses the problem of hunger, malnutrition is a phenomenon that is on the rise. For instance, type 2 diabetes, obesity, and anemia are only a few of the metabolic diseases induced by nutritional imbalances that affect different groups of people.

To address the lack of balanced and nutritious diets, the term “nutritional security” was coined. Nutritional security ensures that everyone has access to nutrients from all food groups, including proteins, carbohydrates, fiber, vitamins, and minerals (FAO, IFAD, UNICEF, WFP, and WHO, 2020). Fresh and healthy foods such as vegetables, fruits, nuts, grains, and oilseeds, amongst others, are at the forefront of the food candidates to lower the risk of obesity, type 2 diabetes, cancer, cardiovascular disease, and other disorders (Melotto et al., 2020). In the next 50 years, it is expected that food and fiber applications will increase by up to 70% in the future. This will therefore result in increased demand for agricultural outputs, which is likely to put additional strain on the existing water quality, cultivable land, and weather changes (Singh and Trivedi 2017).

Food and nutritional security are not about setting food at the table. On the other hand, food and nutritional security is part of a complex food system that further includes production, input supply, storage, distribution, processing, consumption, retail, marketing, sustainability, governance, economics, and cultural identities (Loboguerrero et al., 2020). Food systems have always changed to adapt to the demands of modern populations. For example, traditional farming has expanded to multinational large scale and/or vertical farming, crop breeding is at its highest performance to create crops resistant to pathogens and abiotic stress, and the presence of pollutants in soil and water affects the safety and toxicity of the food we consumed and the nutritional content of the produced and processed food (Hueston and McLeod 2012).

Omics technologies play a major role in food and nutritional security. The word ‘omics’ emphasizes the terms whole or all. Unlike targeted biomolecule analysis, the omics-based method provides a better understanding of biological processes at a system level (Prasad 2020). The application of modern omics tools to the food system to improve customer confidence, health, and wellness is known as foodomics (Herrero et al., 2012; Lee Nen That et al., 2022). Rapid advances in omics research have resulted in high-throughput tools for profiling the make-up of crops, environmental samples, food, and human biofluids. Multi-omics has been a successful technique for the plant system for several decades. Multi-omics is the aggregation of all the complex data gathered to grasp better what is going on at a macroscale. This multi-omics data largely consists of information derived from the epigenome, genome, and metabolome, as well as a complete technique for finding and understanding essential components of yield, senescence, and stress response in a variety of crops, including millet, soybean, and wheat (Yang et al., 2021).

The multi-omics technique utilizes recent developments in genomes, transcriptomics, proteomics, metabolomics, and bioinformatics. The tools of genomics, transcriptomics, proteomics, and metabolomics are all used to figure out how different systems work. Moreover, proteomics and associated platforms are significant for food safety because they may provide absolute quantification of food allergens and mycotoxins contained in plant-based foods (Jain et al., 2019). Metabolomics is a platform that analyses all of the metabolites found in food systems quantitatively and qualitatively (Pandohee et al., 2015). This information can be used to predict the nutritional content, sensory quality, and safety of food products (Settachaimongkon et al., 2017). This chapter presents the applications of multi-omics technologies in several factors crucial to contributing and improving food and nutritional security.

Agriculture, Crop Breeding, and Microbiome

Crop breeding is an evolutionary process that involves both plants and people. Humans alter plant species that can be used in agriculture, and those newly developed plants, in turn, alter human populations. In developed countries, agriculture employs only a small percentage of the population (Breseghello and Coelho 2013). This field of crop breeding can benefit from applying several inventions to overcome modern limits in plant breeding and phenomics while simultaneously developing plant genomics. The participation of a global and interdisciplinary team will aid in the improvement of the methodology's competence. As a result, more options and facilities will become accessible for successful robotization, standardization, and automation of large-scale phenotyping for crop breeding (Fasoula et al., 2020).

Depending on the species, plant breeding can be a time-consuming task, and it can take up to 10 years to fully develop, hybridize, and test new crop species for consumption, safety, and acceptance. Thus, breeders constantly require new procedures and technologies to generate crops resistant to pathogen attack or environmental resilience in a logical and high-throughput manner. In recent years, various advances in research have been made, including genomics, phenomics, trait physiology, genetic modification, molecular genetics, and geographic information systems (Lenaerts et al., 2019). These plants and crops were bred using gene-edited crops and genetically engineered organisms. This technique is beneficial because it uses fewer pesticides and chemical fertilizers, generates greater crop yields, is more resistant to post-harvest losses and climate change, and produces more nutritious food. They are, however, still underutilized. Organisms that have been precisely genetically created are typically considered negatively.

Consumer awareness of food quality and safety is another factor to consider when developing new technologies and food products for human consumption. An example is food containing or made of genetically modified organisms (GMOs). It has been observed that even after 30 years of research in the production of genetically modified food, evidence of its safety and increased nutritional content, the skepticism and awareness of the effect of genetically modified food on human health is widespread among most consumers around the world favoring food made from conventional crop breeding (Qaim 2020). Therefore, it is becoming more and more important that appropriate and complete labeling reports of the safety, toxicity, and manufacture of food are made available. In countries where food poisoning and intoxication have occurred, there have been increased calls for hazardous microbial testing to be routinely performed in the food processing area.

The microbiome is another aspect of food and nutritional security that could be used to enhance the health of the general population. The microbiome, or microbial community, consists of millions of microorganisms living in plants and animals. In the last few years, there has been a greater focus on elucidating the function and makeup of soil and plant microbiomes. As a result, it was considered that plants are not self-contained entities but rather cohabit with a variety of microbes. Plant tissues can have bacteria on the outside or inside of them. Plant–microbe interactions have typically been researched using cultured microorganisms, although unculturable bacteria are still being studied (Dastogeer et al., 2020). In agriculture, the control of pests and phytopathogens, improved nutrient uptake, and improved plant growth are all advantages of a healthy microbiome. As a result, there is a compelling need to incorporate the functional potential and creativity of the plant-associated microbiome into agricultural production (Ray et al., 2020).

Food spoilage from microorganisms and foodborne pathogens can be detected using DNA-based assays, which identify almost all microorganisms through a universal DNA marker. Multi-omics were used, including 16 rRNA gene sequencing of the rumen microbiota and milk metabolomics, to examine the effect of rumen- protected methionine supplementation on nutritional milk content and investigate the related important bacteria in the rumen. The findings reveal that Rumen-protected methionine supplementation can be used to create milk fat and AKG-enriched milk in dairy cows, as well as new and improved approaches for developing functional dairy products (Gu et al., 2021). The latter employed the iTRAQ comparative proteomic technique in another work to explore unexpected effects on proteomic profile in genetically engineered soybean lines versus nongenetically modified soybean lines. Thus, proteomics and other omics-based approaches play an important role in producing biofortified staple crop varieties in a timely and cost-effective manner (Liu et al., 2018). Consequently, technical improvements in the field of food quality are focusing on the development of universal and more accurate analytical methods, as well as their compatibility (Ferri et al., 2015).

Improving Nutritional Status

High-Quality Plant-Based Food

With a growing world population and increase in food demand, there is immense pressure on the agricultural industry. Dependence on major crops such as wheat, maize, and rice must be reduced as it is believed that their yields have peaked. Moreover, uncontrolled urbanization in some countries has in turn affected farmland resulting in severe weather conditions such as drought, floods, and extreme heat. Altogether these problems have been mitigated by high-resistant and high-yield crop varieties. The challenges concerning land use, crop yields, future food demand expectations, and employment of a more intensive cultivation strategy must be attended to immediately. To meet these challenges, phenotypic and genetic varieties of the plants need to be well characterized (Scossa et al., 2021). Genomics, transcriptomics, proteomics, metabolomics, and ionomics used in combination can help elucidate the networks of genes, proteins, and metabolites. This understanding and the usage of different gene editing technologies can accelerate crop breeding programs, resulting in crops with nutritionally superior traits and environmental stress resilient traits among many others.

In recent years, the number of crop improvement programs that use OMIC-assisted breeding approaches has expanded significantly. The knowledge of the whole genome sequence of rice, sequencing techniques, analysis tools, and high-throughput technology has accelerated attempts to enhance nutritional and agronomically significant features in the crop. Several genes that regulate nutritional quality-related features are currently being investigated in depth. For instance, it was found that the genes sd1 and GA20ox2 regulate the protein content in the grain in the semi-dwarf mutant allele, whereas the gene OsYSL2 regulates the long-distance transport of manganese and iron. One of the extensive integrated omics projects included the discovery of 3000 metabolites in 10 kinds of cooked rice through metabolomics. Employing functional genomics, the genetic variants that created this metabolite diversity were examined. This resulted in the determination of phenolic compound genetic diversity and identification of SNPs in their untranslated regions, which have a key role in gene expression regulation. The study discovered nutritionally important discriminating compounds as well as yield-related differences between three rice varieties (Rana et al., 2020).

Elaeagnus or silverberry has increased lycopene (10 times higher than tomatoes) and hence, is a valuable crop. These berries are noted for their drought tolerance and high magnesium, protein, and ascorbic acid content. Analysis of total protein content, lycopene, organic acids, and soluble sugar quantity through proteomic studies of *Elaeagnus umbellata* showed that phytoene synthase (EutPSY) expression is related to the accumulation of lycopene. As a result of this study, it was understood that EutPSY could be targeted to enhance the content of lycopene in other fruits so that their quality can be improved further (Kumar et al., 2021).

Nutritional Crops for Food Security

Micronutrient deficiencies have become more common, with more than 2 billion individuals with severe clinical problems. Micronutrient levels have usually been altered through food variety and dietary supplementation. However, because of the absence of social and economic infrastructure in developing nations, such efforts have met with limited success. A diet-based strategy, biofortification, is being employed to increase the micronutrient content of staple crops by traditional breeding or transgenic approaches. Biosynthetic pathways of nutrient assimilation, composition, and food quality can be studied using proteomics and its tools. A number of different biotechnological platforms, along with genomic and proteomic methods, are being employed to investigate changes in the abundance of gene/protein in relation to various macro and micronutrients. Integration of different omics technologies to produce nutrient-rich staple crops such as soybean, potato, rice, maize, and wheat with an aim to eradicate malnutrition and micronutrient deficiency, thereby ensuring long-term nutritional security is the need of the hour (Jain et al., 2019).

Enhancement of food quality by integrating bioactive molecules into crops or by improving the macro and micronutrient bioavailability in fruits, vegetables, and cereals can be accomplished by nutrigenomics. It helps to understand the implication of nutrients and diet regimens on gene expression (Rana et al., 2020). It employs functional genomic methods to identify genes that are impacted by food constituents, resulting in changes in homeostatic pathways. It is a challenging field to investigate because a single nutrient can have varied specificities, affinities, and targets depending on the population being studied (Kumar et al., 2016).

Finger millet is a member of the Poaceae family and is a common staple meal for those living on marginal lands. It is an agronomically sustainable crop that can survive in saline and drought conditions and high altitudes while maintaining optimal yields. It requires minimal irrigation and other inputs. From a nutritional standpoint, finger millet is significantly higher in mineral content and micronutrient density than the largest global cereal grains, rice and wheat. A hypothetical model demonstrating the transport and distribution of calcium in embryonic finger millet seeds was developed using metanalysis of gene expression data.

Banana belongs to the family Musaceae and plays a major role in food security in tropical areas. They offer agrarians a substantial source of revenue through local and worldwide trade. To maintain postharvest losses of the fruit and extension of shelf-life, ripening-associated softening is targeted. Development and ripening stage phenol extracts of proteins from different tissues of bananas were obtained. Global proteome approaches were used to resolve these proteins, and further analysis was done. The protein and gene sequence information were used to generate RNAi constructs to firstly determine the individual role of identified important proteins and genes, and to secondly extend the shelf-life (Mohanty et al., 2017).

Traditional Medicinal Plants for Food and Health Security

The objective of “Health for All” can be achieved by using medicinal plants that are economical, safe, free of side effects, and nonnarcotic. Consequently, both developing and developed nations are seeing an increase in demand for medicinal plants. As people’s perceptions of traditional medicines change, more people in rural and urban areas are recognizing and using medicinal plants for primary health care. As a result, traditional practitioners and new pharmaceutical outlets have exerted dual pressures. Despite this threat, medicinal plants help in providing health security to millions around the world (Rekha Singal 2005).

Traditional plants are consumed by native groups around the world, particularly in rural regions, for nutrition as well as health. For sustainable food production, nutritious traditional plants need to be considered. Recognizing the significance of traditional plants, researchers have started sequencing traditional plants to develop, cultivate, and mainstream them. Using a combination of omics and gene editing technology, many advantageous traits have been incorporated into traditional plants.

Corylus avellana L. or Hazelnut is a good source of minerals like phosphorous, potassium, magnesium, calcium, etc., as well as vitamins C and E, lipids, proteins, and starch, and is cultivated worldwide. It is an especially good source of unsaturated fatty acids and malic acid. Through different omics technologies, it was found that it contains a high concentration of palmitic acid that can prevent diabetes and other such metabolic syndromes (Kumar et al., 2021).

Improvement of Food Crops Towards Food Security

The rising demand for healthy and sustainable food has the food industry keep an eye out for any novel technologies or food items. Aquatic plant cultivation (like seaweed) is a rapidly expanding traditional industry, where algaculture for consumption is now being exercised in around 50 countries. New food products that have a reduced impact on the environment are being created due to evolving technologies and innovations. For instance, protein alternatives like mycoproteins are derived from fungal biomass. Cellular agriculture is a growing industry that uses plant and animal cells to produce food in vitro, such as gelatine, eggs, milk, and meat. Despite their potential, more research on the benefits and drawbacks of newly emerging food products and their involvement in nutritional and sustainable food systems is required (Lindgren et al., 2018).

Ethnobotany is a science that studies the interactions between humans and plants. It is centered at the intersection of natural and social sciences. Bioprospecting for new botanically derived drugs and new food crops has traditionally relied on ethnobotanical data. For the identification and detection of bioactive compounds from medicinal plants, bioprospecting that is ethnobotanically-led is becoming more effective than random assays. For example, oseltamivir was derived from *Illicium* *verum* Hook.f., amid the epidemic of bird flu, based on ethnobotanical information from Chinese traditional medicine. Developing novel molecular tools have given rise to new perspectives. DNA sequencing allows for phylogenetic reconstruction with clustering of hot nodes of prospective plant species known traditionally for medicinal value.

The use of identical or closely linked species of plants in varied cultures suggests that the knowledge was gained independently by distinct and noninteracting groups of humans. This is attributable to the reality that certain plants have similar morphological traits as a result of their close placement in terms of phylogeny, a concept called "evolutionary convergence." It was also proposed by certain research groups to label plants in one phylogenetic node, having resemblance in uses, with the expression "ethnobotanical convergence." Plants that have evolved in the same lineage have far more uses medicinally as compared to species that have evolved separately, and the variation in medicinal use is linked to the species' evolutionary history. The use of spices as a seasoning for two foods from distinct cultural and geographical regions acts as a good example of ethnobotanical convergence. *Origanum vulgare* L. is used to season pizza in Western cultures, while another species of the same genus, *Origanum syriacum* L., is used to season a similar dish in the Near East called Manousheh. Along with the approach of phylogeny, "omic" techniques producing large data sets and their analyses with the help of bioinformatics are valuable in the detection of plants with established ethnobotanical uses, the plant taxa with the most potential in culinary or medicinal use (Garnatje et al., 2017).

General metabolite profiles, both quantitative and qualitative in nature, are presented by metabolomics studies. When these profiles are derived from plants susceptible to a variety of environments, the information produced helps in evaluating the temporal and spatial distribution of phytochemicals in the study and the impact of geographical and plant developmental cues. The impact of complex mixtures, like those commonly used in Chinese traditional medicine, can also be better understood with the help of metabolomics (Pandohee et al., 2021). The major challenge in the research of natural products is understanding complex mixtures and designating the bioactive compounds. Untargeted metabolite profiling along with fractionation based on bioassays enhances bioactive compound identification. Detection of loci of a significant metabolic quantitative trait through metabolomics can improve breeding techniques of medicinal plants. Thoroughly researched medicinal products and culinary ingredients may be developed through this approach of metabolomics. The combination of the two approaches—metabolomics and functional characterizations of products of genes based on genomic studies—enhances the speed of discovery of significant bioactive compounds and their novel biosynthetic pathways. This way, the production and discovery of culinary and medicinal products are also improved. An example of this combination of ethnobotanical approach with the "omic" technique is the FAD2 phytochemicals discovery, which took place in a bioengineered, non-plant host.

In an era of decreasing water resources and diminishing agriculturally productive lands, sustainable crop production can provide for the growing world population. Crop enhancement employing OMICS technologies could ensure food security.

Crops with additional beneficial traits can also fight against malnutrition which is prevalent around the globe (Faryad et al., 2021).

Recent advances and revolutions in omics technology have made it possible to conduct research at a wider scale and in a shorter period of time for organisms at the levels of gene, genome, proteome metabolome, and ionome. Technologies for structural genomics are being used to build genetic and physical maps of genomic regions that a particular trait of an organism. The genome structure, polymorphism profile, and chromosomal organization of crop plants are explored. Furthermore, functional genomics technologies allow researchers to better understand the roles of genes that control these characteristics. Transcriptomics allows for the determination of transcripts, and the linkage of these transcripts with phenotypic data allows for elucidation of gene–trait associations. Proteomics and metabolomics techniques are used to measure the quantitative and qualitative composition of protein metabolites. Likewise, a plant's whole mineral and elemental makeup can be deduced using ionomics tools. Integration of all these omics tools will ultimately help in the identification of the connections between elemental composition, transit, and storage, as well as the set of genes that control diverse activities in these crop plants. The merging of multi-omics technologies offers a significant potential to speed up the task of identifying genes that control traits that are agronomically important in plants and to accelerate improvement programs using both traditional breeding and cutting-edge CRISPR/Cas9-mediated and other gene editing technologies (Kumar et al., 2021).

Feedomics

Feedomics is a new research domain that uses high sensitivity and resolution omics technology to study the science of feed and food, nutrition of animals, and production/health of animals that are economically important on a global molecular and systematic scale. The prospect of feedomics is promising as an established targeted method to enhance the functional properties, safety, quality, and quantity of food animal products. It also can help to minimize the use of antimicrobial growth promoting factors and limit the emissions of greenhouse gas from the food and animal sectors. This is accomplished by using traditional or alternative feed resources and novel feed supplements (e.g., miRNAs) (Sun and Guan 2018).

Food Safety and Environmental Effects on Food Safety

Food safety in the food market is one of the most important areas of concern in public health because it impacts individuals of all ages, races, genders, and income levels all over the world. Food advertising on a local and international scale continues to have a substantial impact on public food safety and health. Food supply systems today span many national borders, putting health concerns on an international scale. To protect the public's health and safety, robust national risk-based food control systems are required. Countries must also ensure that their goods entering international trade are safe and of high quality and that imported foods meet national standards (Gizaw 2019).

Agriculture progress is critical to the stability of the food supply. Even though pesticides have ensured consistent agricultural production, their increased use has had long-term negative effects on the environment and food safety. As a result, researchers are paying more attention to pesticide risk evaluation and management. In the pharmaceutical sector, multi-omics is a promising method for determining the mode of action of bioactive substances. However, just a few studies have used this approach to assess pesticide risk and toxicity causes. Some critical biomarkers and their corresponding regulatory pathways were identified based on prior research, and the potential manner of harmful effects and application of pesticide risk management by various omics techniques were investigated.

Health Status Versus Food Intake

A healthy lifestyle usually starts with a nutritious, well-balanced diet and regular physical activity. To maintain a healthy and active life, humans require a wide variety of nutrients, good nutrition, or the right consumption of food in accordance with the body's nutritional needs (e.g. gender, age, lifestyle, and physical activity). Healthy dietary practices have changed drastically from earlier civilizations till today. Conventional plant-based foods are rapidly substituted by a high-fat diet, which includes a large amount of animal-based meals (Sabaté and Soret 2014). Diet has long been recognized as a significant risk factor for chronic diseases. Lack of physical activity has been identified as a rising factor in health and is the outcome of a growing trend in habits more toward sedentary lifestyles in both emerging as well as developing countries (Nothwehr and Yang 2006).

At all stages of life, nutrition is essential to one's health. Food and nutrition offer energy for our bodies, and we must replenish nutrients daily to keep our bodies moving (Bilali et al., 2019). Food science has advanced significantly in recent years, and the importance of diet in human health is not a new idea, as it is known, "Let food be thy medicine, and medicine be thy food." Foodomics is a holistic, high-throughput strategy for utilizing food technology in the context of improving health and nutrition. Foodomics is a novel concept in diet and nutrition that investigates the food domain in combination with the nutrition domain to achieve the fundamental goal of optimizing population health aspects using omics techniques (Capozzi and Bordoni 2013). Transcriptomics, proteomics, and metabolomics are dynamic domains that are influenced via interactions between the organism and food. As a result, to achieve the ultimate aim of improving population wellbeing, researchers must take into account most of these potential dynamic interactions using an omics strategy (Subramanian et al., 2020).

The Challenges for Maintaining Adequate Nutrition

Noncommunicable diseases (NCDs) are the major global public health problem. Malnutrition, obesity, cardiovascular complications, hypertension, cancer, and diabetes mellitus are caused by nutrient inadequacies in the diet. Diet and chronic disease have a complicated relationship because many ailments emerge with time, and identifying a specific food cause is extremely challenging (Sun et al., 2021). Ethics surrounding the sharing of created genomic data, greater variations in "omics" data samples, and a shortage of skilled people for the development, analysis, and processing of large data are all issues that must be addressed (Dove et al. 2014). Multi-omics techniques must be used to gain a better understanding of food microbial diversity. Nevertheless, because of the necessity for appropriate biostatistics and bioinformatics expertise for data analysis, this strategy has limitations that are inaccessible to the common people. Multi-omics has become a novel concept and a challenge that must be met by changing existing age-old concepts. Researchers in the fields of clinical sciences, biochemistry, analytical chemistry, molecular biology, microbiology, food technology, and food chemistry can finally collaborate using omics methods in the multi-omics sector (Jain et al., 2019).

Nutrition, Diet, and Its Associated Diseases

People's diets in their cultural variations affect their wellness and development to considerable proportions. The food patterns, combined with decreasing levels of physical activity, have resulted in significant increases in childhood obesity, yet undernutrition problems remain unaddressed. Undernutrition or overnutrition is identified as a major contributor to non-communicable diseases. Malnutrition has devastating effects like death, disability, and impaired mental and physical development. Along with malnutrition, iodine deficiency and vitamin A deficiency are also common, which cause brain damage and mental impairment, infant blindness around the globe (Brahmbhatt et al. 2001). One of the comorbidities that contribute to higher mortality in extremely malnourished individuals is severe anemia. Under and over nutrition often coexist, which reflects the diet pattern of the increasing proportion of people, which is linked with many chronic diseases.

Obesity is a problem in almost all countries (both high- and low-income) (Templin et al., 2019). The concept "obesogenic environment" has gained scientific discourse, implying that the external environment's potency is responsible for a major part of the elevations in food consumption that are one of the primary agents causing the obesity epidemic (Frank et al., 2012). Moreover, the interaction between diet and non-communicable disease development differs between persons, and the processes underlying this interindividual response are poorly known. Multi-omics research provides greater insights into the mechanisms that support various illness susceptibilities in individuals as they are influenced by diet composition as well as food intake (van Karnebeek et al., 2018).

Omics-based technologies allow us to acquire the molecular knowledge of human metabolism. Studies based on single omics technologies, like genomics, transcriptomics, proteomics, or metabolomics, resulted in the discovery of biomarkers for diseases, as well as a better understanding of disease pathophysiology and etiology, and used to investigate the impact of the environment on wellbeing. Nonetheless, the tools incorporating these technologies are required to properly capture the complexities of human interindividuality (Maruvada et al., 2020).

A Multi-omics Strategy to Study the Role of Nutrition

Current nutritional studies are concerned with promoting health and avoiding or delaying illness development (Gomes et al., 2018). Tailored nutrition, which involves personalizing diets to individual needs, is a concept parallel to personalized medicine (Qi 2014). Recent developments in "omics" technology have enabled to forecast, diagnose, and recommend solutions to food crops with poor protein content and bioavailability of important micronutrients (Van Emon 2016). A range of omics subdisciplines, such as epigenomics, interactomics, lipidomics, diseasomics, metallomics, have evolved in addition to the four basic categories of omics measurements genomics, transcriptomics, proteome, and metabolomics (Fontes et al., 2018). Food scarcity is a huge global issue, as demand for food will keep rising because of population growth. High throughput technology has been utilized for omics research to detect novel bioproducts. Researchers are now confronted with the possibility of linking food components, food products, nutrition, the individual, well-being, and ailments. Success can be attributed to the omics approach (Kumar et al., 2021).

Nutrigenomics is an integration of the study of nutrition, genetics, and omics (Braicu et al., 2017). Nutrigenomics and nutrigenetics aid in the knowledge of human diversity in dietary preferences and demands, which might be future techniques for consumer assessment prompted by individualized nutritional counseling for disease prevention and management (Braicu et al., 2017). Transcriptomics enables researchers to demonstrate how nutrients affect global gene expression patterns, relate them to disease management, and develop microbial risk controls for ready-to-eat packaged food (Braicu et al., 2017). Proteomics, alongside genomics, transcriptomics, and metabolomics, is an important element of the omic family of disciplines (Daniel 2002). It will help us get a better understanding of the complex systems that control nutrient consumption (especially dietary efficiencies), explore various biomarkers for nutritional status and disease development, and design a modern approach for disease prevention with the help of a balanced diet. As a result, proteome analysis has a promising prospect for providing better health (Braicu et al., 2017). Furthermore, metabolomics, the scientific study of the distinct chemical fingerprints obtained as a result of certain cellular processes, is quickly becoming a basic technique in food research and nutrition science (Alawiye and Babalola 2021). It enables the determination and analysis of biological components of the sample, providing effective tools for obtaining complete and systematic images of food content. Nutritional metabolomics strives to improve nutrition for better health maintenance and homeostasis (Wishart 2019). The MEATabolomics method aims at identifying possible biomarkers that can be used to monitor meat safety and quality. Meat quality is primarily determined by its appearance (especially color), and the presence of myoglobin is primarily responsible for the red color of meat, while other elements like tissue structure, pH, muscle source, antioxidant content, and lipid oxidation also play a key role (Muroya et al., 2020).

Conclusion and Future Perspective

The holistic use of certain dietary components or their combination and nutritional contrivance is an important approach in complementary medicine. In diet and health science, the need for an omics-based approach will continue to grow. As a result, more collaboration among researchers with diverse experiences and backgrounds is needed. The contemporary omics era, with its high-throughput molecular data generation, is making way for these complexities to be accepted and is profoundly changing the entire branch of nutrition. From both a translational and clinical standpoint, it is critical to understand the future directions of nutrition science. Deciphering the high interindividual variability of reactions to food consumption is the most difficult aspect of unraveling the links between food and health. The ability to predictably design our physiologic networks for food, wellness, and illness is a growing area of nutritional science. This will allow nutrition modification and health monitoring to be fine-tuned in the future.

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Chapter 6
Applications of High-Throughput Phenotypic Phenomics

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Introduction of Phenomics

Phenomics is the study of the phenome with the goal of characterizing phenotypes in a rigorous and formal fashion and linking them to the genes and gene variations that cause them (alleles). The study of plant development, performance, and composition is known as plant phenomics. Phenotyping technologies are used in forward phenomics to “sieve” collections of germplasm for important traits. The

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sieve or screen may be high-throughput, completely automated, and low-resolution, with higher-resolution, lower-throughput measurements following. Abiotic or biotic stress challenges may be used in screenings, which must be repeatable and physiologically relevant. Reverse phenomics is the detailed dissection of attributes that have been found to be valuable to disclose mechanistic insight and use this mechanism in new techniques. A physiological characteristic may need to be reduced to biochemical or biophysical processes and subsequently to a gene or genes.

High-Throughput Phenomics (HTP)

Phenomics is the study of multidimensional phenotypes with high throughput and correct achievement at different (cell, tissues, organs, individual plant, plots, and field) levels during the developmental stages of a crop. The phenotypic performance of crops is totally dependent upon the interaction between genotypes and environmental factors such as climatic factors, biotic and abiotic factors, and management methods of crops. In other words, phenomics can be defined as the whole study of high dimensional phenotypes. To improve the crop and understand the plant biology, its necessary to obtain data on all main features of phenotype in detail, as shown in Table 6.1.

Phenotypic Technologies

The phenotypic traits can measure exactly through the fast growth of harmless or nontoxic senses and advanced techniques of imaging in which visible, thermal infrared, fluorescence, 3D, and hyperspectral imaging, as well as tomographic imaging using magnetic resonance imaging (MRI) or X-ray computed tomography (CT) are involved. The number of high-throughput phenotyping (HTP) platforms can increase with the help of different technologies like sensing technologies, automatic controlled technologies, computers, robotics, and aeronautics for crop phenotypic traits inquiry. Numerous phenotypic platforms for the traits of the crop at numerous application scales are developed by scientists. In this chapter, there are three types of phenotypic platforms: microscopic, ground-based, and aerial phenotyping platforms split on the bases of imaging levels, which permit the representation of phenotypic traits at the different levels (tissue level, individual plant level, plot level, and field level). For the highly developing field of phenomics and giving rise to an increasing amount and diversity of data, high-throughput technologies are generally used. Turn off the extent data into the

Table 6.1 Various imaging tools and techniques used for different traits phenotyping

Sr. no.	Imaging tools and techniques	Traits to be measured/phenotype
1	Visible light	Shoot biomass, yield attributes, leaf morphology, ear traits, and root traits are all factors to consider
2	RGB imaging	Growth dynamics, chlorosis and necrosis, simple pigmentation, senescence, projected structures, surfaces, shoot colors, roots, seeds, leaf spots, growth dynamics, chlorosis and necrosis, simple pigmentation, and senescence
3	MRI, CT (X-ray)	Internal structures, in-soil structures, volumetric data, root development, and metabolites are all examples of internal structures
4	Thermal infrared	Index of the surface of the leaf, temperature of the leaf, insect state infestation, sheet and canopy water status
5	Laser, stereo imaging, time of flight imaging	Deep 3D structure, leaf angle, leaf area, plant height, cover, biomass amount, and structural composition are all factors to consider
6	Hyperspectral	Moisture content, leaf wellness, panicle health, wheat quality, pigments composition, and other factors.
7	Multispectral imaging	Minimal pigmentation, discontinuous spectrum reflection, includes NIR
8	PAM-fluorescence, wet chemistry, destructive sampling	Advanced studies of plant physiological functions and processes, including photosystem II activity, metabolite and phytohormonal profiles, enzyme activities
9	Fluorescence imaging	Leaf disease severity ratings, leaf health status, and more. Photosynthetic performance, quantum yield, non-photochemical quenching, leaf disease severity ratings, leaf health status, and more. Photosynthetic system (indirect), biomass development, nitrogen content, and senescence are some of the topics covered in this paper
10	Thermal imaging	Temperature of the leaf or canopy surface, relation to stomatal conductance, and biotic and abiotic stress responses
11	3D imaging	Turning structure; sheet corner distributions; canopy structure; root architecture; height
12	MRI	Water content, morphometric parameters, etc.
13	Hyperspectral imaging	Senescence, water, chlorosis/necrosis, continuous spectral reflectance, including NIR, immediate physiology, advanced pigmentation, and specialized specific pigments, such as xanthophyll related to biochemical composition and photosynthesis, senescence, water, chlorosis/necrosis
14	PET	Solute content, metabolites content, etc.
15	Fluorescence imaging	Photosynthetic machinery and associated metabolism, predicted leaf area, research of leaf stress owing to biotic and abiotic causes, snapshots of photosynthetic performance, and senescence

beneficial forecast and perception the artificial intelligence (AI) acts as a game changer. Although we need specialized programming skills and deep knowledge about machine learning, deep learning and ensemble learning algorithms are used to understand this artificial intelligence.

HTP Methods

RGB Imaging

RGB camera or RGB imaging method is mostly used to measure the morphological effects of plants (caused by its cost efficiency and ease of insertion). RGB cameras consist of an infrared blocking filter (VIS camera) that can detect the light wavelength of 400–700 nm instead of customer cameras. For the measurement of the color of every pixel, this camera used different color sensors (red, green, and blue). Scientists concluded that the model's prediction accuracy could be enhanced by adding other elements such as the growth date (Golzarian et al., 2011) or more anatomical or physiological characteristics in a more complex model (Chen et al., 2014). Because of its cost-effectiveness and ease of installation, the RGB camera approach is the most extensively used technology for measuring plant morphological features. RGB cameras (VIS cameras) include an infrared blocking filter that detects light wavelengths between (400 and 700 nm), unlike consumer cameras. The VIS camera measures through red, green, and blue color sensors as shown in Fig. 6.1.

Near-Infrared Imaging

In different topics, the highest reflectance is in the near-infrared wavelength range (700–1400 nm). This near-infrared imaging (NIR) attribute is employed to confirm plant transformations during drought stress.

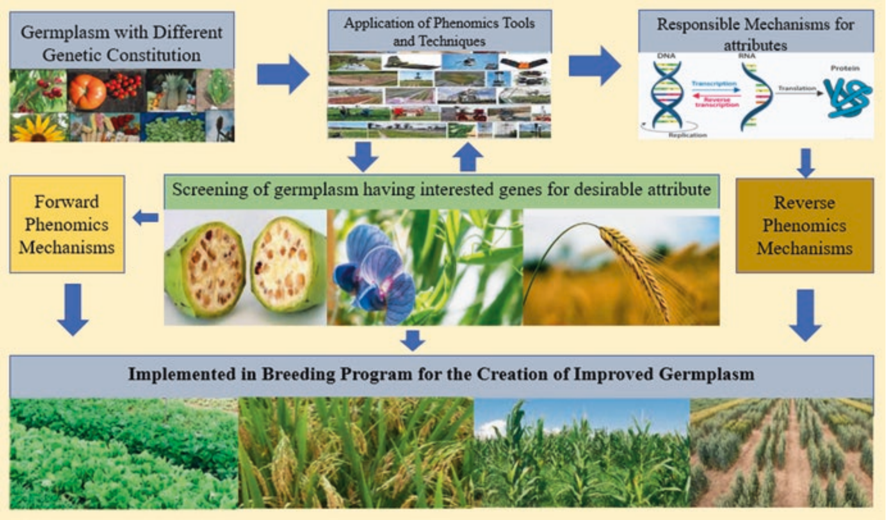

Fig. 6.1 High-throughput phenotyping technology concept

Hyperspectral Imaging

Hundreds of thousands of bands per pixel are detected by hyperspectral sensors, which cover the visible (400–700 nm), NIR (700–1000 nm), and SWIR (1000–2500 nm) wavelength ranges. Even though the data's physical complexity mandates the use of high-performance analytical processors, sensitive detectors are required, and also pictures may be recorded at high resolution with restricted spatial coverage to distinguish reactions to different pressures and large data storage capacity.

Fluorescence Imaging

With the fluorescence sensors, we can easily test the photosynthetic ability of the crop, and it can be tested by the estimation of chlorophyll fluorescence. For example, the excretion of unessential energy by the plant in the form of fluorescence.

Applications of High-Throughput (HTP) Phenotypic (Phenomics)

According to Soulé (1967), the word phenome implies an entire phenotype like genome manifestation in a certain location (Houle et al., 2010; Chen et al., 2014). As a result, a plant phenotype in an agricultural system must be viewed as the outcome of complicated G*E*M interactions (Houle et al., 2010). The word phenomics was coined in 1997 (schork, 1997) and was described as the methodical study of phenotypes at an organism-wide ranging like genomics and the various further omics technologies (Houle et al., 2010). The set of morphological, physiological, and recital-associated features of a genotype in the environment is known as the phenotype (Dhondt et al., 2013). In other disputes, phenomic is an inclusive wide-ranging study of high-dimensional phenotypes that is vital for the generation of meticulous data on all important aspects of phenotypes and for an improved understanding of plant biology and crop improvement. Therefore, the phenotyping system not only includes tools for performing phenotypes on its own but also plants in a specific environment, from the tightly controlled condition of the climatic chamber to the natural environment of the field it also means to grow (Dhont et al., 2013). The throughput of a plant phenotyping system relates to the number of individual units at a given organization. Steven A., a UC Berkeley and LBNL scientist, invented the term phenomics to describe the scientific study of phenotypes. As a result, it is a multidisciplinary field of study that includes biology, data science, engineering, and other disciplines.

Phenotypic Technologies

Multidisciplinary collaboration and some of the initial developments were targeted toward assessing genetically modified crops on a large scale (Reuzeau et al., 2005). Plant refurbishment approaches, Trait Mill, a suite of proprietary bioinformatics tools, a high-throughput gene engineering system, Crop Design (Belgium) established a HTP stage that was utilized to detect morphometric characteristics. (aboveground biomass, plant shape, and plant color) that might have an impact on yield. TraitMill's details, as well as the trial methodology and results, were inappropriately kept confidential (Reuzeau et al., 2005).

High-Throughput Phenomic Methods

Scanalyzer 3D platform:

Hairmansis et al. (2014) and Neilson et al. (2015) reported that the Scanalyzer 3D platform was created by Lemna Tec in Germany, and it has been implemented in numerous countries. Computer-controlled conveyor systems are installed at the Plant Accelerator (Australian Plant Phenomics Facility, University of Adelaide, Australia), automated weighing–watering devices, imaging stations, *Near-infrared (NIR), fluorescence* (at near-infrared wavelengths between 700 and 1400 nm, the green portions of plants had the maximum reflectivity), and *Hyperspectral imaging* (hundreds of thousands of bands per pixel are detected using hyperspectral sensors, which cover the visible spectrum) (Mathieu et al., 2015).

RADIX Imaging Marié et al. (2016) and Jeudy et al. (2016) examined the RhizoTubes (an automated "plant-to-sensor" platform including 1200 rhizo tubes to acquire the RSA in about 6–8 weeks) and RhizoSlide (a rhizoslide platform used to screen the shoots and roots of 200 maize plants) (Le Marié et al., 2016).

Quantitative Plant Morphology Detection Through Phenomics

To maintain future food security, it is critical to developing crop tolerance to abiotic stresses and new pests brought on by climate change. The growing use of gene editing, as well as the continuous utilization of natural genetic diversity, present excellent prospects for producing novel alleles and selecting natural sources of genetic variation for crop development. This necessitates the examination of hundreds of lines growing in a variety of environments. At the same time, breakthroughs in DNA marker assays and sequencing technology have enabled genotyping to achieve this throughput at a reasonable cost, similar innovations provide an urgent demand for high-throughput and meaningful phenotypic data. The purpose of plant phenomics, which we describe as the study of the development of plants, is to achieve this and implementation of a set of tools and methodologies that are used to achieve three key objectives—gathering data on the structures, functions, and performances of huge groups of plants, as well as their surroundings; analyzing, organizing, and storing the generated datasets; and constructing models that can untangle and recreate plant activity in a variety of settings. Plant phenomics has advanced significantly in the last decade, with new sensors and imaging approaches being developed for a variety of features, organs, and conditions. When it comes to turning sensor data into knowledge, however, data handling and processing remain significant obstacles (Tardieu et al., 2017).

Plant phenotyping is the study of complex plant characteristics such as growth, development, tolerance, resistance, architecture, physiology, ecology, and yield, as well as the fundamental assessment of quantitative parameters that serve as the foundation for more sophisticated aspects. Photosynthetic efficiency, root shape, biomass, leaf features, fruit traits, and yield-related aspects direct measuring parameters in the plant phenotype, including biotic and abiotic stress response. To enable current genetic crop development, there is a necessity for more effective and reliable phenotyping data, given the fast development of high-throughput genotype screening for associated growth, yield, and resistance to various biotic and abiotic stresses in plant breeding and genomics. Currently, expert visual evaluation is used to assess phenotypic features for disease resistance or stress in breeding programs (Yang et al., 2020). This takes time and may result in prejudice between experts and experimental duplicates as shown in Fig. 6.2.

It will be a huge challenge for plant science and crop development to ensure that crop production is sufficient to meet the needs of a human population that is predicted to reach more than 9 billion by 2050. This aim is difficult to achieve because crop output increases at a 1.3% annual rate, which is insufficient to keep up with population growth. High-yielding, stress-tolerant plants can be selected

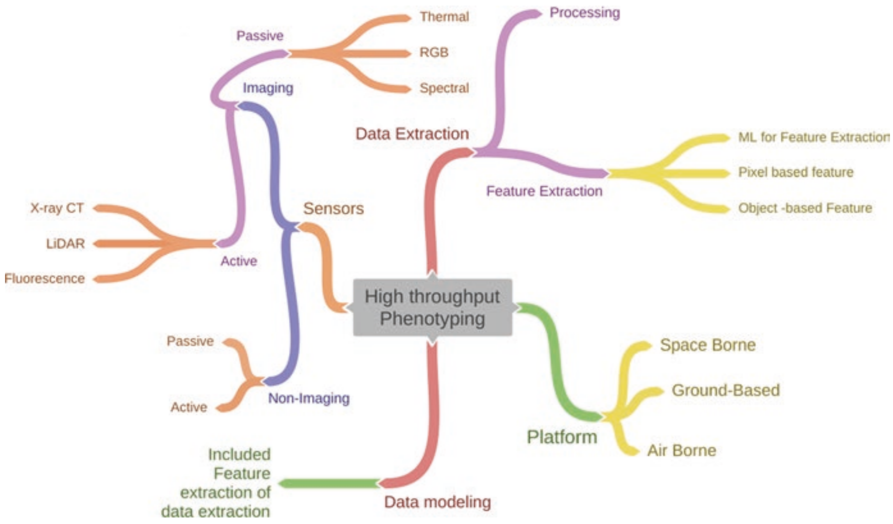

Fig. 6.2 Application of phenomics implemented in breeding program

significantly more quickly and efficiently than is now possible by connecting the genotype to the phenotype. Breeders can benefit from advances in technology such as next-generation DNA sequencing, which might potentially boost the rate of genetic improvement through molecular breeding (Jaradat, 2018). However, our capacity to unravel the genetics of quantitative variables relevant to growth, yield, and stress adaptability is limited because of a paucity of phenotyping skills. Long before the discovery of DNA and molecular markers, plant breeders and farmers made decisions based on phenotypes. The more crosses and habitats that are used for selection, the better the chance of finding a superior variety.

It is necessary to improve breeding efficiency to fulfill future demands. The establishment of huge mapping populations has been aided by high-throughput genotyping and phenotyping panels made up of hundreds of recombinant inbred lines and the creation of massive mapping populations and phenotyping diversity panels made up of hundreds of recombinant inbred lines. Although molecular breeding techniques place a greater focus on genotypic information, phenotypic data is still required. Phenotypes are used for selection and to train a prediction model in genomic selection. A single phenotyping cycle is utilized in maker-assisted recurrent selection phenotyping to develop markers for future selection through generations to find potential events in transgenic investigations (Chaerle & Straeten, 2001).

Breakthroughs in phenotyping are critical for capitalizing on advances in traditional, molecular, and transgenic breeding (Li et al., 2014). Plant phenomics is concerned with defining the plasticity of the plant phenome when subjected to a variety of environmental variables rather than just correlating a genotype with one phenotype in a specific state (e.g., in a controlled environment). In contrast to most animals, which maintain roughly the same structure regardless of their environment, plants can take on a variety of architectural forms depending on the circumstances. After being exposed to either short- or long-day circumstances, the same type of Arabidopsis thaliana can produce a huge 30-leaf plant or a small 8-leaf plant. Water deficiency, nitrogen deficiency, and poor light all have a significant impact on the quantity and size of plant organs. As a result, plant phenomics research concentrates on the study of variation in organism structure, whereas animal phenomics research is primarily concerned with metabolism (Tardieu et al., 2017).

Quantitative Plant Morphology Detection Through Phenomics

Analysis of plant development, production, and formation is called Phenomics. According to (Soulé, 1967), the word “phenome” implies an entire phenotype like; genome manifestation in a certain location; collection of expression in a body belongs to phenome. To get valuable characters from assembled germplasm, forward phenomics act as a tool. Complete analysis of traits exposes systematic understanding and permits the development of this mechanism in new methods.

Measurable and qualitative characteristics of a fused at a certain phase of ontogenesis in certain living conditions of a living system. At a considerable range, a superior proportion of phenotypic data find through phenomics. Skelly, Lobos, Orgogozo, and some other scientists proved that phenomics is usually considered parallel to genomics.

The procedure of development, transformation, and regulation of phenotypic expressions in living systems are examined in phenomics, then decreased to regularities (Skelly et al., 2013; Orgogozo et al., 2015; Lobos et al., 2017) though varies from genomics. Because of differences in phenotypic manifestation of characteristics upon the ecological circumstances, the entire classification of a genome is feasible in genomics, while the entire classification of phenome is not easy in phenomics, have been proved by Houle (Houle et al., 2010). Achievement of molecular genetics and breeding efforts particularly in field crops ever more defines through phenomics (Afonnikov et al., 2016). Plant phenotyping is considered an advanced and practically designed method of plant physiology (Furbank et al., 2011). Phenotypical modification that detect structural variation is quite easy to identify and examine. Stress response phenotypes depend on structural markers to compute stress responses in both ways. Phenotypes of numerous plants were recorded by Parent in 2015 because of the development of visual imagination and remote sensing skills and can easily be calculated at once mechanically and constantly (Parent et al., 2015).

Several sensors are utilized by extraordinary-data examining systems to access some structural characteristics like: plant height, canopy size, leaf area, green leaf pigment, shoot angle, virus spot size, and plant wilt degree. An individual can widely explain the significance of characters through this data like plant manner, nutritional value, drought acceptance, and virus resistance. A number of technologies are applied in various plants. By utilizing the Tomato Analyzer image, fruit shape traits were evaluated in 21 eggplant accessions from four varieties (Hurtado et al., 2013). A total of 23 fruit form parameters were calculated for agreement for fruit shape index, blockiness, homogeneity, proximal fruit end shape, asymmetry, internal eccentricity, and slenderness. (Hui et al., 2018). Three-dimensional (3D) canopy of cucumber, pepper, and eggplant based on multiview stereo (MVS) about plant canopy. By utilizing Crop Circle ACS-470 technique, Jaradat (2018) has isolated phenotypic information and some factors involving less heat tolerance during propagation, premature plant, etc. (Jaradat, 2018). Brassica napus verities that commonly grow in the Midwestern United States having extraordinary yields, and these can be measured through attributes. Tomato biomass and important linear association among expected shoot area, plant numeral biomass was analyzed by a scientist Laxman et al. (2018) using Scan analyzer 3D large scale imaging platform (Laxman et al., 2018). Bernotas et al. (2019) brief about photometer stereoscopic (PS) images, which consist of phenotype arrangement and its eudicot variety, including some vegetables like cabbage, tomato, and oilseed rapes (Bernotas et al., 2019).

Phenotyping of Roots in Plants Through Phenomics

Root system architecture (RSA) explains the spatial configuration of the root system rising from root morphology, topology, and distribution (Lynch, 1995). Root architecture, domestication environment, and techniques may be used to direct breeding programs to connect a root system with a life strategy and agroecology, increasing system adaptability (Bullock et al., 2017; Schmidt et al., 2016). Hypocotyl roots, as well as basal roots in some species, initiate epigeal germination between the radical and the cotyledons, which are elevated out of the soil. Above the basic root, there are three groups of epigeal germinators (Zobel, 2011). Basal roots of common beans can be divided into three types (Zobel & Waisel, 2010). Hypocotyl, main, and basal roots are among the types of roots. Basal roots emerge from the hypocotyl's base. Root morphology (Zhu et al., 2011), leaf features (Micol, 2009), biomass (Tackenberg, 2007; Golzarian et al., 2011), yield-related traits (Duan et al., 2011), photosynthetic proficiency (Clark et al., 2011), and abiotic stress response (Rellan-Alvarez et al., 2015) are the most widely considered phenotypic traits Here, we will go through some of the most important plant phenotyping instruments, as well as some of the most promising photonics-based technologies.

As a result, despite notable breakthroughs, there are relatively few publically available root phenotyping datasets. Laboratory investigations benefit from improved levels of control, and at least in a few cases, loci with fundamental RSA in early root development have been identified. Nonetheless, the growth flasks utilized in these experiments, which were filled with actual or artificial soil (Rellan-Alvarez et al., 2015), limited geographic and temporal explanations for small or immature root systems (Judd et al., 2015; Lobet et al., 2013). Individual tools offer varying degrees of computational automation, ranging from manual to semiautomatic to fully automatic, making this software assembly an exciting prospect. None of these, however, give a combined stage that can (a) combine secondary root images with environmental and phenotypic metadata, (b) provide nontechnical users with continuous access to supercomputing resources, and (c) communicate content within a cooperative team and with the general public.

We formed DIRT in order to speak about these issues. The DIRT stage includes several key features that enable researchers to: (a) manage root picture collections and metadata, (b) interactively standardize dimension pipelines, (c) calculate crop root traits on available high-throughput compute platforms; and (d) analyze computation outcomes. DIRT enables researchers to process thousands of root images, complete the pipeline with routine parameters, and display and analyze calculated RSA output connected to the raw images. As a result, our stage allows researchers with few practical capabilities to access high-throughput computational stages. Thus, automation, remote control, and data (image) investigation pipelines agreeable to HTP stages acceptable showing of large plant populations, germplasm collections (core collections), breeding material, and mapping populations with increased accuracy and precision in phenotypic trait achievement attached with decreased labor input attained by high-throughput (Junker et al., 2015).

More study is needed to fully utilize genomics and molecular breeding methods in crop improvement, which address the creation of phenotyping tools and technologies, phenomics for a specific trait, phenotyping requirements, ongoing initiatives, and obstacles (Furbank & Tester, 2011; Cobb et al., 2013; Lobet, 2017). This review study aims to: emphasize the importance of phenomics and phenotypic constraints in crop improvement in the genomics era, (i) review the current status of phenomics stages and accommodations worldwide, (ii) emphasize the use of high-throughput phenomics platforms for trait separation in different crop plants and detection of genes/QTLs for a variety of traits in different crop plants, and (iii) emphasize the need for phenomics files and phenotyping. Responsible root function in soil, as well as root structure and growth screening, has long been a fascinating area (Gregory et al., 2009). For cereal species growing on stored soil moisture, access to water at penetration is critical for drought tolerance, and a study using model species to identify genes relevant for root characteristics is now underway. Small, short-lifecycle crop models, which are better suitable for cereal species, have recently been produced and are great systems for phenomic display (Watt et al., 2009).

This topic denotes a few technologies ranging from imaging in thin layers of soil or reproduction media to MRI and X-ray CT-scanning (Faget et al., 2009; Nagel et al., 2009). Root crown phenotyping occurs at the apex of crop root systems and can be utilized for marker-assisted breeding, genetic mapping, and a more sympathetic understanding of how roots inspire soil resource acquisition. There are a number of imaging methodologies and picture series available, but none of them are optimized for high-throughput, reproducible, and vigorous root crown phenotyping. The RhizoVision Crown stage includes an imaging unit, picture detention software, and image analysis software that have been upgraded to remove measurements from huge numbers of root crowns in a uniform manner to identify that root crown shapes. The hardware platform uses a backlight and a monochrome machine vision camera. The RhizoVision Imager and RhizoVision Analyzer are free, open-source applications that improve picture capture and analysis by incorporating spontaneous graphical user boundaries.

Physical validation of the RhizoVision Analyzer was done using copper wire, and feature validation was done with 10,464 ground truth simulated images of dicot and monocot root systems. The soybean and wheat root crowns were then phenotyped using this platform. The researchers phenotyped 2799 soybean (Glycine max) root crowns from 187 lines and 1753 wheat (Triticum aestivum) root crowns from 186 lines in both species; principal component analysis revealed comparable connections between characteristics. The greatest heritability was 0.74 in soybean and 0.22 in wheat, demonstrating that species and population variations must be taken into account. The RhizoVision Crown platform enables HTP of crop root crowns and establishes a benchmark against which open plant phenotyping platforms can be measured.

The total volume of soil that roots can investigate is influenced by RSA, which is shaped by interactions between genetic and environmental components (Schmidt et al., 2016). The number, length, growth angle, elongation rate, diameter, and branching of axial and lateral root phenes (or elemental units of phenotypic) shape the final RSA. Weaver and colleagues (Weaver, 1925; Weaver & Bruner, 1926) pioneered root-digging, diagramming, and photography methods that have been widely utilized for almost half a century (Böhm, 2012). These classical methods were since changed by Stoeckeler and Kluender (1938) with the use of water to remove soil particles from the root systems on a large scale and the use of high-pressure air to enter soil pores while leaving roots complete (Kosola et al., 2007). Devised hydro pneumatic root elutriation to give a rapid and repeatable method for extracting roots from the soil of field-composed soil important samples with minimal harm (Smucker et al., 2009).

Traditional digging methods are best for trees and shrubs because the root systems of woody plants are often stronger and more resistant to breakage than grasses or annual crops. The existing inability to quantify root architecture in the field is a major hindrance to current “phenomic” technologies’ claims of marker-assisted selection for better root system features. Traditional field approaches for root phenotyping, such as digging up soil cores and using standard digging techniques to control root depth, root branching density, and root angle, are still considered the best (Trachsel et al., 2011; Nielsen et al., 1997). Such approaches, however, do not reveal the finer aspects of root architecture, anatomy (for example, root hair densities), or function (e.g., nutrient uptake as a result, identifying crops with increased root architectural traits, as well as developing appropriate instruments for studying root growth in the soil, largely under field settings, remains a major challenge for current plant biology. Under drought conditions, root architecture changes dramatically, favoring the formation of more long lateral roots and root hairs to increase total surface area for better water absorption (Osmont et al., 2007). The increase in root quantity, mostly deeper in the soil, results in an enhanced plant water status, which is required to promote biomass production and yield when combined with techniques that limit water loss, such as stomatal closure, leaf systematic, and leaf abscission.

A new machine vision-based facility for the automated evaluation of yield-related characteristics in rice has been developed. This work resulted in the creation of an integrated facility for completely automated yield trait scoring. The facility can thresh rice panicles, evaluate rice production attributes, and pack loaded spikelets automatically. The accuracy (mean absolute percentage error is less than 5%) and efficiency of this unique machine vision-based facility were demonstrated in tests (1440 plants per continuous 24 h workday) (Table 6.2).

Future Perspective Phenotypic–genotypic integrated breeding as the sequencing technology of crops has advanced. With the emergence of phenomics, breeding has entered a new age. It allows breeders to accurately phenotype many samples. Breeders may be able to correlate a lot more traits with accordant genotypes if it is combined with NGS technology. In current years, even more innovative attempts in the phenomics field have been made; computational approaches like machine learning (ML), deep learning (DL), and artificial intelligence (AI) have been integrated with HTP analyses to anticipate the population of many crops.

Table 6.2 Advantages and disadvantages of HTP application in field crops

Sr. No.	Technology and tools	Advantages	Disadvantages
1	GROWSCREEN-Rhizo	In soil-filled rhizotrons, high-throughput methods for obtaining shoot and root traits were used	In 2D rhizotrons, root development is restricted (rhizobox)
2	PhenoBox	Affordably priced and simple to maintain	Large-scale screening requires a lot of work
3	Field Scanalyzer, a rail-based gantry phenotyping system	High picture resolution is achieved by the integration of many optical sensors	Expensive; small image area; fluctuating ambient light
4	MRI–PET; PET–CT; MRI–CT	3D root system topologies may be obtained in soil-filled tubes	Costly; time-consuming; no specialist prototype for agriculture research
5	TraitMill; Scanalyzer3D; PHENOARCH; HRP	For big populations, dynamic and automatic methods are used to acquire shoot growth, biomass, and a wealth of information	It is expensive to maintain and upgrade; it need the involvement of diverse professionals
6	CPRS, a fixed phenotyping tower	Simple to set up and maintain	There was only a limited amount of agricultural information available in fixed regions

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Chapter 7
Basil (*Ocimum basilicum* L.): Botany, Genetic Resource, Cultivation, Conservation, and Stress Factors

Mahmut Camlica and Gulsum Yaldiz

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Introduction

The basil name is derived from the Greek word as basileus, and it means “king.” It includes over 150 species distributed in the tropics and subtropics region of the world. *O. basilicum*, *O. gratissimum*, *O. xcitriodoru*, *O. americanum* L., *O. minimo* L. and *O. tenuiflorum* L. are the most cultivated species of basil. These six species are grown widely in the course of temperate and tropical regions for the essential oil

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(EO) (Blank et al., 2004). It is an open and insect-pollinated plant. The inflorescence of basil goes on from June to September, and it excretes a considerable nectar amount (Nurzyńska-Wierdak, 2000).

It was reported that morphological properties of Ocimum species had a high level of diversity in previous studies. Morphological properties such as leaf color, leaf shape, blooming, flower, seed, and stem have important roles in the identification of Ocimum species (Svecova & Neugebauerov, 2010; Agarwal et al., 2013). Moreover, taxonomic classification and identification of Ocimum genotypes could depend on morphochemical and molecular analysis (Chowdhury et al., 2017).

Ocimum basilicum is an important species economically in the Ocimum genus, and it has extremely varied depending on obtained morphological and chemical properties (Salles Trevisian et al., 2006; Nurzyñska-Wierdak, 2007a, b; Zheljazkov et al., 2008; Abduelrahman et al., 2009). Distinct morphological diversity within basil has been heightened by centuries of cultivation with great variation in pigmentation, leaf shape, size, herbage, and EO yield. The EOs in the leaves contribute to the fragrance and refreshing taste, being a particularly rich source of phytochemicals. Quality and quantity of basil EO change depending on many different factors such as seasonal variation, harvest method, leaf development stage, and ecological conditions (Raseetha Vani et al., 2009).

Besides the genetic diversity of basil genotypes, growing conditions and environmental factors are important topics in basil cultivations. Using and managing fertilizers were the main important factor in growing medicinal and aromatic plants. Suitable fertilizers should be chosen to help obtain desired properties such as quantity and quality of plant (Larimi et al., 2014). It was reported that the studies on herbage and oil yield of basil on the effect of the organic and inorganic fertilizers are insufficient (Al-mansour et al., 2018).

Refaat and Saleh (1998) suggest that the manure mixtures as multi-composition of manures to plants because of including microelements and affect the herb yield and EO quality. It is noted that basil needs quite significant nutrition and fertilizer (Nurzyńska-Wierdak et al., 2012). Different organic manures affect the morphology, yield, and quality properties of basil (Yaldiz et al. 2019a, b). Besides organic manures, basil replies strongly against inorganic fertilizers such as nitrogen and potassium fertilizers (Golcz et al., 2006; Sifola & Barbieri, 2006; Daneshian et al., 2009; Biesiada & Kuś, 2010). Arabaci and Bayram (2004) and Golcz et al. (2006) reported that nitrogen fertilization increased the basil leaf weight and effected the chloroplast dyes and yield of EO. Moreover, increased nitrogen doses increased the basil yield (Biesiada & Kuś, 2010).

So, organic manures and biofertilization are very important for medicinal and aromatic plants to produce the best product in both quantity and quality, and it is safe for humans, animals, and the environment.

When considering these features, this study provided information on botany, origin, distribution, agricultural systems, agronomical studies, growing conditions, and genetic diversity of basil. Also, it was aimed to contribute to the cultivation and utilization of basil with this study.

Botany and Distribution

The Ocimum genus was identified firstly by Linnaeus (1753), and it was listed as five species. Secondly, in 1832, this genus was broadened until 40 species were grouped into three sections: *Ocimum* (*Ocymodon* Benth.), *Hierocymum* Benth., and *Gymnocymum* Benth. (Paton et al., 1999; Carović-Stanko et al., 2010). Existing interspecific hybridization within the Ocimum genus has caused a taxonomic challenge, and very few taxonomic studies have been published on basil which follows by the International Code of Botanical nomenclature in the early twenty-first century (Grayer et al., 2002; Labra et al., 2004). That is why Labra et al. (2004) combined the analysis of morphological traits, EO composition, and molecular markers, which should be optimized to overcome the species identification difficulties.

Some known techniques such as morphological, chemical, karyological, and molecular means have been used to solve the genetic diversity and relationship among the basil species through the years. At the end of the studies, some basic chromosome numbers were recorded. The basic chromosome numbers were reported as x = 12 for Ocimum species. *Ocimum basilicum* clade *is* tetraploid and *O. americanum* clade is hexaploids. Chromosome numbers could be found x = 10 for *O. gratissimum* and x = 9 for *O. tenuiflorum*. The molecular breedings showed that *Ocimum* spp. has different genome sizes and chromosome numbers, and these differences have the most important role in the evolution process by using sequence deletion/amplification, chromosome rearrangements, and polyploidization (Gupta et al., 2018).

Generally, commercial basil cultivars belong to the species *O. basilicum*, and these are divided into seven categories as shown in Fig. 7.1 (Darrah, 1980).

The International Union for the Protection of New Varieties of Plants (UPOV) (2003) improved a standardized descriptor list to determine the genetic diversity based on the morphological properties of basil. This descriptor list was approved as a strict selection of morphological properties, and attentive analysis contributed to routine screening and managing the basil germplasm accessing as cheap and reliably. A similar small study was conducted by Carović-Stanko et al. (2011) to add literature for recognition and categorization of basil morpho types to solving morphological properties of basil accessions. It was noted that the Ocimum genus was classified into six clusters. One of them was basil. It was created from two clusters within these six clusters depending on EO components. These two clusters are separated according to rich linalool and estragole contents from other Ocimum genera.

Origin, Domestication, and Spread

Basil may be originated from North-West India, North-East Africa, and Central Asia (Randhawa et al., 1992) and is widely cultivated in India, Iran, Japan, China, and Turkey (Sadeghi et al., 2009). Another study reported that the widest genus of

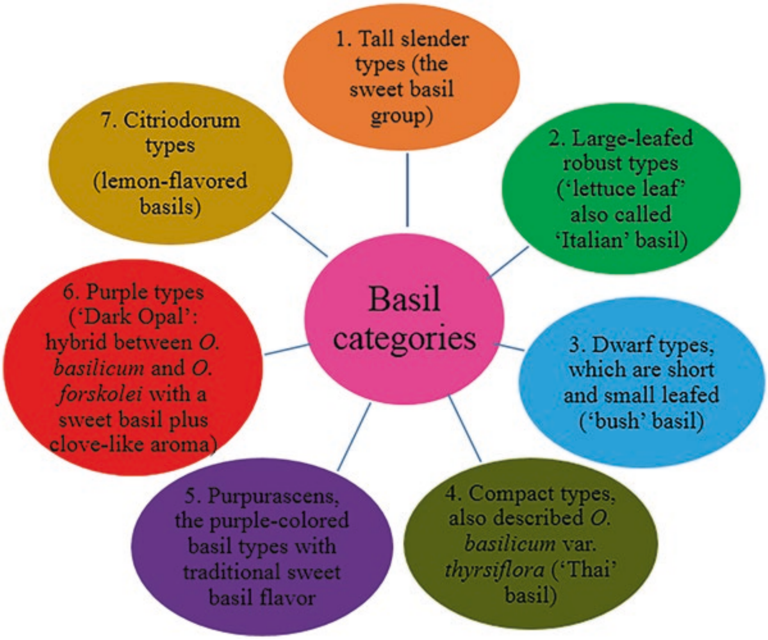

Fig. 7.1 7 divided categories of commercial basil cultivars

the Labiatae family is Ocimum, and it is native to tropical and hot temperature regions. It is symbolized by the annual and perennial aromatic bushes from the largest species number in the African continent (Mukherjee & Dutta, 2007; Chowdhury et al., 2017). Likewise, Kurd et al. (2017) noted that basil is grown in some countries of Asia (Iran, India, and Turkey), warm and subtropical regions of Africa, and central South America.

Choi et al. (2020) conducted a study to determine the origin of basil seeds using hyperspectral imaging analysis (HIA). HIA used the basil seeds to discriminate their origins as Singapore, India, Pakistan, or Vietnam. The dominant wavelengths were useful in classifying the basil seeds, which were approximately 1449–1457 nm, 1242–1254 nm, 1380 nm, and 1696 nm (associated with moisture content, crude lipid content, total phenolic compounds, and fatty acids). The results of the study showed that seed color values were found to be very similar in different origins, and these properties did not provide the basis for good discrimination. Other properties (moisture and crude lipid content) were found significantly different between groups. They recommended the HIA can be used to discriminate against basil seeds.

Plant Genetic Resources

Plant genetic resources (PGRs) are the main source of agro-biodiversity (Upadhyaya et al., 2008). The plant genetic source is an important topic in determining the wild and cultivated species. Ocimum grows in six continents, and it is cultivated in different countries such as Turkey, China, India, Japan, and Iran (Sadeghi et al., 2009). Landraces, cultivars, breeding lines, wild species, and weedy types comprise the PGRs for the traditional plant species (IPGRI, 1993). These species are used as the genetic materials for plant development programs. Some factors such as urbanization, environmental factors, and cultivation practices affect the PGRs. Over the years, many countries have collected the conservation of these PGRs in the world in over 1750 gene banks with more than 7.5 million gene bank accessions (FAO, 2013). In this context, PGRs are collected to achieve food security and reduce poorness in developing countries (Upadhyaya et al., 2008).

Many gene banks for basil can be listed, but the most important gene bank can be described by the United States Department of Agriculture Department from the USA. Also, many official and special institutions and universities developed many basil cultivars and lines in different countries such as Turkey and other countries. In Turkey, it was developed the varieties such as Large sweet, Compact, Moonlight, Midnight, Dino, Morfes, and Limoni.

Collections and Conservation

Different basil species seeds are collected to evaluate the yield and quality properties and desired properties and are grown for the breeding programs and basil producers. The basil seed should be collected in mature time to obtain high seed viability. Information on some collection, gene banks, and conservation was given in Table 7.1 for basil.

The different conservation strategies should supplement each basil plant and help protect maximum diversity as much as possible. Four strategies called such as ex-situ, on-farm, in-situ conservation, and breeding adopted are identified. These strategies should be classified into two groups: static (seed, tissue, pollen, DNA, and cryobanks) and dynamic (in-situ and on-farm conservations, community and household seed banks, and field gene banks) (Joshi & Ghimire, 2017).

Characterization and Evaluation

The genetic pool in plants is an important factor for breeding programs. The existing genetic sources can be used for diversity studies. The Ocimum genus has different genotypes, which have been used in genetic diversity studies. Also, different

Table 7.1 Information of collection, gene bank, conservation of basil in some different countries

Number of accession	Basil species	Collection site, gene bank and conservation	Reference
38 accession (different Ocimum spp.)	*O. americanum* L. var. *Americanum, O. americanum* L. var. *pilosum* (Wild) Paton, *O. basilicum* L. 'Dark Opal', *O. basilicum* L. 'Purple Ruffles', *O. basilicum* L., *O. basilicum* L. *var. Difforme, O. basilicum* L. *var. Thyrsiflorum, O. basilicum* L. *var. thyrsiflorum, O. campechianum* Mill., *O. gratissimum* L., *O. kilimandscharicum* Guerke*, O. minimum* L., *O. selloi* Benth., *O. tenuiflorum* L., *O. ×citriodorum* Vis.	Embrapa, Brazil-Kew Garden, U.K.-USDA, U.S.-Richters Co., Canada-Purdue Univ., U.S. Companion Plants, U.S.-Nichols Garden, U.S.	Vieira et al. (2003)
25 commercial varieties-two hybrids	*Ocimum basilicum* (26 genotypes)-*Ocimum x citriodorum*	Research Farm of Universidade Federal de Sergipe, Brazil	Alves et al. (2019)
7 populations of two species of Ocimum	*Ocimum basilicum* var. grand vant (3 genotypes), *O. basilicum* var. *porpuroscens* or dark opel basil, *O. basilicum* var. *finoverde*, and *O. kilimandscharicum*	A number of localities representing natural populations of two species-Botany Department, Faculty of Science, Tanta University, Egypt	Mustafa et al. (2006)
46 basil genotypes-four local cultivars	*Ocimum basilicum*	Collection from USDA gene bank and Bolu Abant İzzet Baysal University, Agriculture Faculty, Field Crops Department, Turkey	Yaldiz and Camlica (2021)
12 basil samples from 9 cultivars	*Ocimum basilicum*	Stazione Sperimentale per le Industrie delle Essenze e dei Derivati dagli Agrumi (SSEA), Reggio Calabria, Italy	De Masi et al. (2006)
Nine landraces	*Ocimum basilicum*	Sultan Qaboos University, Oman	Al-Maskri et al. (2013)
Four cultivars	*Ocimum basilicum* L. (Kardinal, Litra, Minette, Petit, Anis Blanc	Maria Curie-Skłodowska University Botanical Garden, Lublin, south-eastern Poland	Nurzyñska-Wierdak (2014)
28 basil accessions	*Ocimum basilicum* L.	19 accessions from biodiversity instute, 9 accessions from wondo genet agricultural research center from different part of the country, Ethiopia	Egata et al. (2017)

(continued)

Table 7.1 (continued)

Number of accession	Basil species	Collection site, gene bank and conservation	Reference
Nine genotypes	Two varieties from *O. tenuiflorum* L. (Purple and Green type, commonly known as Krishna and Radha tulsi respectively), two varieties from *O. basilicum* L. (Babu and Marua tulsi), two varieties from *O. gratissimum* L. (Ram and Ajowan tulsi) and single species from *O. africanum* Lour. (Lebu/Bon tulsi), *O. americanum* L. (Bon tulsi) and *O. kilimandscharicum* Guerke. (Karpur tulsi)	Collected from different place of the district of Dakshin Dinajpur, West Bengal, India	Chowdhury et al. (2017)

genotypes provide the required raw materials for the next breeding studies to select the desired properties such as morpho-chemotypes and disease resistance and to obtain the highest agronomic properties (Gupta et al., 2018).

Egata et al. (2017) determined the genetic diversity of 28 Ethiopian sweet basil accessions. The genetic diversity was found by using the standardized descriptor developed by International Union for Protection of New Varieties of Plants (UPOV, 2003; Nassar et al., 2013).

Similarly, Srivastava et al. (2018) carried out a study to find the genetic and chemotypic variability in basil germplasm towards future exploitation consisting of 60 accessions using Mahalanobis D^2 analysis. The results revealed seven groups, and differences were found between genetic divergence and geographical origin in basil germplasm. Plant height changed between 71.33 and 118.86 cm, primary branch ranged from 4.53 to 8.03 number/plant, days to 50% flowering was found between 65 and 74 days, inflorescence length varied from 12.92 to 23.39 cm, leaf area ranged between 3.94 and 9.05 cm^2, herb yield/plant was found between 363.33 and 3228.33 g, EO content ranged from 0.40 to 0.84%, and EO yield was found between 2.06 and 12.81 ml in basil germplasms.

Chowdhury et al. (2017) conducted a study to determine the genetic diversity in nine basil genotypes by using morpho-molecular and chemical analysis. Totally 35 traits (18 qualitative and 17 quantitative morphological traits and chemical compositions) were assessed. Bract length, seed color, seed mucilage, bract width, petal length, petal width, inflorescence length, inflorescence type, leaf tip, leaf shape, leaf area, leaf width, the number of whorls/inflorescence, leaf length, petiole length, anther color, inflorescence color, and leaf surface were determined in Ocimum species. Principal component analysis (PC) was used in these species, and eigenvalue, variability (%), and cumulative (%) were reported as 13.226, 8.817, 5.299, 40.079, 26.717, 16.059, 40.079, 66.796, and 82.855 in PC1, PC2, and PC3, respectively.

Molecular markers were used for the genetic diversity of basil genotypes. Vieira et al. (2003) revealed the genetic diversity of 37 basil accessions based on randomly amplified polymorphic DNA (RAPD) markers. 14 *Ocimum basilicum* accessions among the 37 Ocimum accessions showed acute genetic variation.

Yaldiz and Camlica (2021) determined the agro-morphological and phenotypic variability of sweet basil genotypes for breeding purposes, and 13 morphological yield properties and 23 UPOV criteria were evaluated in different origin basil genotypes. Constellation plot analysis was carried out to determine the genetic diversity by using the reported properties in 50 basil genotypes. It was noted that most of the same origin genotypes were found in the same group, and other same origin genotypes were found in different groups.

When the previous studies were examined, the basil genotypes had different variations. Genetic diversity in basil genotypes can be used to improve new varieties adapted to changing environmental conditions and obtaining specific desired properties.

Use of Basil Genetic Resource

Ocimum genus has a large of species, and these species have different properties as morphological traits and chemical compositions. Basil has many different uses worldwide as scientifically and socially. It is used as a sign of love, and women place a pot of basil on the balcony to show ready for marriage to their suitors. If men bring a sprout of basil, the intention is seen as a seriously signal in Italy (Darrah, 1972). This genus is used as a spice, medicinal treatment, and oil-producing plant. The EO of basil is one of the most important oil because of its use in aromatherapy. In addition, it is used as antidepression, antimicrobial, and antioxidative properties (Muralidharan & Dhananjayan, 2004; Gora & Lis, 2005; Murbach Freire et al., 2006).

Basil has been used in both Ayurvedic and Unani systems of medicine. Different parts of sweet basil have been widely used in traditional medicine. Its leaves and flowers are used in folk medicine as a tonic and vermifuge. Basil tea is good for treating nausea, flatulence, and dysentery. The oil of the basil has been found to be beneficial for the alleviation of mental fatigue, colds, spasm, rhinitis, and as a first aid treatment for wasp stings and snakebites. Also, previous studies on basil revealed that basil possesses central nervous system (CNS) depressant, anticancer, cardiac stimulant, hepatoprotective, hypoglycemic, hypolipidemic, immunomodulator, analgesic, anti-inflammatory, antimicrobial, antioxidant, antiulcerogenic, chemomodulatory, and larvicidal activities (Mueen et al., 2015).

Basil was used in human studies for potential health benefits in metabolic disorders, cognitive enhancement, strengthening the immune system, and oral and skin health (Singletary, 2018).

The reports are limited to the typical human intake and bioavailability of basil. Intakes of fresh and dried basil were determined as 2.2 g/mo by Norwegian adults (Carlsen et al., 2011). Volunteers who consumed an infused basil in a 5 ml beverage prepared from 500 g basil and analytes levels revealed that the extracts did not show any carcinogenic effect on humans (Barfi et al., 2016).

Ecology and Cultivation of Basil

Agricultural Systems

The sown area of the world is limited, and the present soil is not enough for crop production, such as soil degradation and water deficiency. So, new developing and modified agricultural systems should be developed for the urban area (Lehman et al., 1993; Lal, 2013). In this context, alternative food production systems can undertake an important role in the agriculture future to resolve the lack of land, soil, and water limits.

Aquaponics consists of the unification of hydroponics and recirculating aquaculture in a single water cycle (Diver & Rinehart, 2010). In other words, aquaponics is a combination of hydroponics and aquaculture, and produced plants and aquatic species can be grown together in a soilless water-based system (Seawright et al., 1998; Rakocy et al., 2006). Nutrients are supplied by fish waste entirely or partly for the nutrition of the plant, and the fish waste turns into nitrogen (N) form from ammonia (NH4) form (Wilkie, 1997; Rakocy et al., 2006). Levels of nutrients are generally lower in aquaponics compared to conventional hydroponics (Bittsanszky et al., 2016).

It was reported the use of hydroponics and aquaponics agricultural systems are suitable for the soilless agricultural system in urban areas, and they are highly productive in meeting food demand and eliminating soil deficiency. Moreover, plant selection in the soilless system is a crucial topic. Basil can be grown under these systems. It had a higher yield in the soilless system compared to the conventional system (Medina et al., 2016).

According to previous studies, Rakocy et al. (2004) conducted a study on basil to compare the batch and staggered cropping system. It was reported that grown under aquaponics had more yield with 1.8 kg/m^2 than grown under field conditions with 0.6 kg/m^2. Similarly, Saha et al. (2016) reported basil growth, yield, plant quality, and nutrition under soilless agricultural systems (in aquaponics and hydroponic systems). They said that the aquaponics system had higher basil values than the hydroponic system in height, fresh weight, and dry weight at 14%, 56%, and 65%, respectively. But, the chlorophyll content or leaf nutrients found similar values between the aquaponics and hydroponic basil systems.

Similarly, Yang and Kim (2020) determined the nutrient composition and concentration in basil in aquaponic and hydroponic systems. It was reported that plant height was found as 39.9 and 49.8 cm, leaf length was noted as 11.4 and 12.9 cm, leaf number was found as 118.3 and 140.7 per plant, total fresh mass was reported as 306.3 and 545.9 g/plant, and total dry mass was found as 29.8 and 52.4 g/plant in the aquaponic and the hydroponic system, respectively. Average mineral nutrient concentrations of basil in these systems, NO_2-N, NH_4-N, Na, and Cl rates, were noted to be higher in hydroponics than in aquaponics. Other mineral nutrient concentrations such as PO_4-P, K, Ca, Mg, and SO_4-S were found higher in aquaponics. Other aquaponics and hydroponics studies on basil were summarized in Table 7.2.

Table 7.2 Results of different agricultural systems on basil

Growing system	Examined Traits in basil	Values	References
Hydroponics	N	3.28 g/m^2	Bulgari et al. (2017)
	P	0.48 g/m^2	
	K	3.24 g/m^2	
	Ca	0.88 g/m^2	
	Fe	242.8 mg/m^2	
	Mn	13.93 mg/m^2	
	Chlorophyll a	0.610 mg/g FW	
	Chlorophyll b	0.205 mg/g FW	
	Phenols	298 GAE µg/g FW	
	Anthocyanins	14.41 µg/g FW	
	Carotenoids	0.126 mg/g FW	
Aquaponics+ non-inoculated and inoculated basil with strains of *Azospirillum brasilense*	Stem diameter	3.13–3.20 mm	Mangmang et al. (2016)
	Leaf area	108.6–141.8 cm^2/plant	
	Shoot fresh weight	4.63–5.32 g/plant	
	Root fresh weight	3.12–4.97 g/plant	
	Shoot dry weight	416.1–466.6 mg/plant	
	Root dry weight	214.3–265.4 mg/plant	
A closed hydroponic system + plant density (10, 16, 20, 25, and 40 plants/m^2)	Leaf fresh mass	1.27–3.48 kg/m^2	Maboko and Plooy (2013)
	Leaf area index	2.88–7.49 m^2/m^2	
	Leaf dry mass	0.26–1.29 kg/m^2	
	Shoot fresh mass	1.05–2.85 kg/m^2	
	Shoot dry mass	0.13–0.31 kg/m^2	
	Total plant fresh mass	2.45–6.32 kg/m^2	
	Total plant dry mass	0.39–1.61 kg/m^2	
Hydroponic system + salinity (0, 40, and 80 mmol/L)	Plant height	Between 15 and 30 cm	Santos et al. (2019)
	Stem diameter	Between 3 and 10 mm	
	Root length	Between 15 and 30 cm	
	Leaf number	Between 30 and 100 number	
	Total dry matter mass	Between 1.5 and 8 g/plant	
	Water comsumption	Between 15 and 25 L/plant	

The present studies revealed that basil could be produced in both hydroponic and aquaponic systems together with restricted water and without soil. These systems affect the morphological and yield properties of basil genotypes positively under different growing conditions.

Agronomic Studies on Basil

Different agronomical applications or genetic diversity were conducted on basil germplasm by many researchers. In this context, basil cultivar and genotypes showed differences based on their agro-morphological (leaf and flower color, leaf size and shape, plant height and habit) and their chemical contents (Nurzyñska-Wierdak, 2007a, b; Hussain et al., 2008; Abduelrahman et al., 2009). Many studies were carried out by researchers to find morphological and chemical properties of basil genotypes and cultivars in different parts of the World. Especially, morphological and chemical properties were determined using different data, and described properties were used to catalog and characterize 80 basil genotypes in different geographical regions of the world such as India, Singapore, Tanzania, Thailand, and the Slovak Republic. Obtained morphological properties showed a large variation among themselves. It was reported that plant height changed between 56 and 126 cm, primary branch number was found to 4–8, internode length ranged from 2.5 to 7.9 cm, inflorescence length varied from 16 to 25 cm, leaf area changed between 1.81 and 8.11 cm, per plant herb yield found between 500 and 3125 g, EO content reported between 0.2 and 0.98%, and EO yield in per plant varied from 0.5 to 12.21 ml (Gupta et al., 2018).

It was reported that a study was conducted to determine the morphological variability and EO composition of four basil cultivars (Nurzyñska-Wierdak, 2014). These cultivars had 24.8–64.5 cm plant height, 32.0–49.1 cm plant diameter, 34.6–70.2 mm leaf blade length, 15.1–38.5 mm leaf blade width, 9.2–10.9 number of the main branches, 19.9–29.0 inflorescences number per plant, 13.4–29.0 mm inflorescence length, 11.2–14.2 mm verticils number in the main inflorescences, 5.8–6.0 flower number in the verticils, 185.9–358.8 g fresh weight per plant, and 28.7–58.2 g air-dry weight per plant. Also, EO compositions of the basil cultivars were noted as 29.6–77.5% for linalool, 0.3–32.5% for methyl chavicol and followed by eugenol (null to 27.6%), (E)-methyl cinnamate (null to 11.2%), 1,8-cineole (1.0–9.4%), α-cadinol (2.8–6.5%), and germacrene D (2.1–4.1%) as most abundant components.

Raseetha Vani et al. (2009) conducted a study to investigate the variability of EO composition of basil in seasonal variation. The highest EO composition was reported as 80.95% in methyl chavicol (estragole) in the month of April 2003 for basil species.

The green and purple basil genotypes were introduced in the northern parts of Turkey by using the herb yield, EO content, and EO components under different growing conditions (open field and underneath or between kiwi plantations). The properties were determined and compared for the response of different growing conditions. It was reported that plant height ranged from 36.0 to 98.7 cm, fresh herb yield was found between 516.3 and 6184.2 kg/da, dry herb yield varied from 68.9 to 426.7 kg/da, EO content changed between 0.47 and 1.83%, and major EO components were found as linalool (6.9–42.7%), methyl cinnamate (8.2–19.2%), and farnesene (0.2–21.1) in identified 12 EO components. The study reported that the highest fresh herb yield, dry herb yield, EO content, and EO components such as methyl cinnamate and linalool were found in open field conditions in green basil (Yaldiz et al., 2015).

The agronomic studies revealed that basil genotypes had a large variation depending on their examined properties.

Use of Organic Manure and Inorganic Fertilizers in Basil

Organic manures are one of the most important topics in soil fertility management in agriculture. It was reported that the effects of the available organic manures were evaluated on the yield of basil produced in different regions. In this context, many studies have been reported to determine the response organic manures on yield, yield components, and quality properties in basil (Anwar et al., 2005; Sharafzadeh & Alizadeh, 2011; Safaam et al., 2015; Yaldiz et al., 2017, 2018a, b, 2019a, b; El-Ziat et al., 2018).

Yaldiz et al. (2019a, b) conducted a study on basil to determine the effects of poultry manure on the yield and nutrient composition of sweet basil. Different organic manure doses as 7.5, 10, 12.5, and 15 t/ha chicken and turkey manures and with a control (no manure) and conventional fertilization were used. At the end of the study, it was reported that the highest fresh and dry weights were obtained from the plants grown with turkey and chicken manure at a rate of 10–12.5 t/ha, and most of the nutrient content varied significantly with organic poultry manure applications. It was also noted that chicken manure affected the nutrient as potassium (K) and magnesium (Mg) concentrations of basil.

A study was conducted by Treadwell et al. (2011) to determine the organic fertilization programs for greenhouse fresh-cut basil in a soilless media trough system. Four national organic program-compliant manure treatments were used compared with conventional control. Treatments and their analyses in nitrogen (N), phosphorus (P), and potassium (K) contents are as follows: conventional hydroponic nutrient solution (150 ppm N, 50 ppm P, and 200 ppm K), granular poultry litter (4 N-0.9P-2.5 K), granular composite (4 N-0.9P-3.3 K), granular meal (8 N-2.2P-4.1 K), and granular meal plus a side-dress application of 5 N-0.9P-1.7 K fish emulsion. Macronutrient concentrations (N, P, K) from whole basil leaves at final harvest were similar among the treatments, whereas micronutrients (Ca, Mg) showed significant differences.

Safaam et al. (2015) determined the effect of some fertilizers on improving the growth and oil productivity of the basil Genovese genotype. Organic fertilizer such as compost (10 m^3/fed), biofertilizer (nitrobein+phosphorein), and inorganic fertilizer like algreen 600 foliar spray (1 g/L) were used on some growth parameters, photosynthetic pigments, minerals, and oil yield of basil. Plant height, number of branches, and fresh and dry weight of plant (g) of basil significantly increased depending on used organic and inorganic fertilizer and their combinations. However, the highest EO yield of basil leaf was determined in the interaction of fertilizer compared to individual applications.

Moreover, a study was conducted to determine the effect of organic and inorganic fertilizer on basil yield and quality properties in 2015 and 2016 years. Nine treatments as farmyard manure (FYM) (10 t/ha) + 100% recommended N through FYM, FYM (10 t/ha) + 100% recommended N through FYM + biofertilizer, FYM (10 t/ha) + 75% recommended N through FYM, FYM (10 t/ha) + 75% recommended N through FYM + biofertilizer, FYM (10 t/ha) + 50% recommended N through FYM, FYM (10 t/ha) + 50% recommended N through FYM + biofertilizer, recommended FYM (10 t/ha only), recommended NPK (160:80:80 kg/ha) only, and recommended FYM (10 t/ha) + recommended NPK (160:80:80 kg/ha) were used. Fresh herb yield was found between 22.92–39.95 t/ha, EO content ranged from 0.31 to 0.48%, and oil yield changed between 73.8 and 199.7 kg/ha. The result of the study showed that recommended FYM (10 t/ha) + recommended NPK (160:80:80 kg/ha) had the highest values in terms of examined properties (Al-mansour et al., 2018).

Another study was conducted by El-Ziat et al. (2018) to find the organic manure and humic acid versus chemical fertilizers on red Rubin basil. These applications applied the growth, EO production, and external secretory structures of basil compared to the recommended dose of chemical fertilizer. Control, NPK, organic manure (100 g/pot), and different humic acid doses (1 and 2 g/l) were evaluated. The highest plant height, number of branches, leaf area, fresh herb weight, dry herb weight, the steam ratio of leaf, oil percentage (%), and EO yield (ml/plant) were found between 35.22 and 46.67 cm in the first cut, 5.83–12 number/plant, 1.37–3.74 cm^2, 176.6–300.3 g/plant in the second cut, 16.94–22.22 g/plant in the first cut and 5.90–12.16 in the second cut, 0.01–0.19 in the first cut and 0.09–0.50 ml/plant in the second cut, respectively.

A study was conducted by Biesiada and Kuś (2010) to determine the effect of nitrogen fertilization doses (50, 150, and 250 kg N/ha) and irrigation on the yielding and nutritional content of basil. The total yield means ranged from 2.65 to 4.34 kg/m^2 in nitrogen fertilizer doses. It changed to 4.29 kg/m^2 and 2.55 kg/m^2 in irrigation and without irrigation conditions, respectively. Nitrate content varied from 284.00 to 1047.00 mg/kg in different nitrogen doses; it changed between 373.33 and 712.33 mg/kg in irrigation conditions and between 534.33 and 902.00 mg/kg without irrigation conditions. P, K, Ca and Mg contents changed between 0.33–0.55% d.m., 4.59–5.48% d.m., 1.14–2.00% d.m., and 0.19–0.28% d.m., respectively. They reported that basil gave a positive response to irrigation conditions. The highest yield values were noted in 150–250 kg N/ha doses. While the basil yield increased, nitrates, potassium, and phosphorus content decreased in irrigation conditions.

Nurzyńska-Wierdak and Borowski (2011) were carried out to determine the dynamics of sweet basil growth affected by cultivar and foliar feeding with nitrogen. Plant height, plant diameter, number of branches, leaf blade length, and leaf blade width were found as 51.7–78.8 cm, 36.4–41.6 cm, 6.3–12.2 number per plant, 6.0–9.3 cm, and 3.5–5.5 cm, respectively. While the urea application increased the plant height, leaf blade length, and width significantly in basil, plant diameter and branch number did not show any response to urea.

Similarly, Nurzyńska-Wierdak et al. (2012) reported the growth response to nitrogen and potassium fertilization of common basil. Different nitrogen doses of 0.2, 0.4, 0.6, and 0.9 g N dm^3 and potassium doses of 0.4 and 0.8 g K dm^3 were given in the form of sulfate. Plant height changed between 69.7 and 89.5 cm, branch number ranged from 11.3 to 13.5, fresh weight was found between 145.6 and 189.2 g/plant, and air-dry weight varied from 13.6 to 22.5 g/plant. They reported that the effect of the nitrogen and potassium doses did not show significant differences in basil.

Munnu-Singh and Singh (2002) reported that in India, application of N in 150 kg/ha or vermicompost of 2.5 tons/ha with 75 kg N + 25 kg P_2O_5 + 25 kg K_2O/ha application gave similar plant height, grass, and oil yields, and this values were found to be significantly higher than control application.

El-Naggar et al. (2015) investigated the effect of different doses of organic cattle manure ((CM) 0, 40, 60, and 80 m^3/fed) and four biofertilizer concentrations of active dry yeast ((ADY) 0.0, 2.0, 4.0, and 6.0 g), and they reported that the highest vegetative growth values (plant height, branch number, leaf number, leaf area, and leaf dry weight), total chlorophyll content, and oil percentage were obtained from CM at 80 m^3/fed with and ADY at 6.0 g/l biofertilizer. In addition, it has been reported that the main chemical components of the leaf EO (estragole, eucalyptol, linalool, and trans-4-ethoxycinnamaldehyde) have increased significantly in the application of 2.0 and/or 4.0 g/l biofertilizer (ADY) together with 60 m^3/fed CM.

Many studies stated that basil EO components were changed by organic manure combined with ADY treatments, and also methyl chavicol (estragole), β- (E) -ocimene, linalool, eugenol, 1,8-cineole (eucalyptol), and α-(E)-bergamotene components have been found to be the main components in basil EO (Simon et al., 1990; Khatri et al., 1995; Sajjadi, 2006; Telci et al., 2006; Chalchat & Özcan, 2008; Pripdeevech et al., 2010). On the other hand, Anwar et al. (2005) indicated that the application of vermicompost and compost increased the linalool and methyl chavicol content in basil EO.

In another study, according to soil analysis, 135 kg/ha each N, P_20_5, K_20 as base fertilizer and 22–34 kg/ha N application after the first harvest were sufficient for basil in Hawaii's ecological conditions (Hamasaki et al., 1994).

A combined fertilizer of N-P_20_5-K_20 (120–100-100 kg/ha) was found to be optimum for basil production in sandy soils with low nutrient content (Wahab & Hornok, 1982). Tesi et al. (1995) reported that fresh basil weight decreased with increasing dose in the application of soluble fertilizer between 1 and 5 g/l in peat potting mixture. It is reported that excess N fertilizer reduced the postharvest quality of basil (Hamasaki et al., 1994; Davis, 1997). It has been noted by Tesi et al. (1995) basil had the same yield as ammonium and nitrate applications. Tesi et al.

(1997) observed that plant growth and leaf nitrate content increased with increasing N applied at rates of 0–80 kg/ha.

In a study investigating the effect of nitrogen fertilizer on the yield of basil EO, it was found that the rate of EO increased with increasing nitrogen dose, and after a certain nitrogen level, the yield and EO ratio decreased (Youssef et al., 1998).

In a study investigating the effect of fertilizers on the number of leaves, the highest number of leaves was obtained from compost application (56.66) and the lowest number of leaves was obtained from control (no fertilizer applied). In the fertilizer and humic acid application, 22.55 leaves were detected. Generally, using any fertilizer resulted in an increase in the number of leaves per basil (Sirousmehr et al., 2014a, b).

As with all cultivars, the growth and yield of basil depend on the availability of nutrients in the nutritional medium, and the yield and quality are closely linked to the intake of macro and micro elements (Zheljazkov et al., 2008; Dzida, 2010a, b; Sharafzadeh et al., 2011).

It has been reported that increasing the potassium dose did not significantly affect the fresh weight of basil (Sharafzadeh et al., 2011). Also, the interaction of N and K was not found significant in all three harvests of basil (Rao et al., 2007).

The organic and inorganic fertilizers affect the basil genotypes as positively. Different organic manures such as farmyard and poultry manure and different inorganic fertilizers such as nitrogen fertilizer showed positive effects on the yield and quality properties of basil genotypes.

Influence of Some Abiotic Factors on Basil Growth and Secondary Metabolite Production

Abiotic stresses like drought, flood, salinity, and cold significantly affect plant growth, development, and productivity. Plants have developed numerous small organic molecules called osmolytes to protect their cellular machinery against such stresses (Chen & Jiang, 2010; Liang et al., 2013).

So, it is important to understand the various mechanisms controlling different metabolic pathways and their interplay during different abiotic stresses and develop improved crop varieties for realizing complete yield potential.

Furthermore, these stresses can affect the bioavailability of many food ions, and this is an important regulatory factor for EO content and composition. Although the effects of stress factors on EO-containing plants are complex, the effects can be additive, antagonistic, or synergistic. Since there are few studies available so far, it is necessary to conduct studies at the molecular level and to elucidate the genetic basis of tolerance and response in EO species. There is an enormous diversity of EO species, and many types of ecotypes have been identified, such as genes responsible for the secretion of certain components and mutants for salt, water, and metal stress tolerance (Biswasa et al., 2011).

Salinity Stress

Salinity is one of the serious abiotic stresses that negatively affect most field crops; it causes serious product losses economically. Under saline conditions, osmotic and ionic stress lead to the production of reactive oxygen species (ROS) in chloroplasts, mitochondria, and the apoplastic space (Miyake, 2010). This oxidative stress causes membrane peroxidation, ion leakage, and damage to nucleic acids, cell membranes, and cellular structure (Noreen et al., 2009) and ultimately reduces the quality and total yield of the affected crop (Hessini et al., 2015). Additionally, it can adversely affect water quality and soil structure. To overcome salt stress, agriculture needs to look for suitable methods that are economically feasible and cost-effective.

In a study conducted under saline conditions, it was reported that yield values differed between basil genotypes. Although the yield decreased in all genotypes under saline conditions, some genotypes (Isfahan III, Birjand I, and Malayer genotypes) were found to have the highest fresh and dry plant yield values under saline conditions. However, the EO content was higher in all genotypes under salinity conditions. The highest EO ratio in salinity was determined with 2.2% and 2.3% values in Malayer and Birjand I genotypes, respectively (Akbari et al., 2018). Similarly, it was stated that the EO content was affected by environmental and agricultural practices, and salinity can increase the EO content (Charles et al., 1990; Hendawy & Khalid, 2005; Neffati & Marzouk, 2009; Ben Taarit et al., 2009; Estaji et al., 2018). The EO increased due to a higher oil gland density as a result of the leaf being reduced with salinity (Charles et al., 1990; Neffati et al., 2011). Also, net assimilation, segmentation, and differentiation processes can indirectly alter the EO content (Charles et al., 1990).

In many studies, it has been stated that salty conditions reduced the yield values (Neffati & Marzouk, 2009; Yu et al., 2015; Estaji et al., 2018). In addition, salty conditions reduced the plant's water and nutrient intake and caused a decrease in plant growth (Partridge & Jenkins, 2002). Therefore, as the decrease in water and nutrient intake reduced cell division and cell elongation, it decreased plant growth and yield (Estaji et al., 2018).

In one study, basil was grown in non-saline (EC = 0.64 dS/m), low salt soil (EC = 5 dS/m), and high saline soil (EC = 10 dS/m), and they reported that there were differences between arbuscular mycorrhizal (*Glomus deserticola*) colonized plants (+AMF) and non-colonized plants (–AMF). They found that mycorrhiza increased K, P, and Ca intake because of salinity, and also the balance between +K/Na and Ca/Na in AMF plants was improved. Furthermore, they reported that basil plants increased chlorophyll significantly with mycorrhizal inoculation and increased water use efficiency under salinity conditions. This result is based on AMF, which improves gas exchange in basil under salinity stress, and it also increases plant growth, photosynthetic yield, and water use efficiency (Elhindi et al., 2017).

Similarly, Agüero-Fernández et al. (2019) reported that basil plants inoculated with arbuscular mycorrhizal fungi (AMF) showed some degree of infectivity and efficacy of the AMF strain in the rhizosphere region and growing conditions and also showed increased in their morphophysiological variables even under NaCl stress conditions. As NaCl concentrations increased, the colonization percentage decreased, peaked at control (0 mM), and decreased at 50 and 100 mM NaCl.

There have been many studies that have shown that the application of AMF to saline soil fields can increase plant growth and tolerance by alleviating the negative effects of salinity (Al-Karaki, 2006; Daei et al., 2009; Kumar et al., 2010; Abdel-Fattah & Asrar, 2012; Asrar et al., 2014; Allen & Cunningham, 1983; Evelin et al., 2012).

Hasegawa et al. (2000) and Yaldiz et al. (2018a, b) reported that higher ratios of toxic ions such as Na^+ and Cl^- reduced the plant's ability to absorb other ions such as K^+, Ca^{2+}, and Mn^{2+}, damaging the balance between ions.

Similarly, in another study, Na^+ and Cl^- content increased in leaves under salty conditions. Moreover, it has been reported that Na accumulation has less effect on leaf hydration and photosynthetic activity under mild salinity conditions, whereas there is no Na accumulation in control (Attia et al., 2021).

Likewise, in basil genotypes grown with different doses of $CaCO_3$, the highest yield was obtained from high dose $CaCO_3$ administration (12 g/dm^3 substrate). They also found that both variety and calcium carbonate dosage significantly affected the vitamin C content in fresh basil herb (Dzida, 2010).

In a study investigating the response of various doses of nitrogen and potassium in basil, it was concluded that increased potassium did not have a significant effect on plant yield.

Similarly, the increasing level of potassium did not cause significant differences in fresh basil herb weight (Sharafzadeh et al., 2011).

The other studies found a decrease in carotenoid levels in basil leaves under stress conditions. It has also been stated that basil can tolerate salt stress up to 150 mM NaCl, and chlorophyll fluorescence can be used as a criterion to evaluate sweet basil salt tolerance. However, it shows that basil can be grown in soils with salt effect up to a medium level, which may have potential importance in its production (Khaliq et al., 2017; Camlica & Yaldiz, 2017).

Kaya and Inan (2017) reported that salicylic acid (SA) applied to basil leaves increased yield parameters. However, in previous studies, a decrease in plant height, root length, root weight, and fresh weight was reported (Kordi et al., 2013; Fatemi & Aboutalebi, 2012; Bekhradi et al., 2015; Bernstein et al., 2017; Delavari et al., 2014).

Similarly, Angooti and Nourafcan (2015) reported that the application of 1–0.1 mM SA concentration caused reductions in fresh leaf yield depending on the salt concentration in basil. Also, salt stress reduced the number of basil leaves (Fraj et al., 2016).

However, Gharib's (2006) finding that SA application increased the number of leaves in basil. Talaat and Youssef (2002) reported that the application of tryptophan (Trp) increased the yield parameters in basil. Kahveci et al. (2021) reported that using PGE at 1.0 g/L as a foliar application strategy for basil plants grown under DI stress provided higher RWC and WUE and lowered EL. They also found that plant growth and EO productivity increased by limiting the immune effects of DI. They also reported that salinity (100 mM NaCl) reduced shoot and root length, root and leaf weight, leaf number, plant weight, and photosynthetic pigments. However, priming treatments (0.025 and 0.05 mM salicylic acid, 0.25 and 1 mM tryptophan, and 0.1 and 0.2 mM β-carotene for 24 h) partially alleviated the negative effects of salinity on the shoot and root length, root, leaf, and plant weight. However, they found that it had no significant effect on photosynthetic pigments in plants grown under salty conditions. Furthermore, it has been reported that in salty conditions, the percentage of linalool and eugenol from EO components decreased, but the percentage of methyl eugenol increased in non-primed plants. However, prime has been found to increase the percentage of linalool and eugenol while decreasing the percentage of methyl eugenol in plants grown under saline conditions. It has also been reported to cause significant decreases in DPPH activity, total phenolic, flavonoid contents, caffeic acid, chicoric acid, and rosmarinic acid content in salty conditions. However, it made a positive contribution to gas exchange parameters under prime salty and salt-free conditions.

Drought and Low-Temperature Stress

Drought, an important abiotic stress factor, is closely related to salinity. It has an important effect on the growth, development, and survival of plants. Therefore, understanding the complex mechanisms of drought and salinity is essential for agriculture and breeding programs.

In the study conducted under drought conditions, it was found that the maximum number of side branches was not significantly different with control and field capacity of 80%, while the minimum number of side branches was obtained at 60% field capacity (Sirousmehr et al., 2014a, b). This reduces the number of leaves during drought stress because of the aging plant, and the high concentration of ethylene is a way to reduce sweating and allow the plant to escape stress faster. Furthermore, the reason for the decrease in the number of leaves in the plant as drought increases is that drought weakens the cell wall and decreases the cell volume and causes a decrease in growth due to low-pressure potential. These factors reduce the number of leaves and leaf size per plant (Sumner, 2000).

These results were supported by basil studies by Asgharipour and Armin, (2010) and Asgharipour and Rafiei (2011a, b) and also indicated that the high branch was considered an undesirable feature because of the unnecessary consumption and waste of soil moisture in drought conditions. In addition, they explained that the plant creates limited branches to cope with drought in more critical development stages, such as flowering when the plant needs more water.

Drought stress is suggested to increase the EO compositions and gene expression levels included in its biosynthesis. A study was conducted to determine the effect of drought stress on the expression level of four genes (chavicol O-methyl transferase = CVOMT, eugenol O-methyl transferase = EOMT, cinnamate

4-hydroxylase = C4H, 4-coumarate coA ligase = 4CL, and cinnamyl alcohol dehydrogenase = CAD) involved in the phenylpropanoid biosynthesis pathway in *O. basilicum* c.v. Keshkeni luvelou. Changes in the expression levels of CVOMT and EOMT are significantly correlated with methylchavicol and methyleugenol content. Thus, drought stress can be increase the methylchavicol and methyleugenol content, through increasing the expression levels of CVOMT and EOMT (Mandoulakani et al., 2017).

The EO content and chemical composition of sweet basil were determined at different irrigation regimes (100%, 85%, 70%, and 55% of field capacity). The EO was obtained by hydro-distillation of herb and was analyzed by GC and GC-MS. The EO content of herb increased from 1.12% to 1.26% as plant water deficit increased (to 70% of field capacity). The number of components of the oil of *Ocimum basilicum* increased as water stress increased. The main constituents of the oil, such as linalool, methyl chavicol, 1,8-cineole, and trans α-bergamotene significantly affected by water stress (Omidbaigi et al., 2003).

Khakdan et al. (2017) conducted a study to determine the effect of water deficit stress on methyl chavicol and methyl eugenol and compounds together with the expression profiles of five critical genes (4Cl, C3H, COMT, CVOMT, and EOMT) and three basil cultivars at the flowering period. All genes showed different transcription ratios under water deficit stress except for some expectances. It was reported that regulation of genes and production of methyl chavicol and methyl eugenol depend on cultivar and drought stress-dependent mechanism.

In a study investigating the effects of using organic fertilizers such as humic acid and compost on the basil plant in drought stress, it was determined that organic fertilization reduces drought stress. For this reason, it has been determined that compost should be extended for in vitro use in agriculture (Sirousmehr et al., 2014a, b).

Increases in proline concentrations have been noted in basil exposed to water stress, which plays a crucial role in regulating cellular osmoregulation. On the other hand, although the total sugar concentrations of basil plants exposed to stress decreased, it was observed that it was able to synthesize high antioxidant compounds. Also, both temperature and water stress treatments caused oxidative stress in the basil, as shown by a significant increase in Malondialdehyde (MDA) concentrations. An increase in total phenolic and flavonoid concentrations and a rather significant decrease in carotenoid concentrations in basil leaves were observed in response to water stress. On the other hand, it has also been noted a negative correlation was found between temperature and carotenoid content in basil leaves. It has been reported that heat stress leads to a significant increase in total phenolics and flavonoids in the leaves of plants subjected to heat treatment and the strengthening of the former in plants experiencing water stress (Kopsell et al., 2005).

Basil is very sensitive to cold temperatures. It is most susceptible in early spring, slowing its growth and increasing its susceptibility to diseases. When temperatures fall below 5 °C, it causes significant leaf necrosis. Moreover, serious damage has been observed in leaves when temperatures fall below 10 °C (Lange & Cameron, 1997; Ribeiro & Simon, 2007). Therefore, it is cultivated in arid and warning arid countries such as France, Egypt, Greece, and Hungary, especially mostly in India, Indonesia, and Morocco (Padalia et al., 2017). Chang et al. (2005) reported that the best growth of 19 basil genotypes occurred at 25 or 30 °C in their study with different temperatures. In another similar study, Fallah et al. (2015) reported that purple and green basil seeds showed maximum germination at temperatures of 15–30 °C. According to their results, the base, optimum, and ceiling temperatures for basil were 7, 25, and 42 °C, respectively. Similarly, Mijani et al. (2013) stated that the cardinal temperatures (base, optimum, and ceiling or maximums) for sweet basil were 5.7 (base), 30.0 (optimal), and 46.4 °C (ceiling).

The effect of cold stress on gene expression patterns linalool synthase (LIS), β-myrcene synthase (MYS), γ-cadinene synthase (CDS), germacrene D synthase (GDS), and geraniol synthase (GES) of mono and sesquiterpene biosynthesis and their contents were determined in *Ocimum basilicum*. It was noted that the LIS expression increasingly occurred to 4.86-fold at 10 °C after 12 h while that of GES reached 5.7-fold at 10 °C after 48 h. Temperature 4 °C for 12 h increased the expression levels of MYS and GDS genes to 41.5- and 14.2-fold, respectively, while the expression level of CDS increased to 25.5-fold at 4 °C for 48 h (Senji & Mandoulakani, 2018).

Looking Forward or Future Perspective

Planned combinations of organic-sourced manures with inorganic fertilizers provide an integrated nutrient supply to plants, and their importance is gradually increasing in environmentally sensitive agriculture.

The use of organic manure alone or in combination with chemical fertilizers in basil increased herbal and EO yield and nutritional quality and also had a positive effect on nutraceutical properties. Different breeding techniques were used for the improvement of basil cultivars by previous researchers, and in this context, new cultivars were obtained. The Ocimum genus continues to contribute to basil's new cultivars because of including a wide genetic diversity as an open platform for the breeders. Obtained these cultivars should have new skills with improving newer chemotypes for EO content and composition and herb/biomass yield.

Soilless technologies such as hydroponics and aquaponics should be supported for plant production in inefficient and small soil areas by understanding the dominant soil properties. According to previous studies results, basil has a wide genetic diversity as the morphological and chemical profile depending on the agricultural systems and agronomical applications.

Aquaponics and hydroponics systems contribute the sustainable agricultural production in both global and urban areas. They reduce the environmental effects and provide the recirculating wastewater pouring to recirculate in the system.

From this point of view, different agro-morphological and chemical contents are foreseen in the same region, field, and ecological conditions in the same basil genotypes. Genetic diversity has been revealed in different basil cultivars and genotypes by the investigated previous works of literature. It can be reported that basil genotypes have high variations depending on the tested morphological, yield, and chemical traits.

New studies should be conducted on basil in different alternative agricultural systems, including aquaponics and hydroponics agricultural systems, organic and inorganic cultivation, and genetic diversity, to determine the high basil genotype/genotypes as quality and quantity properties in the future. These growing conditions should be carried out together, and the best system should be determined.

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Chapter 8
Multi-Omics Approaches for Breeding in Medicinal Plants

Afifa Younas, Nadia Riaz, and Madiha Rashid

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Introduction

The survival of humanity on earth is dependent on about 12% of the total 300,000–400,000 plant species (Costion et al., 2011; Jiao et al., 2011). Herbal remedies made from plants have been used for over 5000 years. Approximately 60% of plants were claimed to have medical effects in the post-Neolithic age (Chen et al., 2015) (Table 8.1). Active metabolites or chemicals of pharmacological relevance

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Table 8.1 Omics of medicinal plants

Medicinal Plants	References
Genomics	
Brassica napus L.	Chen et al. (2019)
Limonia acidissima	Pradhan et al. (2012)
Capsicum annum L.	Velasco et al. (2007)
Momordica charantia L.	Cai et al. (2013)
Transcriptomics	
Curcuma longa L.	Dinda et al. (2008)
Ophiorrhiza pumila Champ. ex Benth.	Zhang et al. (2012)
Withania somnifera (L.) Dunal	Singh et al. (2015)
Proteomics	
Coptis chinensis Franch.	Valdiani et al. (2013)
Garcinia hanburyi Hook. f.	Talei et al. (2014)
Garcinia oblongifolia Champ. ex Benth	Fang et al. (2012)
Metabolomics	
Aloe vera (L.) Burm.f.	Hartmann-Boyce et al. (2018)
Panax ginseng C.A. Mey	Hao et al. (2014a)
Panax notoginseng (Burkill) F. H. Chen	Hao et al. (2014a)

for humans are produced by medicinal plants. Only 5000 plant species of 250,000–300,000 were fully researched for their therapeutic usefulness (Payne et al., 1991). Trees and tiny, narrow-ranged species both contain medically important chemicals (Cámara-Leret et al., 2017; Nilsson et al., 2011).

Traditional medicinal herbs are extensively used in numerous parts of the world or across continents, such as in South America, Asia, and Africa, as well as in various civilizations (Briskin, 2000; Brusotti et al., 2014; Suárez & Chávez, 2018). Ethnomedicine has been used by roughly 60% of the world's population for centuries to meet their healthcare requirements (Busian & Kasilo, 2010). When the therapeutic characteristics of plants were being investigated in prehistoric times, their scientific significance, the experimental potential for life, molecular mechanisms of medical uses, and the application of new omics technologies were unknown. In the early 1800s, the bioactive component "morphine" was isolated from the medicinal herb Papaver somniferum L. for the first time (Schmitz, 1985). Korea, China, India, and Japan were heading the scientific examination and confirmation of traditional medicine's essential concepts (Joshi et al., 2010).

The World Health Organization (WHO) has compiled a list of 21,000 therapeutic plants from all around the world. Among them, 2500 species were indigenous to India, making it the world's leading producer of medicinal plants (Modak et al., 2007). According to the WHO, up to 80% of the population in underdeveloped nations is reliant on plant-based medicines (WHO, 2015; Kaushal et al., 2018). Plant-based medications are estimated to offer primary healthcare for 3.5–4 billion people worldwide, according to the WHO (Farnsworth, 1988). Over 50,000 plant species are used for therapeutic purposes worldwide, according to the International Union for Conservation of Nature and the World Wildlife Fund (Chen et al., 2016;

Kumar et al., 2018). Around 25% of medications have a plant-based basis, either directly or indirectly (Bandaranayake, 2006; De Luca et al., 2012). According to Global Industry Analysts (GIA), the global plant and herbal supplement industry will be valued at USD 115 billion by 2020 (GIA, 2015). According to the WHO, the current annual demand for herbal pharmaceuticals is USD 14 billion, and by 2050, it will be USD 5 trillion (Aneesh et al., 2009). From the beginning of time, medicinal plants have been utilized in Indian therapeutic systems. Ayurveda (2559 herbs), Siddha (2267 herbs), Unani (1049 herbs), Sowa-Rigpa (671 herbs), Yoga, Naturopathy, and Ethno-botanical Folk (6403 herbs) are examples of Indian medical systems, as are those that came to India from other countries and were enriched and incorporated into Indian cultures, such as homeopathy (460 botanicals), which showed up in the eighteenth century (Prasad, 2002).

Ayurveda, or Indian traditional medicine, is gaining popularity across the world. In India, about 25,000 effective plant-based medicines are being used. The global trade in authorized medicinal plants and their products is estimated to be worth USD 60 billion. Ayurveda-based medications generate roughly USD 813 million in annual earnings on the worldwide market (Biswas & Biswas, 2014). As a result, with a commutative trade of roughly USD 140 million per year, the Indian market is the epicenter of medicinal plant dealing. Plant-based and natural ingredient exports were valued at roughly USD 33 billion in 2010 and were expected to reach USD 93 billion by 2015, while Indian medical plants and their products were expected to be around USD nearly equal to 0.2 billion. Apart from international trade, India's global medicinal plant economy produces USD 1.6–1.8 billion in income (Mishra et al., 2016). India accounts for just 2.5% of the global herbal market's yearly revenue of USD 60 billion.

Despite its rich Ayurvedic literature and wide medicinal plant variety, India struggles to meet prospective market needs (Mishra et al., 2016). About 86 genera from 29 families of herbaceous plant species from Jammu province and 105 medicinal species from 36 families from various niches in the district of Samba in the Union Territory of Jammu and Kashmir are being used for their medicinal importance (Pandita et al., 2013, 2014). In the Union Territory of Jammu and Kashmir, around 41 dicotyledonous species from 29 families and 13 monocotyledonous species from 5 families were found effective in treating diabetes mellitus (Pandita et al., 2015).

Multi-Omics in Technologies

Modern omics approaches, such as genomics, transcriptomics, proteomics, and metabolomics, are increasingly useful in the identification and description of critical gene-protein-metabolite networks, new drug metabolites, complete genomes, transcriptomes, and proteomes of medicinal plants, and human cell responses to drugs or whole ethnobotanical plants for medicinal use and large-scale production of plant-derived medicines (Mehta & Hasija, 2018; Chakraborty, 2018; Swamy

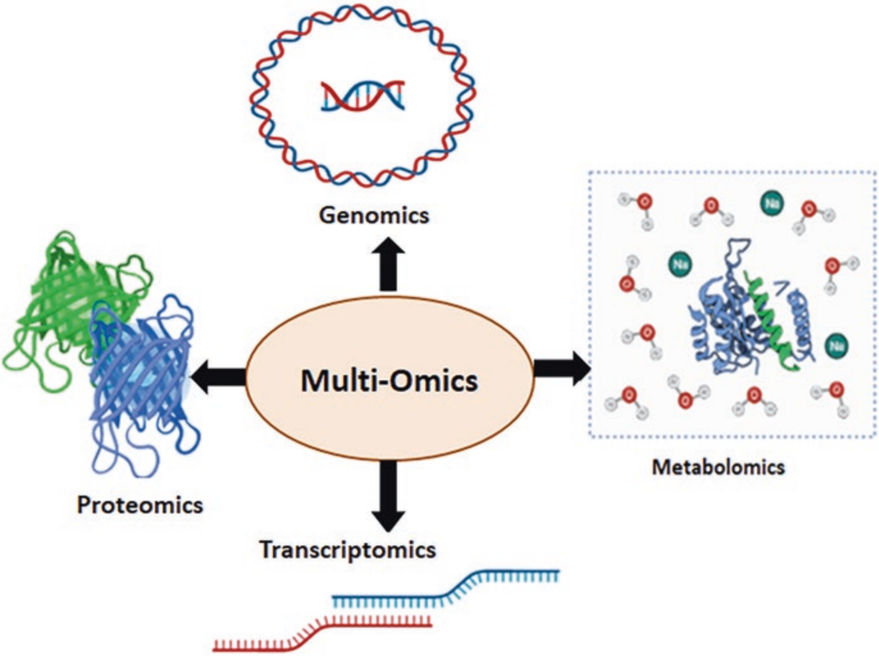

Fig. 8.1 Multi-omics in medicinal plants

et al., 2018a, b) (Fig. 8.1). Deep transcriptome investigations of medicinal plants, crosstalk between gene expression co-responses, and metabolite accumulation have all benefited from the next-generation sequencing (NGS) method. The idea of "guilt-by-association" suggests that genes that co-express lead to the manufacture of metabolites that accumulate at levels similar to the levels of gene co-expression (Yonekura-Sakakibara et al., 2013).

The combination of metabolomics and functional genomics of gene products from ethnomedicinal plants has sped up the discovery of novel biosynthetic pathways for a variety of bioactive compounds. This has facilitated the potential identification and development of pharmaceutically significant products. Artemisinin (an antimalarial drug) was first improved by breeding Artemisia annua L. with novel hybrids with high-yielding qualities to obtain a sturdy cropping system, and then in a re-engineered microbial host by the restoration of artemisinin's biosynthetic pathway, which was procured after the genome was sequenced (Hale et al., 2007).

The discovery of a string of FAD2 phytochemicals following transcriptomics and metabolomics of growing seeds that accumulate aberrant fatty acids was explored in a non-plant bioengineered host via the crosstalk of omics with the ethnobotanical technique (Sumner et al., 2015). UV-C irradiation induced the expression of 238 genes and 2012 metabolites in *Vitis vinifera* L. multi-omics (transcriptomics, metabolomics, and genome-wide microarray study). This provides support to the theory that stilbene production promotes transcription factor-mediated regulation (Suzuki et al., 2015). Omics techniques have largely been used to identify genes involved in the morphine biosynthesis pathway in *Papaver somniferum* L. (Hagel & Facchini, 2010; Gesell et al., 2009). An alkaloid biosynthetic gene cluster was discovered in the genome of *Papaver somniferum* L. using a combination of transcriptomic and genomic techniques (Winzer et al., 2012). High-throughput technologies were used to investigate phytochemicals such as carotenoids, flavonoids, lignans, and phenolic acids (Chen, 2020). The molecular insights into the bioactivities of these chemicals, as well as their involvement in medical therapies, screening, extraction, identification, and biotechnology, have all been investigated, with a particular focus on gene transfer and nanoparticles.

Another study employed bioinformatics-assisted methods to investigate medicinal plants. Their research focused on a multi-omics data-based approach for the Dendrobium medicinal plant, with very little information on the genomes, transcriptomics, proteomics, and metabolomics of other medicinal plants (Ma et al., 2020). Metabolomics, as well as analytical methods, statistical methodologies, and bioinformatics techniques, have potential applications in understanding the system-wide impacts of Thai traditional medicine (Khoomrung et al., 2017). The occurrence and spread of 47,700 microsatellites or simple sequence repeats (SSRs) extracted from 109,609 expressed sequence tags (ESTs) of 11 medicinal plants with antidiabetic potential were investigated for their potential as cross-transferability biomarkers (Sahu et al., 2014).

This piece is unique in terms of both comprehensiveness and details, with an emphasis on multi-omics technologies in medicinal plants. The chemical structures of medicinal metabolites and/or drugs/synthetic derivatives of several major medicinal plants, as well as their therapeutic characteristics, are also highlighted in the study. The goal of this review article is to combine omics-based techniques with scientific investigations on a variety of medicinal plants using case studies as examples. This would bring up to speed on natural-product-based medications derived from a variety of therapeutically important plants, as well as the further exploitation of plant pharmaceutical resources.

Omics in Medicinal Plants

Vast data is being generated by high-throughput omics systems such as genomics, transcriptomics, proteomics, and metabolomics. The data might be used to anticipate secondary metabolic pathways in different medicinal plants, find genes involved in manufacturing of physiologically active compounds, and investigate the plant genome and evolution. Medicinal plants evolve new features to adapt to changing conditions and live longer. To promote man's life, hypothesis-based and big data-based medicinal plant research integrate plant-based analysis, biotechnology, and omics techniques (Kellner et al., 2015, 2019). The Medicinal Plant Genomics Consortium and genome-guided research, as well as the Medicinal Plant

Transcriptome Project (Transcriptome Characterization, Sequencing, and Assembly of Medicinal Plants Relevant to Human Health 2011), the 1000 Green Plant Transcriptome Project (One Thousand Plant Transcriptomes Initiative 2019), and others will aid in the identification and evolution of numerous plant biosynthetic pathways, particularly in the discovery of new pathways originating as gene clusters in *Oryza sativa* L., barley and poppy (Chu et al., 2011).

Anticancer noscapine (alkaloid) synthesis route was discovered in poppy, consisting of a 10-gene cluster spanning 401 kb of genomic sequence (Winzer et al., 2012). The expression patterns of enzymes and transcription factors could be analyzed on a global scale through advanced RNA sequencing methods. For the formulation of a hypothesis regarding the involvement of genes, a database of metabolomics and transcriptomics of 14 medicinal plants is available (http://metnetdb.org/mpmr_public/ accessed on 17 November 2020) (Wurtele et al., 2012). Metabolomics, a scientific field dealing with all of a cell's metabolites was emerged after genomics, transcriptomics, and proteomics (Gahlaut et al., 2013). Two cytochrome P450 genes of the glycyrrhizin biosynthetic pathway were found in *Glycyrrhiza uralensis* Fisch. ex DC. (licorice), controlling the microbial synthesis of glycyrrhetinic acid and triterpene saponin, a natural sweetener (Seki et al., 2011).

Genomics

DNA sequences laid down a decoding foundation for chemical and genomic diversity by integrating the origin, development, and epigenomic regulation of all living organisms (Hao & Xiao, 2015). There are various methods of sequencing, whereas high-throughput sequencing emphasizes biosynthetic pathways of their essential metabolites (Boutanaev et al., 2015), regulatory genes, and enzymes. These days, whole genome sequencing delivers complete genomic information encompassing multiple repetitive sequences, heterozygosity, and non-diploids (Chen et al., 2010). Despite being costly, it provides accessible, detailed information on any genomic sequence. The online available whole genome sequences can be easily accessible for grapes (Velasco et al., 2007; Jaillon et al., 2007), *Phalaenopsis equestris* (Schauer) Rchb.f (Cai et al., 2015), *Brassica napus* L. (Chalhoub et al., 2014), *Capsicum annuum* L. (Qin et al., 2014; Kim et al., 2014), *Momordica charantia* L. (Urasaki et al., 2017), *Coffea canephora* Pierre ex A. Froehner (Denoeud et al., 2014), *Salvia miltiorrhiza* Bunge (Qian et al., 2013), *Ziziphus jujube* Mill. (Mahajan & Chopda, 2009; Li et al., 2014), *Glycyrrhiza uralensis* Fisch. Ex DC. (Mochida et al., 2017), *Dendrobium officinale* Kimura et Migo (Yan et al., 2015), *Azadirachta indica* A. Juss., 1830 (Shivaraj et al., 2015), and *Catharanthus roseus* (L.) G. Don chloroplast and genome (Kellner, 2015; Ku et al., 2013) and the chloroplast of *Pogostemon cablin* (Blanco) Benth. (He et al., 2016). These plants can be considered significant models for investigating plant genetics and their cellular metabolites (Giddings et al., 2011).

Recently, plant genome clustering aided in expanding information on alkaloids biosynthesis i.e., *Salvia miltiorrhiza* Bunge (Danshen) and *Catharanthus roseus* (L.) G. Don synthesizes triterpenes, indole alkaloids, and diterpene quinone. Kellner et al. (2015) sequences bacterial artificial chromosomes and showed clusters denoting genes regulating the biosynthesis of vinblastine and vincristine. Whole genome sequencing for medicinally important plants has also been reported for primary and secondary metabolites (*Ziziphus jujube* Mill.) and antimicrobial potential plants (*Azadirachta indica* A. Juss.). Genome sequencing also gives information about the number and size of encoded genes regulating biosynthetic pathways. The next generation sequencing for Neem depicted repetitive DNA sequences coding approximately 20,000 genes (Krishnan et al., 2012), *Salvia miltiorrhiza* Burge (Danshen) putative genes regulating terpenoid biosynthesis (Ma et al., 2012), and *Salvia miltiorrhiza* Burge (Danshen) having 600 MB genomic size possessing 30,478 genes coding for protein along with genes involved in controlling chronic diseases (Xu et al., 2016).

The comparative genomic sequence of cultivated and hybrid verities of *Capsicum annum* L. (Qin et al., 2014), *Solanum tuberosum* L., *Capsicum annum* L., Atropa and *Lycopersicone Esculentum* was used to explore the evolution information of various members of the Solanaceae family. There are numerous examples of next-generation sequencing giving insights into evolution and polymorphism (*Panax ginseng* cp.) (Zhao et al., 2015), content, genes layout, and RNA editing sites (*Dendrobium officinale* Kimura et Migo) (Luo et al., 2014), expressor genes for phenylpropanoid/terpenoid biosynthetic pathway, cytochrome P450s and transcription factors (*Ocimum sanctum* L. and *Ocimum basilicum* L.) (Rastogi et al., 2015; Rastogi et al., 2014). All these plant species have facilitated new approaches for the mining of biosynthetic pathways of important medicinal metabolites in related species. The potential genes regulating biosynthesis pathways for these metabolites can be used to develop targeted drugs (Upadhyay et al., 2015). This technique has also helped scientists to discover genes involved in morphine biosynthesis (Gesell et al., 2009; Winzer et al., 2012), whereas GBS (genotyping by sequencing) and microsatellites have enabled to assess heterozygosity (Rubin et al., 2012), origin and distribution blueprints of potential anticancer plants (Hao & Xiao, 2015). The genome resources like ISSR analysis assist in the association of genomic variations among 32 native species of endangered medicinal plant *Sinopodophyllum hexadrum* (Royle) Ying showed its genetic diversity by exploring data for conservation and evolution (Liu et al., 2014) and with the origin of new phytochemicals and physiological traits in the therapeutic plants (Jiao & Paterson, 2014). Despite of expensive and demanding process, expansion of genomics of medicinal plants requires information comprising of the genes and regulatory sequences, latent RNA editing sites, origin, evolution, development, domestication, differentiation, polymorphisms, epigenomic regulation, genome heterozygosity, genotyping and biosynthetic pathways of drug molecules, secondary metabolites and their regulatory pathways.

Transcriptomics

A transcriptome is an effective approach for gathering genomic data from non-model therapeutic plants compared to comparative genomics. This technique is highly valuable for characterizing key characteristics involved in the biosynthesis of secondary metabolites at a molecular level (Zhang et al., 2014; Hao et al., 2011, 2012, 2015).

Currently, several plant families constituting hundreds of plants are subjected to go through transcriptome analysis, for instance, Oenothera (Onagraceae) (Hollister et al., 2015), Fabaceae (Cannon et al., 2015), Caryophyllales (Yang et al., 2015), *Polygonum cuspidatum* Sieb. et Zucc. (Hao et al., 2015), *Rhodiola algida* (Crassulaceae) (Zhang et al., 2014), *Taxus mairei* (Hao et al., 2011), *Salvia sclarea* L. (Lamiaceae) (Hao et al., 2015), etc. the sequences can be retrieved from Sequence Read Archive (SRA), National Centre for Biotechnology Information (NCBI), PubMed and Gene Expression Omnibus (GEO) databases. The beauty of transcriptome analysis is gene sequences can be obtained from plants without a reference genome and with them integrated analyses of transcriptomics (transcriptome data) and metabolomics (metabolic profiling data sets) potential for any medicinal plant (Yamazaki et al., 2013). Whereas whole transcriptome shotgun sequencing (WTSS) has made it easier to probe genes coding several metabolites biosynthesis pathways and the relationship of plant metabolites with those genes.

While discussing some recent examples, the transcriptome data of *Podophyllum hexandrum* Royle revealed candidate genes combinatorically expressed in *Nicotiana benthamiana* Domin. In this way, from podophyllotoxin biosynthetic pathway to etoposide aglycone, a chemotherapeutic anticancer molecule, six enzymes were identified (Lau & Sattely, 2015). Similarly, the olivetolic acid cyclase enzyme, a polyketide synthase and an acyl-activating enzyme responsible for the synthesis of olivetolic acid, was identified from the transcriptome data of glandular trichomes (primary sites of cannabinoid biosynthesis) of the female flower of cannabis (*Cannabis sativa* L.) (Gagne et al., 2012; Stout et al., 2012).

Another plant species, *Chlorophytum borivilianum* Santapau and R. R. Fern. has expressed antitumorigenic and anticancer potential (Kumar et al., 2010) because of chloromaloside-A, spirostanol-pentaglycosides-embracing beta-D-apiofuranose, and steroidal glycosides. Ranunculaceae family has revealed medicinal phytometabolites with antitumorigenic potentials, such as saponins, alkaloids, terpenoids, and polysaccharides. On the whole, the expression profiling of various genes and appropriate transcriptomics platforms have exposed the distinct outcomes of phytometabolites in cancerous cells (Hao et al., 2017; Dinda et al., 2009). Iridoids generated from *Catharanthus roseus* (L.) G. Don possesses anti-inflammatory, anticancerous, and antibacterial potential (Geu-Flores et al., 2012; Dinda et al., 2011; Tundis et al., 2008).

Curcuma longa L. transcriptome showed transcripts associated with the terpenoid biosynthetic secondary metabolites such as vinblastine, curcumin, and taxol having the potential to lower the prevalence of gastrointestinal cancers because of secondary metabolite Curcumin (Annadurai et al., 2021). The deep transcriptome analysis of *Ophiorrhiza pumila* Champ. ex Benth. member of the Rubiaceae family yielded a 2GB sequence from which novel genes of plant secondary metabolic biosynthetic pathways (Yamazaki et al., 2013). The genes of chemotypes and the tissue-specific biosynthesis of withanolide from *Withania somnifera* (L.) Dunal, distinct chemotypes were characterized (Gupta et al., 2015). The combined approach of transcriptome and molecular genetics has proposed the existence of a complex gene cluster in the genome used for alkaloid noscapine biosynthesis in the chemovariety of *Papaver somniferum* L. (Winzer et al., 2012). Therefore, advanced genome sequencing and comparative transcriptomics have expanded our understanding of the functions of diverse genes involved in the biosynthetic pathways. From cDNA microarray to RNA-seq data of *Salvia miltiorrhiza* Burge (Danshen), a group of novel genes regulating terpenoid-derived tanshinone and salvianolic acid secondary metabolite biosynthesis was identified (Cui et al., 2011; Wenping et al., 2011). Among the five key secondary metabolic biosynthetic pathways covering almost all bases in the phenylpropanoid and terpenoid pathways, 1539 unigenes were identified. Through differential gene expression profiling of *Lupinus angustifolius* L. chemo-varieties coding quinolizidine alkaloids, it was observed that the gene that encodes the enzyme lysine decarboxylase involved in the first step of the alkaloid biosynthetic pathway (Bunsupa et al., 2012) as well as the putative homolog genes of the iridoid biosynthetic pathway were identified from the transcriptome of a variety of TIA-producing therapeutic plants (Facchini et al., 2012) and were further evaluated, filtered, and validated (Salim et al., 2013).

RNA-seq provides rich sequence information about the full-length gene sequences and assists in recognizing orthologous and paralogous gene cluster families in *Camptotheca acuminate* Decne., *Rauvolfia serpentine* (L.) Benth. ex Kurz, and *Catharanthus roseus* (L.) G. Don for the respective biosynthesis of camptothecin, ajmaline, and vinblastine (Facchini et al., 2012; Gongora-Castillo et al., 2012). For the transcriptome analysis of two *Vaccnium macrocarpon*, Aiton, commonly called cranberry fruits, at different growth stages, online available genomic data were annotated to identify flavonoid biosynthesis regulatory genes, i.e., *CHS*, *F3H*, *CHI*, *F3′H*, and *LDOX* (Stout et al., 2012). The cranberry transcriptome comparing blueberry (Vaccinium sp.) revealed a more characterized type of flavonoid enzyme in cranberry, a *UDP-glucose flavonoid 3-O-glucosyl transferase* (*UFGT*) enzyme of the flavonoid biosynthetic pathway. Later on, the ABC transporters and glutathione S-transferases (GST), WD40, WRKY, and bHLH regulatory transcription factors, involved in flavonoid biosynthesis, were also found in this transcriptome (Sun et al., 2015).

Transcriptome also assessed the metabolic changes that take place in the fruit-ripening of *Rubus coreanus* Miq.1867 rich in anthocyanins (Hyun et al., 2014). The transcriptomic data also identified F3H, CHS, and CHI genes, chief regulatory genes of flavonoids, and anthocyanin biosynthesis in rice, petunia, and tomato (Bovy et al., 2007; Hong et al., 2012; Muir et al., 2001). Initially, the CHI enzyme family was screened from the transcriptome database of Korean black raspberry and authenticated by complementary tests in Arabidopsis transparent testa 5-1 (tt5-1) mutant, which is devoid of CHI potential. Other than these, the comparative transcriptomics of two strains of *Magnolia sprengeri* Pampanini, and Nuovo Giorn with red and white flowers, gave insight into some key enzymes of the flavonoid biosynthetic pathway, such as phenylalanine ammonia-lyase, cinnamate-4-hydroxylase, *F3′H*, *F3H*, and *CHS* (Shi et al., 2014). *Chlorophytum borivilianum*, Santapau & R.R.Fern., an endangered plant sp. characterized as an adaptogen, antiaging agent, and promoter of general health, the transcriptome analysis has enabled insights into the molecular mechanism of flavonoid glycosylation (Kaushik 2005) and gene profile clustering by K-means grouping of RPKM in grapefruit ripening at different growth stages (Grimplet et al., 2009). The transcriptomics of medicinal plants has enabled scientists to gather information on the relationship between genes and plant metabolites, the expression profiling of genes, key characteristics, and the molecular bases of secondary metabolite formation and biosynthetic pathways, such as iridoid, flavonoid, terpenoid, podophyllotoxin, terpenoid-derived tanshinone, and the salvianolic acid secondary metabolite pathway.

Proteomics

Proteomics is a potent platform to compressively investigate protein regulation by drugs. It is a widely used technique for medicinal plants. It comprehends the protein structure illustrations, function, modifications, and protein–protein interactions in vivo and in vitro conditions (Lao et al., 2014). This technique also authenticates post-translations changes like protein phosphorylation, protein acetylation, protein glycosylation, and proteolysis (Zhang et al., 2011). Normally this modification occurs during disease progression or after the treatment of a disease with drugs, or sometimes under natural controlled conditions.

Then any protein alteration in the mechanism of action of drugs is noted by the macro-investigation (Lao et al., 2014). This way, we can call it a multifunctional field of proteomics which helps in the prediction of the protein targets of plant-based bioactive compounds and also provides a logical approach to appreciating the mechanisms of traditional Chinese medicine (TCM) in tumor cells and protein–drug interactions at the molecular level. The natural plant drug molecules cause the suppression of tumors by completely targeting the mitochondria present in the cells of malignant tissue (Wang et al., 2015). Natural flavones such as Luteolin, Baicalein, and Tangeretin show signs of anticancerous activity irrespective of their ambiguous mode of action. The baicalein upregulates peroxiredoxin-6, causing reductions in reactive oxygen species (ROS) generation and hindrances in the cell proliferation of colorectal cancer (Huang et al., 2012). Similarly, Luteolin shows corresponding anticancerous potential against various categories of cancers, along with hepatic cancer in humans. The anticancerous value and intrinsic mechanisms of action of *Tripterygium wilfordii* Hook. F., an extensively used plant for treating several human syndromes, have been analyzed at the proteomic level to demonstrate the effectiveness of metabolite diterpenoid epoxide triptolide (a bioactive compound) in treating colon cancer (Liu et al., 2011).

Another plant spp. *Andrographis paniculate* (Burm. F) possessed diterpene compounds having medicinal applicability against various human disorders, such as cancer, viral, and pathogenic bacteria (Valdiani et al., 2012). Likewise, the proteomic study has been a competent method for attaining a complex understanding of the inheritable traits and the physiological status of plant members of the family Acanthaceae (Talei et al., 2014). Periplocin is extracted from the tissues of the bark and stems of *Periploca graeca* L. and can help fight cancers in the lungs and colon both in vivo and in vitro via the beta-catenin/TCF signaling pathway by means of inducing apoptosis. Later on, tandem mass spectrometry and 2D gel electrophoresis elaborated the outcomes of periplocin's action on the cell line A549 of lung cancer. Further, western blot analysis was used to validate and investigate protein modifications and protein-protein interactions (Lu et al., 2014).

Proteomics has also confirmed the activity of curcumin in diverse cancerous cell lines. It also investigated differentiated 12 proteins with differential expression patterns enhancing functions such as transcription, glycolysis, RNA translation, the splicing of mRNA and lipid metabolism, the synthesis of proteins, protein folding and the degradation of proteins, amino acid synthesis, and the motility of cells in the MCF-7 cell line of human breast cancer (Fang et al., 2011). Gambogic acid, a natural xanthonoid molecule isolated from *Garcinia hanburyi* Hook. f. resin has revealed promising antitumor activity in clinical trials obstructing the growth of cancer cells through multiple signaling pathways (Chantarasriwong et al., 2010; Chen et al., 2012; Anantachoke et al., 2012). In hepatocellular carcinoma, gambogic acid possibly targets Stathmin. Garcinia. *Garcinia oblongifolia* Champ. ex Benth contains the bioactive metabolite 1, 3, 6, 7-tetrahydroxyxanthone, restricting cell proliferation in hepatocellular cancerous cells via the upregulation of p16 and 14-3-3σ (Fu et al., 2012a, b) and 1, 3, 5-trihydroxy-13, 13-dimethyl-2H-pyran [7, 6-b] xanthone (Fu et al., 2012a, b). In another study in 2014, the treatment of primary T cell lymphoma in the central nervous system with fenugreek seeds demonstrated an incidence of tumor regression by cancer cell destruction through cytotoxins (Alsemari et al., 2014).

Differential proteomic analyses of vegetative parts and glands of *Cannabis sativa* L. with different levels of cannabinoids were done by two-dimensional gel electrophoresis (2D-gel) and showed the presence of ~800 proteins followed by mass spectrometry expressing less than 100 proteins (Raharjo et al., 2004). The first comprehensive draft map of the Cannabis proteome has given evidence for the expression of over 17,269 protein-coding regions (Jenkins & Orsburn 2020), while global proteomic profiling of the *Artemisia annua* L. has enabled the identification of 13,403 proteins based on the genome sequence annotation database, and 182 proteins by mass spectrometry, respectively (Chen et al., 2020). Kim et al. (2017) evaluated the detailed proteomics of the Panax species possessing anticancer, antiaging, and protection against circulatory shock and performed comparative proteomics of root and leaf tissues of Oriental, American, and Indian ginsengs. Indian ginseng (*Withania sominifera* (L.) Dunal) was observed to have major secondary metabolites of withanolides representing 56 unique spots, whereas 22 proteins were identified by MALDI-TOF/TOF (Nagappan et al., 2012).

Overall, proteomics is an efficient technique to analyze medicinal plants providing information on the structure, function, and post-translational modifications of proteins, protein–protein interactions, protein targets of plant-based bioactive drugs, protein–drug interactions at the molecular level, and the signaling pathways of cell perturbations. This technique also gives insights into the mechanisms of plant-based medicines against tumor and cancer cell lines. Proteins go through post-transcriptional and post-translational modifications frequently; therefore, proteomics data do not always complement transcriptomics data. Hence, further research is needed for the integration and exploration of the biology of medical plants (Kim et al., 2017).

Metabolomics

In plants, metabolisms are the most important study in which plants produce a huge amount of primary and secondary metabolites (Saito & Matsuda, 2010). This type of study is an efficient tool for identifying novel chemicals for drugs. Metabolomics also helps to find out the medicinal importance and profiling of secondary metabolites in medicinal plants. This study also deals with the reaction of drugs and analyzes the toxic effects of drugs. Because of the high screening of drugs, plants can be helpful for the identification of chemicals for human medicine (Ulrich-Merzenichet al., 2007). The identification and isolation and then fingerprinting of plant metabolites are the basic needs for better quality and quantity of therapeutic plants (Mukherjee et al., 2016).

Metabolomics defines a vast area of medicinal plants that can be helpful for the detection of drugs or metabolites (Kim et al., 2010; Okada et al., 2010). Infect, metabolomics is extensive use of synthetic biology, ayurveda, and medical science. It is also helpful for the analytical modeling of medicinal plants. The low concentrations of secondary metabolites cannot detect easily because of synergistic biological activities which can be produced because of multiple intrinsic components in herbal formulations. Metabolomics is an efficient approach to recognizing the range of medicinally important bioactive compounds (Mukherjee et al., 2016).

The gene clusters are found in biosynthetic pathways and making gene identification much easier (Hagel & Facchini, 2010; Field et al., 2011). It also relates to the metabolites' evolution and their functions. The medicinal plant flora contains approximately 1 million metabolites (Dixon & Strack, 2003), but very few amounts of metabolites are biologically active compounds (Yamada et al., 2008). The secondary metabolites have been verified in plants, and several metabolites arose from the genome duplications that can be the reason for complex characters (Hartmann, 2007; Hao et al., 2014). There are three classes of secondary metabolites of plants that are based on their biosynthetic pathways like terpenoids (~36,000) (Buckingham et al., 2007), alkaloids (~12,000) (Croteau, 2000), and phenolics (~10,000) (Taiz & Zeiger, 2006).

The analytical techniques, e.g. spectroscopy and nuclear magnetic resonance, are useful for data assembling for metabolomics studies. The metadata can be used for detections and amendments (Kim et al., 2010). The procedure of NMR spectroscopy was operating to measure the metabolites profile and inhibitory effects in *Aloe vera* (L.) Burm. f., is an anticancer and antitumorigenic plant. There are some examples of the influence of metabolomics on the genome of hepatocellular carcinoma cells by escalating the gene expressions of p53 and Bcl-2 (Noorolahi et al., 2016). The *Panax ginseng* C. A. Mey., *Panax japonicus* (T. Nees) C.A. Mey., and *Panax notoginseng* (Burkill) F. H. Chen by the UPLC-QTOF-MS technique identified secondary metabolites such as *chikusetsu saponin* IVa and ginsenosides (Rb1, Rb2, Rc, Rg2, and R0) (Xie et al., 2008). In *Persicaria minor* (Huds.) Opiz syn. *Polygonum minus* Huds, 48 compounds via GC × GCTOF MS, 42 compounds via GC-MS, 37 volatile compounds via GC-MS investigation, and 85 flavonoids via LC-TOF were successfully identified (Baharum et al., 2010; Goh et al., 2016).

Natural resources used for anti-cancerous drug in the treatment of cancer (Cragg & Newman, 2013; Newman & Cragg, 2016). In the previous reports, antitumor potential of some Compositae members was determined along with their metabolite profiling. The species of this family express changeable metabolites. Artemisia is most efficient and contains maximum secondary metabolites production. Another example is *Pulcaria crispa* (Forssk.) Benth. ex Oliv. shows proficient in-vitro anticancerous action (El-Naggar et al., 2015). Some techniques are also helpful in improving metabolite profiling and pigments quantification. Mass spectrophotometers are used for flavonoid and anthocyanin groups and reductions in the sucrose, fatty acids, organic acids, and amino acids in *Rubus coreanus* Miq. (Hyun et al., 2014). Plant metabolites of medicinal plants provide complete knowledge about identification, purification, and new chemical entities (NCEs) for the development of drugs, the fingerprinting of plant metabolites, and phytochemistry.

Multi-Omics and System Biology for Plant Improvement

The understanding of cellular components of biological systems and omics approaches is required to visualize organisms under specialized conditions. The previous studies combined the study of metabolomics with genomics, transcriptomics, and proteomics delivered the picture of functions and genomic and phenotypic interactions with the environment (Weckwerth, 2011). The combined approaches have been seen in potato and Arabidopsis in several studies (Urbanczyk-Wochniak et al., 2003; Hirai et al., 2004). These studies analyzed that the omics approaches can be useful and more helpful in identifying the candidate gene. In plants, different approaches are implemented for advancement in omics, computational tools, and integrative omics. Epigenetic integration with multi-omics exposed the biosynthesis of lipid during fiber development (Wang et al., 2016). The addition of GWAS with a metabolite profiling scheme has proved to be a potent technique for investigating the genetic and biochemical processes in several model plants i.e., tomato, rice, and maize (Luo et al., 2015; Matsuda et al., 2015). The abiotic stress tolerant plants phenotypes can be checked by integration of genomics, transcriptomics, proteomics, and metabolomics (Jogaiah et al., 2013). In the breeding system, due to functional genomics and mutagenomics, numerous mutants can be verified with specific variations with reference to growth, development, and stress tolerance in plants, especially cash crops rice, maize, wheat, and barley (Talukdar & Sinjushin, 2015).

The GWAS and HRPF approach used for biomass growth, yield, and genomic traits and combined techniques are most useful than traditional phonemics for plant breeding and genetics (Yang et al., 2014). The new loci, related to leaf color, size and shape were assessed in another combined approach of GWAS and high-throughput leaf scoring (HLS) (Yang et al., 2015). QTL mapping and combined agronomic traits also helped to identify several QTLs in cash crops (Zhang et al., 2017). The phenotypic and genomic information can be used to improve the complex traits of crops (Zhao et al., 2019). All combined approaches could be more efficient and helpful in analyzing the biological processes. These combinations have been validated by regulating metabolites, proteins, and ions related to salinity stress in halophytes (Kumari et al., 2015; Fiehn, 2002). The metabolomics and ionomics techniques were used to assess the photosynthetic growth rates under salt stress conditions (Guo et al., 2017).

Grain and leaves ionomics also exposed the mineral genetic diversification in rice through genetic mapping and QTL identification (Norton et al., 2010; Zhang et al., 2014; Pinson et al., 2015a, b). Genotype and phenotype interactions can provide the whole picture of functional genomics and their mechanisms to improve plants' genetics, growth, resistance, and yield in response to physiological and environmental stress (Kumar et al., 2015). System biology is a powerful tool to combine multi-omics and increase understanding of the adaption and development of an organism (Pinu et al., 2019). These multi-omics approaches deal with plant stress and their system biology (Mosa et al., 2017). So, the broad analyses using three omics technologies, transcriptomics, metabolomics, and proteomics have also increased our understanding of systems biology associated with abiotic stress responses in plants (Cramer et al., 2011).

Metabolomics is the foundation for system biology based data and predict the metabolic pathway and phenotypic responses (Pinu et al., 2019; Kumar et al., 2015). The integration of multi-omics and systems biology approaches has resulted in the identification of molecular regulator networks for salt stress tolerance in grapevine crops (Daldoul et al., 2014). Moreover, systems biology integrated with omics approaches for network and testing models has been proposed for abiotic stress responses in crop plants (Gupta et al., 2013).

Panomics for Crop Breeding Science

The study deals with complex omics such as genomics, epigenomics, transcriptomics, proteomics, PTM proteomics, metabolomics, and phenomics (Weckwerth et al., 2020). The idea of panomics was proposed by Weckwerth et al. (2020). Combine the omics and form a model that can assess the complex traits (Weckwerth, 2011, 2019). The environmental information and multi-omics phenomics would provide better understanding of environmental phenotype dependency at a molecular level. The complex omics dataset reduces the positive outcomes from single data sets for genotype and phenotypes estimation (Ritchie et al., 2015). Panomics and environmental effects can be utilized to identify genes, QTLs, and markers through mathematical models and functional omics to tolerate biotic and abiotic stress. These combinations could be used to create elite lines to improve the germplasm (Weckwerth et al., 2020).

Integrated data analyzed by special tool; PAINTOMICS, KaPPA-view, and COVAIN could be used for the interpretation of multi Omics dataset (Kuo et al., 2013). Paintomics is a tool for the integration of transcriptomics and metabolomics data that displays the data on KEGG pathway maps (García-Alcalde et al., 2011). KaPPA-view is another tool for metabolite data and plants metabolic pathway maps (Tokimatsu et al., 2005). COVAIN is an initial tool for metabolomics data and helps to integrate omics dataset with KEGG pathway and gene analysis (Sun & Weckwerth, 2012).

GWAS with omics dataset transcriptomics (eQTLS), proteomics (pQTLS), and metabolomics (mQTLS) can be used for the identification of new genes (Weckwerth et al., 2020). In this vein, a combined metabolome-based genome-wide association study (mGWAS with eQTL) identified metabolite features associated with kernel weight in maize crops (Wen et al., 2014). There are several genome editing tools TALENs and CRISPR/Cas9, and panomics can be used for precision breeding (Weckwerth et al., 2020). Marker assistant selection and genomic selection techniques have identified important genes and describe less than 40% phenotypic variance. So panomics with genome editing techniques can show better results in identification of phenotypic variance and support the agronomic genes in efficient manner for precision breeding. The method not only improving crops but also increases precision in trait optimization in yield, nutrition value, and plant wellness (Weckwerth et al., 2020).

Potential Applications Translational Genomics to Breeding Design

Translation of genomic information is feasible based on the assumption that blueprints of all living organisms are written with the same chemical language system and genomic knowledge acquired from well-studied models can be projected onto other relatively less-studied crop genomes or orphan species. Such transfer of genomic information may occur at various levels, i.e., gene-to-gene, gene networks, and whole genome-to-genome. Obviously, translational accuracy is affected by evolutionary distances between species; the closer the distance between translated genomes, the more accurate the translated contexts of genomic information. In particular, orthology among translated genes is a strict prerequisite for translating the genomic contexts of specific interests in breeding desired traits without any erroneous understanding. To properly accomplish this, orthologous relationships should be reconfirmed from multi-angled, at least three analyses of genomic data.

Firstly, homology-based identification of orthologs should be preceded as a basic and essential step. In addition to sequence homology, the orthology of genes can be reconfirmed by their similarities in domain architectures, which are identified by the Hidden Markov Model (HMM) algorithm and can be searched at some specialized DBs, such as InterProScan (https://www.ebi.ac.uk/interpro/search/sequence-search; Hunter et al., 2012) and Pfam (http://pfam.xfam.org/search; Finn et al., 2014). Secondly, the orthologous relationships can be confirmed by phylogenetic analysis because homology is not always a one-to-one relationship, and in reality, orthology is frequently confounded by paralogous genes generated by duplication during the evolutionary processes (Freeling & Subramaniam, 2009).

Finally, orthology can further be reconfirmed within the context of syntenic relationships or gene collinearities in corresponding genomic regions in comparison (Paterson et al., 2010). Although chromosomes usually undergo the reshuffling and rearrangement of genomes after speciation, one can detect conserved genomic regions between species diverged from common ancestors and even among distantly related species as well. Based on those synteny analyses, QTL information (involving multiple genes for the QTL traits) of nodal crops, beyond simple gene-to-gene translation, could be transferred to other orphan but phylogenetically related crops.

In recent years, GWAS analysis has been widely used to discover genes, genomic loci, and SNP/InDel associated with useful crop traits of interest. Beyond the capability of genetic map-based QTL analyses in the past, resequencing and/or array-based GWAS is making it possible to a lot more precisely predict or identify the alleles directly linked to certain phenotypic features for breeding, thereby resulting in the revelation of trait-associated single/a few or a combination of nucleotide variations. Many cases of GWAS/array-based identification of trait-associated genomic loci in crop plants.

Additionally, the development of a high-throughput phenotyping system (HTPS) is important to facilitate systematic phenotype-linked genomic analyses. Yang et al. (2014) reported a successful case of the HTPS-integrated GWAS approach in rice. As a result, they could identify a total of 141 genomic loci associated with 15 defined agronomic traits, of which 25 loci contained genes that were previously known for their functions (Yang et al., 2014). Subsequently, these phenotype-linked variations can be developed into trait-associated genetic markers, which are very useful molecular tracers for breeders, and these markers can serve as a powerful tool for genomics-assisted precision breeding. Furthermore, the phenotype-associated genomic information could be translated into other related plant or crop genomes, wherever possible, based on the syntenic relationships. Via these multi-angled and omics-driven approaches, translation of cross-species phenotypic annotation associated with complex traits would be feasible and become more precise as the omics data are more completely integrated.

Conclusion

The multi-omics played an important role in genetics, growth, stress, and breeding process in various medicinal plants. In the last few decades, eminent technologies have appeared for multi-omics approaches for plants. The multi-omics approaches are modern platforms and tools that provide detailed knowledge of genomics, transcriptomics, proteomics, and metabolomics of medicinal plants. The novel gene pathway, proteins, and secondary metabolites biosynthesis pathways of various drugs can be identified from a wide range of plants that have therapeutic significance. This chapter endeavors an advancement in areas of research of multi-omics and is involved in interpreting the significance of utilization of medicinal plants in the present global scenario. The panomics in which multi-omics genomics, transcriptomics, epigenetics, proteomics, and metabolism can facilitate the models to assess the agronomical traits to improve the medicinal plants through precision breeding.

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Chapter 9
Applications of Some Nanoparticles and Responses of Medicinal and Aromatic Plants Under Stress Conditions

Muhittin Kulak, Gulsum Yaldiz, and Mahmut Camlica

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Introduction

Of the plant kingdom members, approximately 20% of the plant species are confined for medicinal and aromatic purposes, which is approximately 50–80 thousand flowering plant species. In particular, the World Health Organization (WHO) reported that 80% of the developing countries and 60% of the total world population are dependent on medicinal herbs (Mahajan et al., 2020), but the biological efficacies of medicinal and aromatic plants (MAPs) are organ-dependent due to the medicinally or aromatically substantial metabolites (especially secondary metabolites) that are confined and specialized in the organs of the plants. Not to be limited to medicinal purposes, those relevant and reputed plant species are also used in the beauty industry as cosmetics, perfumes, and soaps as well as in the food industry (Efferth & Greten, 2012).

Sessile plant species are continuously under the impacts of biotic and abiotic stress factors (Figs. 9.1 and 9.2), which in turn causes a significant reduction in the yield of relevant plants. The effects are dependent on multiple factors, viz. stress type, severity, duration, and frequency (Kulak et al., 2019; Yaldiz & Camlica, 2021a, b). In addition to the external stimuli, the genetic structure, cellular metabolism, growth, and yield properties of plants are also prominent indicators of responses against stress conditions. In this context, genomics and proteomics approach studies have clearly revealed the substantial roles of gene regulation and signal transduction in plants as a response to stress factors, suggesting the potential roles of genes in combating stress factors (Verma et al., 2013).

As stated above, the effects of stress depend on many factors. Especially, the stress periods such as short, mid and long-term critically affect the plant metabolic dysfunction. Plants may recover from short- or mid-period stress factors, but vigorous stress periods lead to the death of plants as it prevent flowering and seed formation and induce senescence (Verma et al., 2013). Hence, plants improve various resistant mechanisms such as adjustment of membrane system, maintenance of cell wall architecture, production of secondary metabolites and antioxidants, phytohormones, and accumulation of osmolytes in response to stress factors. One of the most important factors is the production of secondary metabolites of MAPs. MAPs develop different physiological and biochemical methods against stress conditions (Mahajan et al., 2020). As significantly manifested alterations in growth and crop productivity of MAPs under stress conditions; crucial modifications with respect to the biosynhesis of secondary metabolites (alkaloids, terpenoids, and phenolics)

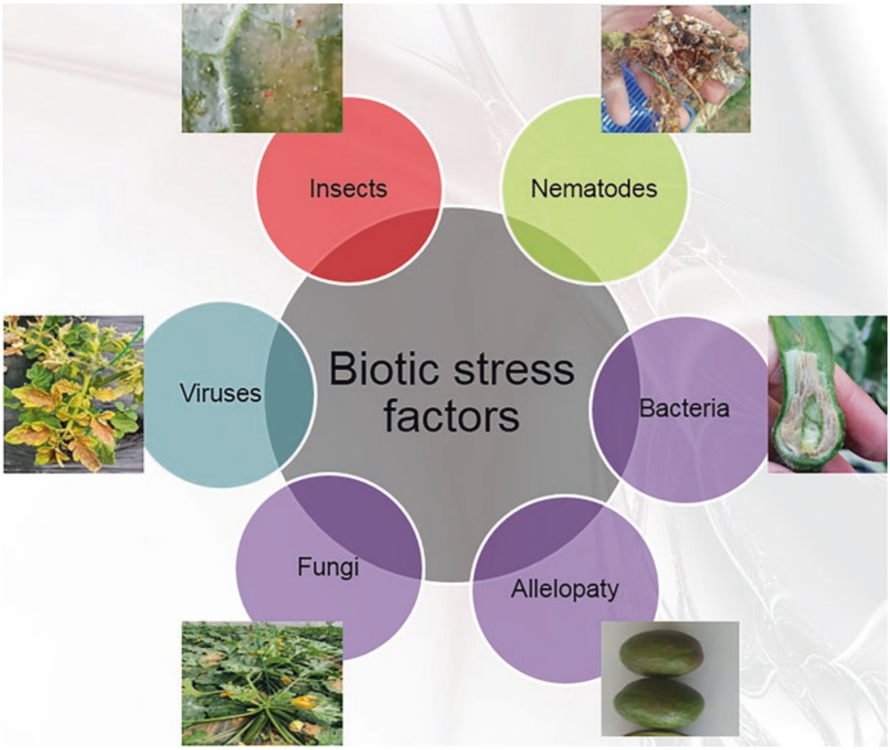

Fig. 9.1 Schematic representation of the biotic stress factors

have been reported as a response to various factors, viz. climatic factors, plant density, fertilizers. However, the effect of different levels of chemical fertilizers on bioactive components and secondary metabolites in MAPs has drawn in less attention (Naeem et al., 2013). Secondary metabolites are important compounds for human beings as they are sources of food additives, flavours, and industrially important pharmaceuticals, cosmetics, nutraceuticals, etc. (Ravishankar & Rao, 2000). Plant secondary metabolites such as digoxin and digitoxin, flavonoids, phenolic morphine, codeine, cocaine, quinine etc. are commonly used in medicine. The synthesis of secondary metabolites is generally increasing when plants suffer from biotic and abiotic stresses. Accumulation of phenylpropenoids and phenolic compounds has been found higher during stress condition in plants (Dixon & Paiva, 1995). The concentrations of various secondary plant products are strongly dependent on the growing conditions and physiology through altering the metabolic pathways responsible for the accumulation of the related natural products (Pradhan et al., 2017, b). The expression levels of certain genes governing the production of such compounds have also been shown to be increased in response to various abiotic stresses.

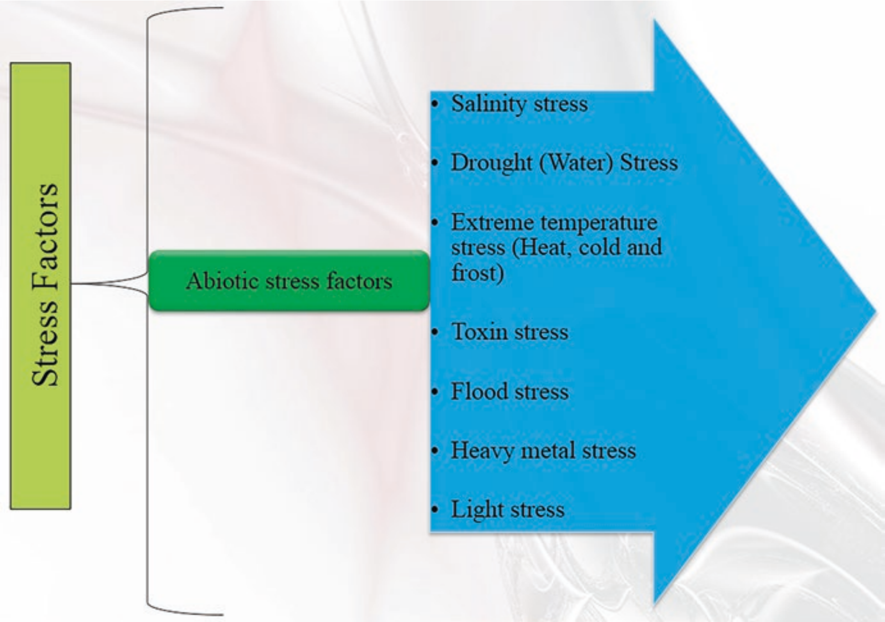

Fig. 9.2 Abiotic stress factors in plants

Biotic Stress Factors

Biotic stress factors contain various pathogens such as fungi, bacteria, oomycetes, nematodes, and herbivores (Fig. 9.1). Biotic stress can also lead to the death of plants by directly depriving their host of nutrients. Insects and viruses crucially affect the photosynthesis of plants by damaging the leaf and leaf area. The changing climatic conditions affect global crop production, and the survival rates of pests and pathogens increase nowadays. Crop productions, including MAPs, are under threat with the increase of these diseases. The cultivation of MAPs is exposed to the damages caused by different bacteria, fungi, viruses, phytoplasmas, and phytonematode groups, and these microbes threaten the production, growth, yield, and biochemical properties of MAPs. The pathogens cause diseases and damages to the plants in the growing stages. Consequently, critical loss in plant yield occurs after infected with the relevant biotic stress factors. However, plants have developed diverse defence mechanisms for combatting and for getting adapted to these negative conditions. These defence mechanisms are controlled via the stored genetic code of plants. This stress factor is entirely different from abiotic stress (Verma et al., 2013; Elad & Pertot, 2014; Singh et al., 2016; Gull et al., 2019).

MAPs are significantly affected by the pathogenic infestation of many fungi that contaminate the aerial part, the leaf, and the root of the plants. Powdery mildews are seen firstly on the leaf and continue to the fresh stems and cover the growing parts of the plant. Rust shows similarity with powdery mildews and affects all plant parts.

In both diseases, pustules on the upper and lower surface of the leaves are observed. Leaf spot and blight are other fungal diseases, and their symptoms are seen on the leaves (Thaung, 2008; Bhandari et al., 2014).

In addition to these major diseases, other various fungal and bacterial diseases such as root rot, wilt, damping off, anthracnose, and dieback affect the growth, yield, and biochemical properties of MAPs (Singh et al., 2016). Many MAPs are exposed to parasitic nematodes. These major nematodes affect the plant negatively and cause yield losses. Some of them are identified as follows: root-knot nematodes (*Meloidogyne* spp.), cyst nematodes (*Globodera* spp., *Heterodera* spp.), reniform nematode (*Rotylenchulus reniformis*), and lesion nematode (*Pratylenchus* spp.) (Singh et al., 2016). Like other biotic factors, viruses also cause vital reduction in biomass yield and production of MAPs worldwide. Many of the MAPs such as *Mentha* spp., *Andrographis paniculata*, *Hyoscyamus muticus*, *Catharanthus roseus*, *Rauwolfia serpentina*, *Hyoscyamus albus*, and *Papaver somniferum* are noted to be contaminated by plant viruses (Postman et al., 2004; Raj et al., 2007; Samad et al., 2008; Khan et al., 2015).

Abiotic Stress Factors

Abiotic stress factors influence the growth and development of plants and cause changes in agroecological conditions (Mahajan et al., 2020) (Fig. 9.2). There are common abiotic stresses such as drought, salinity, high temperature, low temperature, and metal toxicity that adversely affect medicinal and aromatic plants. Depending on the severity of these stresses, the degree of influence of MAPs also varies. In the subsequent sections, the effects of those stress factors were reported separately.

Salinity Stress

Salinity is one of the important abiotic environmental stress factors that limit plant growth and development and play a role in determining the geographical distribution of medicinal plant species (Vriezen et al., 2007). Like other abiotic stress factors, it exerts adverse impact on the three different physiological periods (germination, seedling, and maturation and production periods) of MAPs (Mondal & Kaur, 2017). Salt stress, which limits agricultural production worldwide, is becoming an important global factor affecting approximately 20% of irrigated lands (Khalid, 2006). For this reason, it is necessary to develop products that adapt to salt stress due to the increasing salinity in the soil. Salt stress also affects the secondary metabolites of medicinal and aromatic plants. Therefore, determination of salt tolerance in MAPs is of great importance. Salinity in the plant cells causes ionic, osmotic, and oxidative stresses. Synthesis of different osmolytes like glycine betaine, proline, sorbitol, mannitol, pinitol, sucrose, etc. is one of the important ways to resist saline condition in plants (Yildiz et al., 2020).

Regarding the secondary metabolite production, especially in essential oil production, many conflicts exist concerning whether stress conditions increase/decrease the essential oil production or not. In this regard, while some researchers reported that salt stress reduced the essential oil production (Ashraf & Orooj, 2006; Belaqziz et al., 2009; Said-Al Ahl & Omer, 2011) or the main components of essential oil of MAPs (Said-Al Ahl & Omer, 2011), some of them noted that salt stress increased them (Baghalian et al., 2008).

As clearly reported in a quite number of researches, the effects of stress conditions are correlated with the alterations in ionic balance, metabolites and ionic status in MAPs have been investigated under stress condition. For instance, salinity stress caused the lack or imbalance of ions because of the antagonism of the nutrients (K^+, Ca^{2+}, Mg^{2+}) with toxic ions (Na^+ and CI^-). K^+, Ca^{2+}, and Mg^{2+} concentrations are reduced in the aerial parts and roots under salinity conditions. However, Na^{2+}, Cl^-, and $SO^{2-}{}_4$ concentrations are increased in four MAPs (Yaldız et al., 2018).

Higher Levels of Salinity Stress Alter the Responses of Plants Concerned with Their Physiology and Morphologies

As clearly reported above as well, the previous reports revealed the adverse impacts of salinity on the growth and physiological and biochemical properties of plants at different growth stages (Kausar & Shahbaz, 2013; Shahbaz & Ashraf, 2013). The notable and adverse impacts of salinity have been attributed to a sharp decline in plant-growth-regulating substances under salinity. According to the relevant reports, seed germination is severely affected by increased salinity (Sosa et al., 2005). For example, significant reductions in seed germination of plants such as *Ocimum basilicum* (Miceli et al., 2003; Yaldız et al., 2018), *Petroselinum hortense* (Ramin, 2005), sweet marjoram (Ali et al., 2007a, b), and *Thymus maroccanus* (Belaqziz et al., 2009) were observed under salt stress. In this regard, slow or little mobilization of reserve nutrients by plants, suspension of cell division, and growth and damage of hypocotyls under salt stress have been suggested as the main causes of these effects (Said-Al Ahl & Omer, 2011). Another stage that is negatively affected by salinity is seedling growth. In addition, during the seedling growth stage, medicinal and aromatic plants are adversely affected by salinity. For example, the seedling growth of thyme (Belaqziz et al., 2009), basil (Ramin, 2005), chamomile, and marjoram (Ali et al. 2007a, b) is significantly reduced due to salt stress. In previous studies, it has been reported that there is a significant decrease in leaf, stem, sibling, root, and fresh and dry weights of plants exposed to salt stress (Chartzoulakis & Klapaki, 2000; Hasegawa et al., 2000; Hosseini & Rezvani Moghadam, 2006; Mahdavi et al., 2007; Hamidi & Safarnejad, 2010).

Similarly, leaf number, leaf area, and leaf biomass were negatively affected under salt stress in some MAPs such as *Mentha pulegium* (Queslati et al., 2010), *Majorana hortensis* (Shalan et al., 2006), peppermint (Aziz et al., 2008), geranium (Leithy et al., 2009), *Thymus vulgaris* (Najafian et al., 2009), and *Salvia officinalis* (Ben Taarit et al., 2009). In a study using salt water irrigation, it was reported that vegetative growth and green yield decreased in sweet fennel (Abou el-Magd et al., 2008). Similar results are reported in fennel (Amin, 1994; Graifenberg et al., 1996), *Nigella sativa* (Hajar et al., 1996), *Ocimum basilicum* (Bekhradi et al., 2015), *Coriandrum sativum* (Neffati et al., 2011; Ewase ve ark, 2013), *Salvia officinalis* (Kulak et al., 2020), and some medicinal plants (Yaldiz et al., 2018). Many studies also show that the root is less sensitive to salt stress than the shoot (Munns & Termot, 1986; Lauchli & Epstein, 1990; Graifenberg et al., 1996).

Likewise, increasing salinity and sodicity stresses caused a decrease in both shoot and root yields of citronella (*Cymbopogon nardus*), lemongrass (*Cymbopogon flexuosus*), and vetiver (*Vetiveria zizanioides*) (Basavaraju and Nanjappa 2011). It was determined that root length and root biomass decreased in *S. officinalis* and *S. tomentosa* under high salt stress, while root volume increased under moderate salt stress (Göçer et al., 2021). In a study by Ben Taarit et al. (2009), an increase in root biomass was reported in *S. officinalis* plant grown at high salt levels. The results of previous researches have shown that stress conditions cause physiological and metabolic changes as well as morphological adaptations (decrease in growth rate, deep rooting system, and modification of root/stem ratio to avoid drying out) (Basavaraju & Nanjappa, 2011).

Conflicts in Essential Oil Production as Responses Against High Levels of Salinity

There is conflicting information in previous studies about the accumulation of essential oils and their components in MAPs grown under salt stress. While salinity indirectly affects essential oil production, it directly affects growth and differentiation processes. These conflicting results represent differential responses of MAPs to salt stress with respect to essential oil production; however, the fact that some MAPs can increase the production of essential oil concentration or its main components in response to salt stress encourages the identification of molecular mechanisms of salt stress on the production of secondary metabolites in MAPs. Furthermore, the effects of salt stress on essential oil compounds have been clearly documented (Neffati & Marzouk, 2008; Baatour et al., 2010).

In another study, it has been stated that differences in molecular responses together with changes in the plant transcriptome, metabolome, and proteome affect salt stress tolerance in plants (Banerjee & Roychoudhury, 2017). These transcription factors include several families, such as AP2, ERF, bZIP, NAC, MYB, and WRKY, which exhibit higher correlations with salinity (Kumar et al., 2017).

It was reported that essential oil yield decreased under high salt stress in many MAPs such as *Mentha* × *piperita* (Tabatabaie & Nazari, 2007), *Mentha pulegium* and *Mentha suaveolens* (Aziz et al., 2008), *Thymus maroccanus* (Belaqziz et al., 2009), *Ocimum basilicum* (Said-Al Ahl & Mahmoud, 2010), *Melissa officinalis* (Öztürk et al., 2004), *Majorana hortensis* (Shalan et al., 2006), *Matricaria chamomilla* (Razmjoo et al., 2008), and *Salvia officinalis* (Ben Taarit et al., 2010). On the other hand, carvacrol (main essential oil component) and content of p-cymene and γ-terpinene in *Origanum vulgare* decreased under salt stress (Said-Al Ahl & Omer, 2011). An increase in essential oil content has been reported on *Satureja hortensis* (Baher et al., 2002), *Salvia officinalis* (Hendawy & Khalid, 2005), and *Thymus vulgaris* (Ezz El-Din et al., 2009).

Contrastingly, an increase in essential oil content has been reported under low salt concentration in *Satureja hortensis* (Baher et al., 2002), *Salvia officinalis* (Hendawy & Khalid, 2005), and *Thymus vulgaris* (Ezz El-Din et al., 2009). Also, Baghalian et al. (2008) reported that *Matricaria chamomilla* has high concentrations of essential oil components such as α-bisabolol oxide B, α-bisabolol oxide A, α-bisabolol, and trans-β-farnesene in saline conditions. On the other hand, Singh et al. (1997) stated that geraniol, the main component of lemongrass oil, and monoterpenoids are interconvertible under geraniol dehydrogenase catalysis.

Alterations in Phenolics as Responses Against High Levels of Salinity

Plants control the production of reactive oxygen species (ROS) using both enzymatic and nonenzymatic systems. The activity of enzymes such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) is enzymatic antioxidant processes. The antioxidant defense system consists of nonenzymatic components such as ascorbate, glutathione, proline, glycine betaine, amino acids, and/or phenolic compounds (Dmitrieva et al., 2020). It has been determined that phenolic compounds such as phenolic acids, flavonoids, and tannins, which have antioxidant properties, increase to eliminate ROS formed during stress conditions (Ksouri et al., 2008).

Phenolics are generally produced and accumulated in plant tissues exposed to stress and pathogen attack in their epidermal sublayers (Clé et al., 2008). The concentration of a phenolic compound in plant tissue may vary with the seasons, as well as with different stages of plant development (Dar, 2012). Moreover, various internal and external factors such as injury, drought, and pathogen attack affect the synthesis and accumulation of phenolics (Naikoo et al., 2019).

In a study by Queslati et al. (2010), it was observed that phenol concentration of *Mentha pulegium* increased in different saline applications. Similarly, the biosynthesis of apigenin, quercetin, and trans-cinnamic acid of *Nigella sativa* increased in salt stress conditions (Bourgou et al., 2010). Shahverdi et al. (2018) examined the effects of different saline doses on *Stevia rebaudiana* and stated that low salt stress (30 mM) increased the percentage of stevioside and rebaudioside A. Likewise, a significant increase in phenolic acids such as protocatechuic, chlorogenic, and caffeic acids was noted in *Matricaria chamomilla* grown under moderate water stress (Cik et al., 2009). Similar results were observed in *Nigella sativa* (Bourgou et al., 2010), *Achillea fragrantissima* (Abd ElAzim & Ahmed, 2009), and *Stevia rebaudiana* (Rathore et al., 2014). Drought often causes oxidative stress and was reported to show increase in the amounts of flavonoids and phenolic acids in willow leaves (Bourgou et al., 2010).

Attempts to Diminish the Effects of Salinity Stress

In order to alleviate the salt stress effects on MAPs, of those attempts, employing potassium fertilizers (150, 300, and 450 mg K_2SO_4/kg) against 100 mM NaCl stress was assayed in *Coriandrum sativum*, *Anethum graveolens*, *Ocimum basilicum*, and *Foeniculum vulgare* (Yaldız et al., 2018), suggesting that potassium sulphate applications had significant effects on these four plants at vegetative stages under salinity stress, and additional potassium doses partly reduced the negative effects of salt concentrations (Yaldiz et al., 2018). Similarly, exogenous selenium treatments exerted significant affirmative impacts on dry leaf of sage and fresh root and fresh shoot weights of mountain tea in salinity conditions (Çamlıca et al., 2019). In another study, selenium doses prevent the high salt stress in sage. The selenium and NaCl combinations improved the explicit negative salt stress effects, increased the dry biomass, improved essential oil yield and its components, and increased the nutrient uptake of the sage under salt stress (Yaldiz & Camlica, 2021b). Like other conventional treatments, Naeem et al. (2013) reported that calcium showed to improve the growth, yield, and quality properties of many medicinal plants under different conditions caused by biotic and abiotic stress factors. It was also implied that research of the possibilities to improve and increase the biosynthesis of secondary plant metabolites in MAPs should be carried out.

While the effects of salinity on agro-morphological traits of the plants are, in general, clear; the responses with the respect to the biosynthesis of secondary metabolites exhibit conflict against salt stress. Those conflicting findings suggest more in-depth studies in MAPs, especially under higher levels of salinity. Prior to the alleviative and potential treatments against stress conditions, the clear and exact mechanisms of salt stress on secondary metabolites of MAPs must be understood.

Drought Stress

Drought stress, like other prominent factors, hinders plant growth and development (Xu et al., 2010), emerging when the present water in soil decreases the critical levels and water continues the loss with the help of atmospheric conditions. Corresponding to drought stress, various alterations (mostly damages) in morphology (plant height, leaf number, leaf area branch number, etc.), structure of anatomy (stomata number, chlorophyll content, pollen size), and biochemical properties (enzyme activity, protein content, sugar content) (Pradhan et al., 2017) occur. Those relevant modifications have been attributed to the manifested changes in plant responses against drought stress.

In particular, it was reported that secondary metabolites such as saponin, anthocyanin, and flavonoids increased in low water conditions compared to normal water conditions (Wang et al., 2010). Furthermore, a recent study was carried out to determine the effect of salicylic acid (SA) priming on physiological, biochemical, and yield parameters of water-stressed basil plants. Water content, water potential, quantum yield, shoot height, stem diameter, dry weight, branch number, and leaf number of basil are affected positively by SA priming. Although water stress reduced the element contents except Ca and Mg, SA priming increased the element contents. Also, phenolic and flavonoid content coupled with higher antioxidant activities increased with water stress and SA priming. In addition, essential oil percentages did not show important changes in applied applications (Kulak et al., 2021).

Drought Stress Alters the Responses of Plants Concerned with Their Physiology and Morphologies

Each plant, including the MAPs, is severely affected by water stress. If the plant is exposed to water stress during its morphological growth stage, shoots and roots are most affected. In addition, the survival of plants under stressful conditions depends on the plant species, growth stage duration, and intensity of water deficiency (Jaleel et al., 2007). Drought stress causes significant changes, especially in photosynthetic pigments and components, which severely affects the Calvin cycle. It has been noted that the biomass of roots increases in water-deficient plants because the roots absorb enough water to withstand stressful conditions. So, shoot length was decreased due to the fact that MAPs had to overcome the water and nutrient deficiencies caused by drought. In addition, MAPs cannot accumulate water inside the tissues due to the reduction of internal mass in water-stressed MAPs (Reddy et al., 2004, Uzilday et al., 2012).

Since the water available in the soil is taken up by the plant roots, the root size, density, and the response of plants to drought stress are important for the plant's resilience to stress conditions (Vadez et al., 2013). Under water stress conditions, MAPs complete their life cycles faster than under normal conditions; as a result, crop growth stages have a short duration and fewer days to accumulate assimilates during its life cycle, and biomass production is reduced (Wahid et al., 2007;Nemeskéri et al., 2019). Therefore, MAPs reduce the number and area of leaves in order to reduce water use, and thus yield loss occurs (Schuppler et al., 1998).

A study was conducted on four different irrigation levels applied in *Thymus citriodorus* (lemon thyme or citrus thyme); a decrease was observed in all vegetative plant growth parameters such as plant height, dry and fresh weight of roots and shoots, and root length (Babaee et al., 2010). In a study by Said-Al Ahl et al. (2010), it was stated that the decrease in herb yield in thyme and chamomile under water stress conditions was due to the decrease in photosynthesis and the canopy structure of plants. Accordingly, thyme controls their growth in order to survive under drought stress conditions and ignores vegetative development. Therefore, it can be said that thyme shows a drought prevention mechanism by maintaining root system development through shoot fresh weight reduction, which also increases plant water uptake. On the other hand, Singh et al. (1997) reported that some plants exhibit a mechanism of adapting to water stress by restricting their growth while water scarcity in the rhizosphere continues. The negative effects of water stress were determined on the growth, yield, and quality of various MAPs like mint (*Mentha arvensis*), thyme (*Thymus vulgaris*), yarrow (*Achillea millefolium*), chicory (*Cichorium intybus*), calendula (*Calendula officinalis*), and balsam (*Melissa officinalis*) (Teklic et al., 2021).

Alterations in Essential Oil Yield and Composition as Response Against Drought Stress

The change of essential oils of the plant against drought stress depends on the type of plant but also on the severity and duration of the stress period (Jordan et al., 2003). Phosphoenolpyruvate increases the osmotic pressure of plants by causing the induction of carboxylase activity and lipid biosynthesis. The induction of this enzyme activity can be considered as an adaptive mechanism response to maintain photosynthetic carbon assimilation and water status, and it has been suggested that under stress, increased sebaceous gland density associated with a reduction in leaf area causes a high accumulation of oil (Singh et al., 1997).

The content of artemisinin (major component) was 28% higher in the plant subjected to 38 h of water stress compared to the aqueous control. The researchers stated that the reason for this is that while photosynthesis continues in moderate water stress, with the decrease in growth, excess photoassimilates used in small amounts for growth are directed to secondary metabolism such as artemisinin biosynthesis. They also concluded that not irrigating a day or two before plant harvest caused a moderate water stress condition and resulted in a significant increase of artemisinin without affecting biomass accumulation (Marchese et al., 2005).

Furthermore, thymol showed a significant increase on drought stress, but was not found to be a major component under control and mild stress. Similarly, Aziz et al. (2008) and Said-Al Ahl and Hussein (2010) reported that carvacrol increased under high drought stress in *Thymus vulgaris* oil. However, Said-Al Ahl et al. (2010) and Bahreininejad et al. (2013) reported that thymol content of *Thymus daenensis* decreased upon drought stress. Likewise Jordan et al. (2003) and Said-Al Ahl and Hussein (2010) showed that this component increased under moderate water stress and decreased under severe stress in *Thymus hyemalis*. The essential oil content, leaf photosynthetic pigments, soluble sugars, free proline and ascorbic acid content, and growth parameters were significantly improved in basil (*Ocimum basilicum* L.) plants under drought stress.

The increase in monoterpene concentration in sage (*Salvia officinalis*) due to drought stress is much greater than the corresponding loss in biomass (Nowak et al., 2010). Although the total monoterpene content was significantly higher in sage exposed to moderate drought stress than in well-watered controls, the total content of terpenoids (terpenes g^{-1} biomass) was reduced in *Melissa officinalis*, *Nepeta cataria*, and *Salvia officinalis* plants under high drought stress (Manukyan, 2011). The concentration of monoterpenes in leaves of all ages increased in sage plants grown under stress conditions. Moreover, the total content of monoterpenes in the whole plant increased despite an overall reduction in biomass production.

It has been reported that drought stress may be an important factor in increasing the concentration of secondary metabolic products in some medicinal plants such as *Hypericum brasiliense* L. (Khalid, 2006), *Calendula officinalis* L. (Taherkhani et al., 2011), and *Salvia miltiorrhiza* (Liu et al., 2011). However, there was no significant change in oil yield of *Thymus hyemalis* at four different irrigation levels (Jordan et al., 2003), while Sotomayor et al. (2004) reported that the maximum plant dry matter production and oil yield in *Thymus zygis* were achieved with moderate irrigation.

Changes in Phenolics Under Drought Stress

Aziz et al. (2008) and Said-Al Ahl and Hussein (2010) reported that phenolic compound increased under high drought stress in *Thymus vulgaris*.

Likewise, the total amount of phenolic compounds was found as significantly high in *Hypericum brasiliense* (Abreu & Mazzafera, 2005), *Thymus capitatus* (Delitala et al., 1986), *Echinacea purpurea* (Gray et al., 2003), *Hypericum brasiliense* (Abreu & Mazzafera, 2005), and *Salvia miltiorrhiza* (Liu et al., 2011) under drought stress.

Total phenolic, flavonoid, anthocyanin, and polyphenolic compounds increase during water stress, and these compounds have a protective role during drought condition in *Chrysanthemum* sp. (Hodaeia et al., 2018). In drought condition saponin content was found to be lower in *Chenopodium quinoa* (Soliz et al., 2002). Golkar and Taghizadeh (2018) found that the secondary plant products, i.e. proline, glycine betaine, carotenoids, total phenolic contents, and total flavonoids, were increasing when salinity increases up to 200 mM NaCl concentration in *Carthamus tinctorius* L.

Drought is a common and serious problem for plants in arid or semiarid regions. Although the studies indicate that MAPs develop different morphological, physiological, and biochemical mechanisms (photosynthesis, transpiration, water transport, nutrients, photosynthates, etc.) to withstand drought stress, studies on the effects of water stress on MAPs are still very few (Hall, 2000). Nowadays, when global warming is felt severely, possible regions and/or crop cultivation practices should be evaluated for high-quality and value-added MAPs so that farmers can switch to these products and create profitable and successful farms.

In particular, it is known that there is a close relationship between the secondary metabolism of MAPs and their defense response (Vasconsuelo & Boland, 2007). Among plant secondary metabolites, alkaloids, anthocyanins, flavonoids, quinones, lignans, steroids, and terpenoids have commercial applications as pharmaceuticals, dyes, flavours, fragrances, insecticides, and antioxidants (Jacobs et al., 2000; Verpoorte et al., 1999). Therefore, the secondary metabolites of medicinal plants are very valuable; however, the molecular mechanisms underlying their production have not been widely studied.

Extreme Temperature Stress (Heat, Cold, and Frost Stress)

Heat stress has destructive effects on plant physiology and growth by affecting cellular components and metabolism but its effects and tolerance of the plants are linked to the genetic structure, as well (Qin et al., 2008). Along with the alterations in physiological, biochemical, and molecular processes, temperature stress causes leaf senescence, damage of the membrane, degradation of chlorophyll, and protein denaturation (Pradhan et al., 2017). Cold stress (low temperature) is a very common environmental stress factor affecting plant growth and crop yield (Verma et al., 2013). Previous studies have noted that temperature stress can affect the secondary metabolites and other compounds of the plant production, which are usually the essence for their medicinal activity (Kirakosyan et al., 2003; Zobayed et al., 2005). According to Ebrahimi et al. (2011), the high temperature affects the plant properties, and it significantly decreases most of the growth compounds. However, the low temperature increased the biological yield, flower yield, essential oil percentage, and plant height of chamomile varieties. Corresponding to the temperature ranges, the germination rate and germination percentage of nine MAPs species, except *Ocimum basilicum*, were adversely affected by increasing temperature to 35 °C. The temperature between 15 and 20 °C was found to be the best seedling vigour in most of the species, and the temperature up to 35 °C highly decreased it (Nadjafi et al., 2010).

Heavy Metal Stress

Heavy metal stress is one of the major abiotic stress factors which is increasing in the agricultural soils. Heavy metals (zinc, copper, molybdenum, manganese, etc.) are rarely found in soils, and they increase depending on environmental pollution due to metal working industries and dumping. This stress factor affects both plant and human health. For example, lead (Pb) toxicity affects the enzyme activities, hormonal status, mineral nutrition, and membrane permeability of plants (Sharma & Dubey, 2005). With the pollution of soil, the several undesirable changes are found in metabolic activity of plants. Also, heavy metal uptake by plants can affect the photosynthetic pigments, sugars, proteins, and nonprotein production and can lead to plant death (Mahajan et al., 2020).

Out of the Attempts Available: Uses, Efficiency, and Safety of Nanoparticles Against Stress Factors

In plants, abiotic stress factors cause vital alterations in primary and secondary metabolism, which in turn result in loss of crop productivity, in general, as clearly reviewed in the former sections of the present chapter. As clearly reported, agriculture is under severe pressure of food, feed, fibre, and fuel, and it is being anticipated that the outputs of agriculture have to increase by 70% in order to meet the relevant demands for at least next 40 years (Godfray et al., 2010; Horlings & Marsden, 2011; Rodrigues et al., 2021). In order to fend off abiotic stress and ensure global food security, agrochemicals have been deemed as promising tool in improving the crop yield under ever-changing climatic changes (El-Shetehy et al., 2021). Out of the agrochemicals available, nanoparticles or nanomaterials are widely employed attempts against abiotic environmental factors. The relevant findings of the former studies have been recently well reviewed (Rajput et al., 2021; Singh et al., 2021). Even though a hallmark of those particles has been manifested into the agronomic characteristics of the crops, the effects are clearly dependent on dose, mode-of-treatments, chemical nature and size of the particles (Ghorbanpour et al., 2020; Gohari et al., 2020a, b). The word “nano” is used for materials that have a minimum of one dimension with an estimation approximately between 1 and 100 nm (Ozimek et al., 2010).

An Overview on Nanomaterial Studies Using a Powerful Tool: VOSviewer Visualizing Scientific Landscapes

Due to the high number of documents, VOSviewer has been recently employed in quite a number of disciplines in order to visualize, correlate, and discriminate the core topics of a specific field. With the relevant software, the bibliometric networks are constructed and visualized (Celik et al., 2021; Yuan et al., 2021; Raparelli & Lolletti, 2020). After a basic search on Scopus on September 29, 2021, with inclusion of following criteria: (TITLE-ABS-KEY (nanomaterial OR nanomaterials OR nanoparticle OR nanoparticles)) AND (abiotic AND stress OR biotic AND stress) AND (LIMIT-TO (SUBJAREA, "AGRI")) AND (MIT-TO PUBYEAR, 2021) OR LIMIT-TO (PUBYEAR, 2020) OR LIMIT-TO (PUBYEAR, 2019) OR LIMIT-TO (PUBYEAR, 2018) OR LIMIT-TO (PUBYEAR, 2017)), 515 document results were recorded. The retrieved documents were visualized to reveal the bibliometric networks using VOSviewer visualizing scientific landscapes. In this context, the most used terms and keywords of the retrieved documents were analysed and presented (Figs. 9.3 and 9.4, respectively). Considering the term analysis, two major clusters were observed. The first cluster (green) is addressed on crop productivity and quality under drought, in general. The latter one is mainly concentrated on the estimated parameters, viz. stress indicators, enzymatic and nonenzymatic defence system attributes, and commonly used nanoparticles (Fig. 9.3). Regarding the keyword content analysis, the words "nanotechnology, nanoparticles, and oxidative stress" are of the more pronounced keywords. According to the clusters revealed for keyword analysis, it was easily deduced that the relevant keywords are oriented on

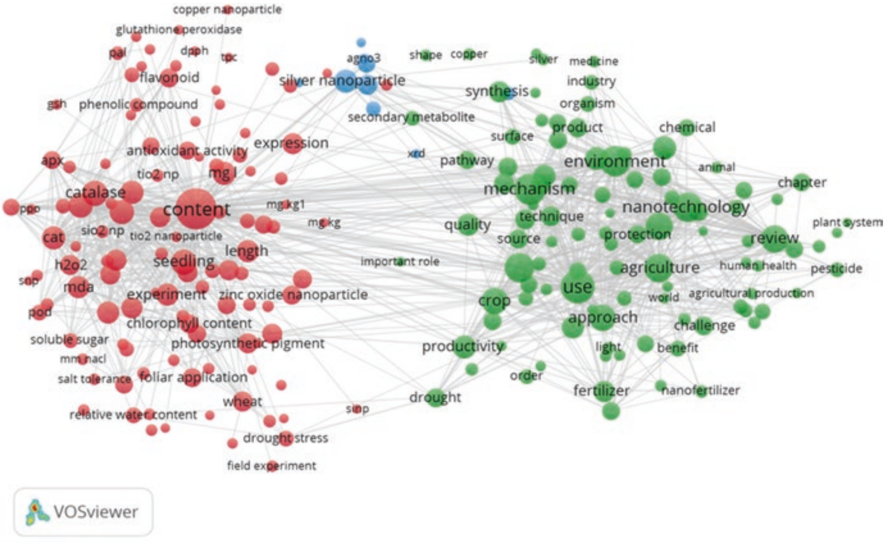

Fig. 9.3 Term analysis of the nanomaterial studies in agricultural areas (2017–2021)

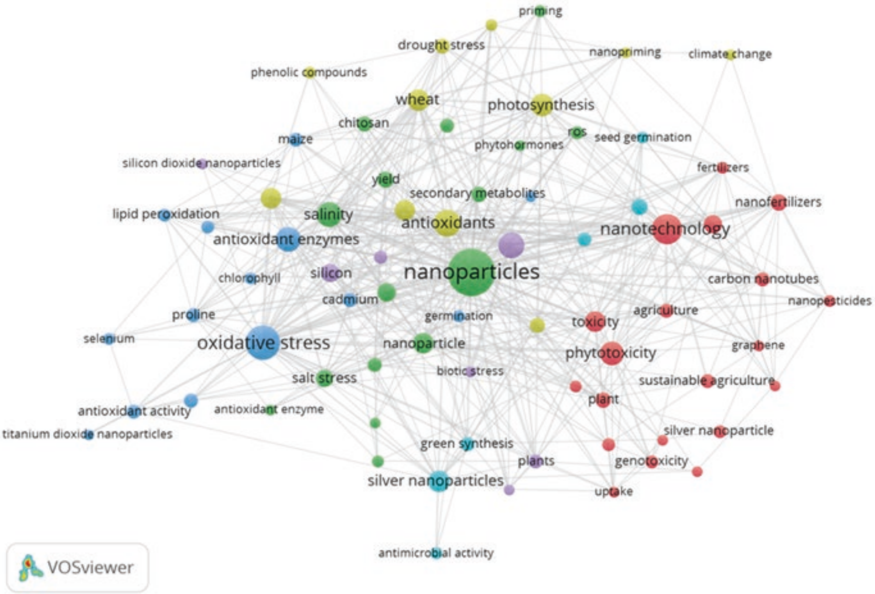

Fig. 9.4 Keyword analysis of the nanomaterial studies in agricultural areas (2017–2021)

the stress markers, antioxidant system, and nanomaterial types (Fig. 9.4). As stated in the following sections, in-depth studies concerned with the mode of action of the nanomaterials are required.

What Should Be the Priority Concerned with the Synthesis of Nanoparticles?

In the era we observe and live in, there are many conflicts between human beings and environments. For instance, there is an ever-increasing number of populations in limited arable agricultural lands coupled with limited water supplies (de la Rosa et al., 2021), which in turn cause dramatic outcomes in social life (Rahdari & Hoseini, 2012). To be compatible in finding the solutions consistent with the problem threatening the food security and ensuring the sustainability of the agricultural lands, the use of more efficient nanoparticles is a requested insight to be employed in increasing crop production under unfavourable environmental conditions. For efficient solutions, prior to the synthesis of the relevant nanoparticles, the complex plant system must be clearly known. In this regard, the interaction between plant organs, viz. root, stem, and leaves, as well as xylem and phloem transport system is a key point. This interaction is addressed on physical, chemical, and biological interactions of nanoparticles and plants, primarily at the atomic and molecular level. The relevant interaction might result in modification regarding initial properties of nanoparticles, suggesting that design and specificity of nanoparticles and their possible modification coupled with the interactions should be considered (de la Rosa et al., 2021).

The Same Nanoparticles But Different Stress Factors: Compatible or Not?

As seen in Fig. 9.3, copper nanoparticles, titanium oxide nanoparticles, silver nanoparticles, and silicon dioxide nanoparticles are the most examined nanoparticles in the relevant fields. Also, drought and salt stress are intensively studied as seen in Fig. 9.4. For that reason, the relevant stress and nanoparticles given above were presented.

Of those particles, the effects of copper nanoparticles on crop plants have been extensively investigated (Pérez-Labrada et al., 2019; Hernández-Hernández et al., 2018; Cumplido-Nájera et al., 2019; Quiterio-Gutiérrez et al., 2019; Van Nguyen et al., 2021) due to the essential roles of copper involved in plant physiology and biochemistry and consequently plant growth (Chibber et al., 2013). Considering the drought-submitted plants, copper nanoparticles enhanced the performance of the plants by a higher level of leaf water content and antioxidant enzyme activity as well as a lower level of malondialdehyde (MDA) content (Van Nguyen et al., 2021; Taran et al., 2017). Regarding the interactions of copper nanoparticles and salinity stress, improved growth as alterations in Na^+/K^+ ratio and activities of antioxidant enzymes and antioxidant substances were noted in tomato plants exposed to the higher level of salinity (Pérez-Labrada et al., 2019). In tomato plants under salinity stress, overexpressed levels of superoxide dismutase and jasmonic acid genes were observed by copper nanoparticles, and the alterations were translated into enhanced plant growth (Hernández-Hernández et al., 2018).

Titanium oxide nanoparticles are prominent nano-engineered materials against drought stress (Salajegheh et al., 2020; Mustafa et al., 2021; Karamian et al., 2020; Ghasemlou et al., 2019; Faraji & Sepehri, 2019; Gohari et al., 2020a, b) and salt stress (Sheikhalipour et al., 2021; Abdel Latef et al., 2018; Shah et al., 2021). As expected, damage in the stress-submitted plants was alleviated through alterations in physiological and biochemical attributes, viz. proline and malondialdehyde (MDA) content, photosynthesis machinery, and antioxidant enzyme activities.

Silver nanoparticles were also employed to enhance the performance of the plants against drought (Hojjat & Ganjali, 2016; Ali et al., 2019; Ghavam, 2019; Ahmed et al., 2021) and salt stress (Khan et al., 2021; Abou-Zeid & Ismail, 2018; Mohamed et al., 2017). Corresponding to the treatments, the physiological attributes such as chlorophyll stability index, leaf succulence, and leaf potassium contents in drought-submitted plants were buffered through silver nanoparticles, and consequently the drought tolerance levels of wheat were enhanced (Ahmed et al., 2021). Silver nanoparticles reduced sodium and chlorine as well as oxidative stress, which were then reflected into the improved growth and development of pearl millet exposed to salt stress (Khan et al., 2021; Khan et al., 2020). Also, engineered silica nanoparticles have been clearly revealed to be effective in combating drought (Aqaei et al., 2020; Mushtaq et al., 2018; Ashkavand et al., 2018) and salinity stress (El-Serafy et al., 2021; Siddiqui et al., 2014; Avestan et al., 2019; Roychoudhury, 2020). As the case observed for the aforementioned nanomaterials, the affirmative impacts of silica nanoparticles are correlated with net photosynthetic rate, stomatal conductance, transpiration rate, water use efficiency, antioxidant enzyme status (Siddiqui et al., 2014), and triggering the nonenzymatic (phenolic and flavonoids) compounds (El-Serafy et al., 2021). Taking into account all findings, it can be deduced that nanoparticles, as exogenous applications of other organic and non-organic amendments, are responsive to trigger the antioxidant defence (enzymatic and nonenzymatic defence system) as well as other osmotic pressure-regulating solutes or metabolites in stress-submitted plants. The mode of action in regulating growth, as the case we observe through literature review, resembles each other but not in comparison. What to be followed in this regard is to find or suggest the nanoparticles with the lowest adverse impacts on human and the environments and the cost-effective ones. Especially, plant-specific compound analysis is of the crucial points to be addressed. As a recent phenomenon, the stress memory of plants through trans-generations is a substantial research topic to be followed up in nanomaterial-treated plant, which deserves to be investigated in ever-changing environmental conditions.

A Hallmarked Indicator for Growth and Crop Productivity: But What Has Been Reported Regarding Metabolite Commodities Corresponding to the Agronomic Improvement Under Stress Conditions?

Accompanying the improvements in physiological and biochemical traits of the plants after treatments of nanoparticles, agronomic traits of the plants were clearly and positively affected, as the case findings of the reports given in the previous sections of the current study. Also, some of those reports available have been addressed on the metabolite alterations corresponding to the interactive effects of stress and nanoparticle treatments. As clearly well known, the efficacy of the plants regarding their communication and response against stress conditions is associated with the secondary metabolites (Ashraf et al., 2018). Of those reports, total secondary metabolites such as phenolics or Krebs cycle acids of barley plants were not affected by treatments of CdO nanoparticles, but individual compounds were affected. Tryptophan and phenylalanine as primary metabolites and ferulic acid and isovitexin as secondary metabolites were the most affected compounds (Večeřová et al., 2016). Corresponding to the roles of those compounds against stress, the roles of tryptophan (as a precursor for melatonin) in plant response and adaptation to stress factors have been reported (Hussein et al., 2014; Zemanová et al., 2014; Arnao & Hernández-Ruiz, 2015). Similarly to tryptophan, the significant roles of phenylalanine in combating stress were also reported for a quite number of plant species (Wen et al., 2008; Gao et al., 2008; Wada et al., 2014). Different nanoparticles at different concentrations (as the case of ZnO nanoparticles at 100 mg/L concentration and CuO nanoparticles at 10 mg/L concentration) have inductive capacity on the secondary metabolites of plants (Javed et al., 2018). Due to chemical plasticity of the secondary metabolites against reactive oxygen species burst (Marslin et al., 2017), in-depth studies to reveal the potential roles of those metabolites would likely to contribute in understanding the responses of the plants against interaction of nanoparticles and stress conditions.

Conclusion

As well known, any environmental stimuli rather than optimal growth conditions of the plants result in adverse effects on agronomic traits of plants, in general, and medicinal and aromatic plant, in particular. The relevant loss or retarded output has been well reported for a quite number of plants. However, the same and clear outputs concerned with secondary metabolites, which are major characteristics of medicinal plants, have not been reported hitherto. Out of the first attempts to be employed, in addition to conventional fertilizer approach, new engineered nanomaterials corresponding a specific target have been recently issued in combating the adverse stress factors, especially salinity and drought stress. Among the nanomaterials, copper nanoparticles, titanium oxide nanoparticles, silver nanoparticles, and silicon dioxide nanoparticles are the most examined nanoparticles. Up to our best knowledge and survey, there are limited reports regarding their mode of application, concentrations, timing, frequency, and in particular safety. As deduced from the studies up to now, future studies might be based on the safety and trans-generational effects on plants, also deserving to be investigated for their impacts on environment.

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Chapter 10
Sustainable Agriculture Through Technological Innovations

Sohaib Sarfraz, Faizan Ali, Akhtar Hameed, Zaheer Ahmad, and Kashif Riaz

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Introduction

Agriculture is a key element of human life providing all the basic essentialities of life, technically the all four F's: food, feed, fuel, and fiber (Spiertz & Ewert, 2009). Originally, the early life of human species was mainly focused on hunting animals in order to satisfy their food requirements, but with the passage of time, mankind became more aware about the domestication of plant crops along with the utilization of different animals for the purpose as well. These events lead to certain spells of rapid food productions over the time period ultimately leading to increased global populations. In the eighteenth and nineteenth centuries, random new discoveries ranging from steam engine to electricity and digital devices created a fusion between the concept of surplus food and innovation which ultimately responded to the explosive growth in birthrates and increasing populations (Mohajan, 2019; Galloway & Cowling, 2002).

The growing population was a concern from the early twentieth century; many of the famines (i.e., Irish famine, etc.) and economic turmoil events (i.e., the Great Depression) exposed the vulnerability of global efforts to satisfy the basic needs of human beings and showed how easily the inability to meet global food demands can become a complete disaster (Romer, 1990; Fotheringham et al., 2013). Lessons learnt from the tragic events of the past prompt a new thinking into the world, and efforts are made into a certain direction so that such catastrophic events are avoided and that enough food supplies can be secured for the world population. This all further led to the initiation of the Green Revolution era of the 1970s, which witnessed an immense increase in crop yield outputs across the globe especially among a large number of middle- and low-income countries, and was instrumental in improving the socioeconomic outlook for many countries. This revolution was mainly powered by the development of such high-yield cereal crop varieties along with the use of modern weed killing, pest control synthetic chemicals, fertilizers, and other agrochemicals (Tilman et al., 2002). This revolution introduced an era of stable food supplies and lesser hungers around the globe for a better half of the last century and in the early years of the twenty-first century; however, the overall impact of these approaches was not assessed properly and resulted in issues including runoff of harmful chemicals, soil degradation, and unintended negative climatic penalty (Pingali, 2012). Now, this in combination with the issue of resource scarcity, less availability of fertile and arable land, and increasing negative impact of climate changes is a terrible threat to global food security.

Climate change and human activities have created some major issues for sustainable agricultural productions, such as higher levels of carbon dioxide, increasing global temperatures, heat waves, drought, and resource depletion, resulting in lower yield outputs across the world (Nelson et al., 2009; Gliessman, 2015). In the meantime, the UN estimates suggest that worldwide population will reach more than 10 billion by the middle of this century, and to satisfy the hunger of this immense population, an increase of more than 70% in production outputs is required (Prosekov & Ivanova, 2018). Securing food for such population is a complex issue as agricultural production systems need to be adjusted in accordance with the different agroclimatic zones and soil conditions to attain the maximum growth (Tubiello et al., 2007).

The central idea of food security is to access healthy, nutritious food as part of daily diet regardless of socioeconomic boundaries, and without a sustainable approach, this would result in major environmental repercussions that leads to stagnant growth in food supplies in face of resources scarcity and global warming (Eitelberg et al., 2015). Sustainability in general is a broader term, and in the field of agriculture, it encompasses socioeconomic, ecological, and production sustainability (Smith et al., 2017). Such approaches focus on increasing yield intensity of crops with aim in sight of productions with lower greenhouse gas (GHG) emissions so that the negative impact on climate can be reduced. GHG emissions tend to have a negative impact on food security, and therefore utilizing plant and animal systems in a sustainable manner for better productions and yield outcomes is crucial in lowering climatic risk and maintaining steady food supplies.

In light of all these major challenges, sustainable agriculture is of great significance as it holds key to the solution of all these issues, to ensure global food security while maintaining balance among changing climate conditions, its socioeconomic impact, utilization of modern technologies, and sustainable productions and securing global food production.

Role of Technology in Sustainable Agriculture

The role of development and degree of innovation in future technologies has greatly inclined the constancy and definitely the productivity of agricultural products (Hutchins & Gehring, 1993). Agricultural technology, in the established logic, comprises the improvement and use of nutrients, biocontrol products, and farm machinery, but it also includes the visualization of genetically improved crops providing greater food quality and use of the latest equipment at farms that emphasize on overall sustainable production, not just seasonal production per hectare.

With the advent of modern technologies, there has been an evolving trend around the globe with dramatic transformation of all walks of life including the field of food and agriculture (Kakkad et al., 2019). Surprisingly, agriculture, though being the least digitized, has seen momentum for the development and commercialization of agricultural technologies. The latest and annually growing technologies are playing a vital role in the sustainability of agricultural growth as a central stage. The development of new technologies will not only lower the burden of farming community but also help in improving the quality of agricultural products. Keeping in view the future challenges and requirements, the development and adaptation of innovative technologies have been considered the most important as far as food availability and security are concerned. With the global increase in population, climate change, global warming, and covid-19-like problems, it has become difficult for the growers to cope with these issues, and thus there is a dire need for technological revolution that overpasses all these challenges and move towards sustainable agriculture. Therefore, the rise of innovative technologies has opened up the ways for a higher productivity with good quality. The share in agriculture-based technology has been accelerating by more than 45% in a couple of years and will extend more in the near future. The latest farm equipment and online data collection can provide growers a better control over crop production, management, and quality, thus enhancing their per acre yield with great efficacy. Now, it is also up to the farmers that they must constantly acquire and adapt new technologies and pursue practices for their effective results to promote sustainable agriculture.

Through smart technology applications, farmers can use accurate data for taking precise decisions, which will improve sustainability performance by increasing production, quality, yield per year, source use proficiency, and farmers' quality of life.

Digitalization of the agriculture sector allows it to expand production boundaries and use resources with great efficiency. Moreover, it also reduces many negative impacts of farming such as soil health and cuts costs for farmers. Here, we go through briefly about some of these emerging technologies that can assist farmers to attain precise data and higher yield.

Wearable sensory technology is an alarming system that alerts farmers towards any symptoms or signs of disease in their livestock and helps in timely and precise treatment well before the infection is established.

Cloud-based data management systems incorporate several information systems altogether that are helpful in reducing farmer labor and data collection and maintenance.

Robotics in agriculture is one more technology that can be used for the transformation of farming. Some of the already adopted systems include automated robotic milking system, robotic feeders, and slurry scrapers that help in reducing the skilled manpower at farms and also improve the comfort for animals like cows.

Laser scarecrow is the latest equipment used in the farm invented to keep away pests and birds like locust and blackbirds respectively that can otherwise destruct up to 70% of crops before and after harvest in the field. It produces a green laser beam up to 600 feet that is not visible to humans in the daylight, hence an eco-friendly and non-disturbing technique. Therefore, it is an important and efficient technology as far as the traditional and labor-consuming methods of pest control are concerned.

Bee vectoring is an excited development in plant disease management as far as the biological control of plant pathogens is concerned. Going through different phases of experimentation in different countries worldwide, it is found to be the proven and effective integrated disease management strategy. This technology can use different organic compounds or naturally occurring beneficial microbes like fungus, used in bio-products (Vectorite), that helps in protecting crops against a number of pathogens like *Botrytis* and *Sclerotinia*. Bee is used as a vector for spreading these important biocontrol-based products to plants, thus a very eco-friendly and natural way of enhancing plant growth and disease management.

Harvest quality vision (HQV) is a new technology that uses computerized system to detect the quantity and quality of harvested fruits and vegetables. This instrument includes a camera with different software for real-time detection and sorting/

grading the produced. Moreover, this software also alarms when any defects or disease symptoms are found on the product and helps in screening for better quality.

Radio-frequency identification (RFID) is a technology that exploits radio waves to detect data labeled to an object. It is very much similar with barcode but has more efficiency and can work systematically at agricultural farms from several feet away even in dirty conditions. It can be reprogrammed and can also be used for tagging harvested and stored crops. Moreover, it also minimizes data errors through computerization and increases traceability and rapid reminder for management whenever needed.

Vertical farming is the future of sustainable agriculture because of many factors like industrialization, climate change, and depletion of land resources and inadequate use of pesticides/chemicals. It is actually the next phase of in-door farming with different techniques of growing crops vertically in huge racks one over the other in a limited space with more than 70% production compared with field. This is really a method for meeting up the future food demands in controlled environment without getting in the climate change factor.

Minichromosome technology is developed by the breeders as a mini plus in genetic engineering to have bio-fortification, the enhancement, or supplementation of the crop's nutritional content. It can also be a part in improving crop resistance with new characters such as drought resistance. It uses a plant's original chromosome, so there is no ethical issues as compared to genetically modified plants. This technology has wide applications and potential in agriculture, but till now it is not fully explored.

Drone technology is one of the more efficient technology adopted at the agriculture farms because of its lesser time consumption with greater output observation and data. This technology is improving day by day as it can carry different cameras and sensors that are found to be very profitable in determining many aspects of crop condition, weeds, disease prevalence, etc. through aerial imaging. This all helps in developing and practicing early, precise, and targeted crop and disease management strategies efficiently (detailed in section Robotics and Drone Technologies).

In spite of the availability of all these and many other innovative technologies, there is still a considerable gap among the science and practices on farm. So, everyone needs to get in for this technological transformation and sustainable agriculture.

Digital Transformation for Sustainable Agriculture

Artificial Intelligence (AI) and the Internet of Things (IoT)

Artificial intelligence is the imitation of making a machine intellect and programming it for impersonating human actions. The term AI is first devised by John McCarthy (1927–2011) also known as the father of AI in 2006. This opens up a new science of learning, through the development of software and technologies getting into each other. An AI system can be developed by engineering machines with computational data power capabilities and using them for the welfare of mankind in one way or the other. Some of the components of AI include m**achine leaning** (**ML**), which guides a machine about implications and assessments depending upon the earlier practices, thus making a self-derived evaluation by using the input data and saving quality time and labor for humans (Patel et al., 2020).

Deep learning (DL) is actually a technique in ML. It imparts machine-to-route inputs through films and translates them into some outcome. Neural networks work on the same principle as human neuron cells by catching a number of data sets and processing it just like the living brain (Kodali & Sahu, 2016; Kulkarni & Deshmukh, 2013). Natural language processing (NLP) uses the natural principle of reading, understanding, and then interpreting an output by a machine. Computer vision and cognitive computing use algorithms for understanding and studying images or speeches in a way to give better output based on observations (Pandya et al., 2020; Sukhadia et al., 2020). There are three major types of AI based on their functionalities including artificial narrow intelligence (ANI), artificial general intelligence (AGI), and artificial super intelligence (ASI) using machine learning, machine intelligence, and machine consciousness respectively. The major purpose of all these types is to facilitate and ease the workload of human beings by developing hi-tech tools and equipment that are fully automated (Parekh et al., 2020; Jani et al., 2020).

AI is playing a vital role in improving our daily lives from Google searches to agricultural farms and in giving perceptions into employer behavior and then recommendations based on the data. It is based on diverse fields including biology, linguistics, computer science, mathematics, engineering, and psychology (Gandhi et al., 2019; Ahir et al., 2020). Moreover, it has various advantages over augmented intelligence and includes all those technologies that made life far better and easy by lessening human error, 24 h availability, replication of work with secure systems, and digital output results.

The **Internet of Things** (**IoT**) includes a group of objects that are implanted with sensing devices, software, and other innovative technologies that link and interchange data with other equipment and systems using the Internet or other communication linkages. Both AI and IoT are linked together to achieve higher levels of innovative and sustainable technologies in different industries.

Artificial intelligence (AI) and the Internet of Things (IoT) increasingly become strengths of agriculture industry as well. With a hopeful prospect, the agricultural technology is getting up, and a recent report indicated that AI agriculture market will grow annually at a rate of 28% from 2019 till 2024. Nowadays, farmers are looking towards AI and the IoT for providing all the latest facilities that are more efficient and sustainable.

AI and the IoT have revolutionized the agriculture sector with the introduction of the latest technologies that increased the crop yield in the presence of uncertain climate change factors, increase in world's population, and other food security issues (Raj et al., 2021). They also provided innovative solutions to the farmers for having a maximum output with lesser or the same input ensuring food quality. There are a number of modern technological applications of AI and the IoT that can be used at agriculture farms like irrigation, spraying, and weeding with the help of sensor-based technologies implanted with software. These innovative technologies can save the use of excess water and chemical pesticides, therefore minimizing the environmental toxicity and maintaining the soil fertility with good-quality food (Kim et al., 2019). Use of agricultural robots and drones with built-in hi-tech software and computer-based systems has made a great contribution in assessing and diagnosing plant data and pest distribution over the large field area in a short time span. These robots are used in sensing through GPS and Google Maps, and their data is processed through different wireless systems for reading (Liakos et al., 2018). With fast-growing technology every day, it is expected that an average farm will create a usual of 4.1 million data sets (Talaviya et al., 2020). Some of the various ways through which AI and the IoT become the best option for sustainable agriculture are as follows.

Monitoring Crops and Climate

Till now, many of the farmers are collecting and recording their data manually, consuming many hours, but still do not have the exact information due to human error. This can be solved by the prime use of AI- and IoT-based scientific technologies for monitoring crops and climate digitally (Mehta et al., 2021). Monitoring devices are of great importance while collecting and interpreting data from farms to the data bank. Cloud-based data like software as a service (SaaS) can be used with IoT platforms to collect records of multiple farms that can be of great value for in-time decision-making and observations like weather conditions, crop stage, soil quality, etc. This cloud-based data collection can improve farming on a large scale and even on national levels with greater insights and advantages. This data then can be deposited in the cloud to make it more systematized and manageable, thus allowing field activities to be monitored from anywhere and anytime. This can also be supportive for running an agribusiness as it helps in maintaining and rationalizing data and financial outputs in real time.

Smart Farm Machinery

From simple tractors connecting with AI-based devices to self-driven tractors, data collection now becomes totally automated and more precise. Nowadays, modern farmers especially in the developed countries use the latest farm tractors which are fully equipped and implemented with AI- and IoT-based sensors that monitor and record its operational data with the Differential Global Position System (DGPS) (Yahya & Ben-Othman, 2009). Performance and navigation of the tractors can be detected by a combination of hardware (sensors) and software (online applications) which measures location, energy consumption, and transfer of data for real-time assessment. Therefore, self-directed farm machinery with sensors, stereo cameras, and DL processes are used for capturing in-depth images for high-level detection (Redmon et al., 2016). Some of the object detection models recently used for agriculture applications include LiDAR (Light Imaging, Detection, and Ranging) (Inoue et al., 2019), RCNN (Ren et al., 2018) and SSDs (Liu et al., 2016). Some self-directed tractors, rice transplanters, and threshers have also been developed in collaboration with AI- and IoT-specialized engineers using DL-built computer visualization devices that can be used efficiently, for instance, in case of detection of fully ripened fruits and vegetables for harvesting (Kim et al., 2019).

Radical Irrigation Systems and Fertilizer Applications

Being humans, we are listening to a very common proverb from years and years that “Water is Life,” but now it is easy to understand this proverb as billions have been already invested in developing technologies that are used in minimum water consumption especially to fulfill future agricultural requirements. Many more efficient inventions have been in line using AI- and IoT-based approaches for better water usage and management taking irrigation systems to an advanced level. A fully automated system providing real-time data on soil moisture, humidity, and temperature through built-in sensors helps in determining soil water requirements precisely. These sensors are linked with MQTT (messaging procedure for the IoT) which helps in observing the real-time data (Nawandar & Satpute, 2019). For effective energy consumption and water intake, controlled water pump with adjustable flow rate is also developed using IoT-based sensors that can be handled from anywhere using mobile or web applications (Al-Ali et al., 2019). Likewise water, less or excess application of fertilizers, and micronutrients are another problem that results in lower yield. Using IoT-based AI technologies like NPK sensor and fog computing, farmers can have access to the availability and requirement of NPK in their soils (Lavanya et al., 2019). Some of the smart and sustainable agriculture-based technologies using AI and IoT include:

Smart elements: It contains a collection of equipment that eliminate manual data gathering by the use of server-based sensors that formulate and forward data to the base camp online and help in rapid and precise decision-making.

allMETEO: It is a gateway to cope with IoT-based weather stations which collect immediate data and create a weather plot prediction.

Pycno: It is a sensing instrument that collects data from the farm and transfers it to a smartphone through software and also contains disease models for risk assessment.

Pest and Weed Control

Regular use of traditional pest control methods and overdose of pesticides are big-time issues for eras, as far as soil, beneficial organisms, and environmental safety are concerned. Numerous approaches have been evolved for protecting crops from pests using the AI- and IoT-built technologies. Similarly, to manage weeds without damaging the major crop is time-consuming, labor requiring, and a challenging task; therefore, particular innovative AI-based site-specific technologies are developed with the combination IoT, robotics, and cutting-edge imaging methods. RGB and infrared (IR) imaging sensors are much used under field conditions for weed detection with pre-processing component that helps in resizing and altering of color spaces followed by separation of plants from soil and collecting only the weeds. To have more effective results, several algorithms like stepwise linear discriminant analysis (SWLDA), principal component analysis (PCA), and linear discriminant analysis (LDA) can be combined. Support vector machines (SVM) and artificial neural networks along with convolutional neural network (CNN) can be used for the final sorting of weeds and respective crop with ML or DL systems giving highly precise results (Akbarzadeh et al., 2018; Wang et al., 2019). Therefore, AI- and IoT-based weed control system can be used efficiently for minimizing the use of herbicides and environmental hazards.

Robotics and Drone Technologies

The agriculture sector is of great importance to the world not only because of the rapid increasing demand for food by the ever-rising population but also due to the economic clout of the sector which already employs a large proportion of the world population and will be further crucial in maintaining steady employments globally (Talaviya et al., 2020). The sector is of key importance for the economic stability and prosperity of developed as well as developing nations and also for the progress of rural communities around the globe making rural progress in line with urban development a reality (Shah et al., 2019). All this put great emphasis on sustainable growth of the agriculture sector worldwide.

Technological advancements are simply revolutionizing all walks of life, and the agriculture sector is no exception in this; over the last decade, digitalization has rapidly increased in agriculture field as integration of electronic devices and computer systems is transforming the sector (kakkad et al., 2019). Automation of agriculture sector involves knowledge and expertise from multiple fields including biology, mathematics, computer science, and engineering, allowing flexibility for the skilled labor force which was originally restricted to specific fields and now is able to work on resolution of certain challenges in various fields (Jha et al., 2019). The latest robot technology is built in a manner to collect information about certain problems from the field and utilize that data for resolving issues via various artificial intelligence (AI)-based approaches. Several high-end robots powered by new AI-based approaches are now being utilized for dealing with numerous field challenges in agriculture including, crop yield assessment, quality monitoring, environmental factors assessment, soil content assessment, real-time image data processing for harvesting, and marketing. As the global population continues to rise, an imminent food crisis is a major threat, and AI-based robotic technological solutions are enabling growers to combat these issues and improve their production outputs on a consistent basis to ensure global food security.

Automated robotic machine solutions are more efficient than conventional field machines and require less labor inputs, thus offering more efficacy; due to this, such automated machines are now becoming more and more integrated in day-to-day farm operations (Dursun & Ozden, 2011). Sustainable food productions with efficient resource utilization have become the motto for machine and robotics application in agriculture; this is crucial in the face of rising populations and ever-squeezing resources and is in line with the sustainable development goals (SDGs) of the UN. Water is the most important resource for sustenance of all kinds of life forms including plants; the agriculture sector accounts for the largest fresh water resource consumption in the world, and this resource is depleting consistently (Talaviya et al., 2020). In this regard, there is a dire need for the development of such system which can assist in conserving water resources along with stable growth in production outputs. Machines and robotics provide a solution for this in the form of modern irrigation systems where the entire program is designed with field robots mounted with sensors which assess the level of moisture, humidity, soil, and air temperature and estimate the amount of water needed by a plant in relation to its growth stage and the future weather forecast; the entire data is processed in real time, and machine-to-machine coordination enables a quick and effective response with least exploitation of resources (Kumar, 2014; Shekhar et al., 2017). Another major challenge for field crops is weed; the assessment for nutrients, water, and other essential inputs is made in a way to maximize yield outputs, but weed effects tend to disrupt this system as they compete with plants for space and nutrients, thus influencing yields. In order to resolve this issue, modern automated systems are being developed; these robot systems are powered by laser range fielder (LRF) for weed management; it consists of a gray-level vision which guides robot motion and a color-based vision which performs image analysis for the detection of weeds and its differentiation from crop plants (Fennimore et al., 2016).

Unmanned aerial vehicles (UAV), or simply known as drones, are sort of aerial robots and are becoming increasingly important in the agriculture industry. These drones have a series of sensors attached on it and work in combination with GPS systems in order to access field health, crop monitoring, weed identification, and assessments. These drones systems are used for recording, assessment, interpretation, and predictive outputs of plants in the field. As these drone technologies are helpful in resource management as well as effective responses, they are becoming more and more popular in agriculture field. These drone technologies are also useful in the management of crops under extreme weather conditions, and for such crops which have tall growth patterns, like maize, sugarcane, etc., drones with attached sensors and high-spectral imaging cameras assess field conditions and provide a broad view of ongoing problems. The collected data can be further utilized for resource distribution, weed management, and disease containment in the field (Sugiura et al., 2005).

Drones are used for spraying purposes in order to disperse chemical treatments over a large field area; these drones assess imaging data and make accurate calculations of how much treatment concentration is needed in a mixture. The resultant chemical mixture ensures lesser spill off into the environment and fewer residual effects as the concentrations are carefully calibrated by the drone machines (Yallappa et al., 2017; Nørremark & Griepentrog, 2004). Use of technology has been around since the dawn of the twenty-first century; with all technologies applied to enhance yield outputs, the introduction of drones has taken this whole operation to a new level. IUAVs with high-resolution cameras and infrared sensors in combination with GPS technology provide real-time field monitoring information; the collected data can be processed to study each growth stage of the crop plant and prepare yield maps to make predictive analysis of yield outputs.

As global population is gradually moving towards nearly a 10 billion mark by 2050, the agriculture and food production sector is coming under an immense pressure (FAO et al., 2017). In addition to the unavailability or lesser supply of fresh water, fluctuating environmental temperatures, less land, and resource scarcity are some of the key challenges to agriculture and global food security. Resolving these challenges is key to the future; for this, cognitive thinking-based automated solutions and application of the latest technologies will be crucial in handling these issues and providing realistic solutions to these challenges.

OMICs and Genome Editing Technologies

Structural identification of DNA and exploring its sequence were a major leap forward in the era of life sciences (Collard & Mackill, 2008; Collins et al., 2003). These discoveries were pivotal for plant research during the twentieth century as it laid the foundation for molecular breeding techniques via probe markers. This marker-assisted approach was of great significance due to the specificity of genetic markers and was supportive for genetic screening efforts which enabled the incorporation of multiple high-value traits in a specific crop cultivar. These genetic approaches were further shifted to the next level by efforts focused on combining genetic information from multiple species in a single plant creating a transgenic plant which imparts specific genetic traits onto the crop, i.e., creation of commercial BT cotton and HT plant cultivars (James, 2007). Now the modern omics technologies are further equipping the scientific community with better tools to resolve issues regarding food security. Reducing costs for genome sequencing has now made reference genomes available for multiple plants species, and in some cases, multiple high-quality reference genomes are available. This genome-wide sequencing results in the detection of key core- and pan-genome regions, and this sequencing power in an integration with new statistical models makes massive genome-wide association studies (GWAS) possible (Luo et al., 2020; Li & Yan, 2020). These OMICS approaches were mostly engulfed together in a single combined era termed as the third breeding revolution, and this information in combination with the latest genetic engineering tools has now initiated the fourth breeding revolution powered by precision breeding techniques.

Transgenic approaches were adopted in the late 1980s followed by the first commercially available transgenic crops coming into scene in the 1990s. The key element of the transgenic approaches is the introduction of some exogenous genetic material encoding for a certain trait into the host plant cell ultimately enabling the production of particular defense-related biochemical compound. Despite the successful achievements of such techniques, the commercialization of transgenically enhanced crop varieties is often limited by lengthy and costly approval procedures, and such time-consuming approval procedures often render the newly established defense vulnerable (Chen et al., 2019; Shrivastava, 2011). With each of the traditionally used approaches being beneficial in one manner or the other, still each of these techniques is held back in their own ways, which in an ever-increasing climate changing scenario poses an increasing risk of food security and is essential to be dealt with. The introduction of the desired site-specific genomic manipulation has become a topic of focus since the early 2000s with the introduction of earlier gene editing techniques like the zinc finger nucleases (ZFNs) and transcription activator-like effector nuclease (TALEN). These earlier gene manipulation technologies faced certain limitations including cost, difficult construction, and off-target mutations making it ineffective for broad-range applications (Chen et al., 2019). Clustered regularly interspaced short palindromic repeats (CRISPR) has become the face of modern gene editing approaches and is the most innovative scientific discovery of the twenty-first century which has brought a revolution in the scientific world. The CRISPR/CAS9 genome editing technology has been applied to mammalian and plant cells with a high success rate in the early 2010s paving the way for broader application of the technology in plants enabling research community leveraging modern sequencing techniques for achieving desired traits (Li et al., 2013). In the light of recent evidences, effective results are achieved by the use of CRISPR/CAS9 in plants. The CRISPR/CAS9 works on a basic principle of cutting and denaturing the gene; for this purpose, CAS9 generates a simple double-strand break on the desired gene location which is later repaired by the natural repairing system of cell. The repair can either be non-homologous end joining (NHEJ) or a homologous directed repair (HDR). In NHEJ the two broken strands are joined together causing mutations in the certain gene and making it imperfect. In HDR the broken strands of DNA are repaired by using a template strand as a reference and repairing the broken strand in reference to the template (Voytas, 2013).

CRISPR/CAS9 genome editing technology is being used in plants for enhancement of various traits and to strengthen the sustainable agriculture approaches with less utilization of resources made possible by using better plants. A major threat to plants is infection resulting from interaction with different types of microorganisms (i.e., bacteria, fungus) and viruses; the resulting interactions cause diseases and

Table 10.1 Genome-edited traits in plants

Plant species	Trait/target	References
Solanum lycopersicum	Powdery mildew disease	Nekrasov et al. (2017)
Nicotiana benthamiana	Bean yellow dwarf viral (BeYDV) disease	Baltes et al. (2015)
Manihot esculenta	Cassava brown streak disease (CBSD)	Gomez et al. (2019)
Oryza sativa	Rice blast disease	Wang et al. (2016)
Arabidopsis thaliana	Virus-induced gene editing (VIGE) system utilization	Ji et al. (2018)
Cucumis sativus	Cucumber vein yellowing virus (CVYV)	Chandrasekaran et al. (2016)
Vitis vinifera	Powdery mildew disease	Pessina et al. (2016)
Theobroma cacao	Black pod disease	Fister et al. (2018)
Oryza sativa	Stem borer attack	Lu et al. (2018)
Citrus	Bacterial canker disease	Jia et al. (2016) and Peng et al. (2017)
Oryza sativa	Bacterial blight	Oliva et al. (2019)
Triticum aestivum	Powdery mildew disease	Wang et al. (2014)
Zea mays	Yield enhancement	Shi et al. (2017)

other physiological implications ultimately making it difficult for plants to grow and reducing yield outputs. In order to resolve such issues, the CRISPR/CAS9-based genome editing has turned out to be a major approach in reducing the impact of such disease threats. Currently, the CRISPR genome editing technology has been successfully used for enhancement of resistance in plants against certain viruses and bacterial- and fungal-based diseases. In addition to the basic efforts focused on better immune response of plants, the genome editing technology is also being utilized for other sort of trait enhancements including such traits that focus on abiotic stress and yield-oriented traits. Table 10.1 gives brief information for some traits that have been engineered in plant using genome editing technology. The corresponding products produced are of great assistance for sustainable food production in face of climate change threat.

Conclusion

The rise in global food demands has led to the intensification of agricultural practices; this ultimately resulted in the production of food to satisfy the hunger of the globe. On one end, these practices secured steady food supplies for everyone on the planet since the last century, but on the other end, these practices also resulted in the exploitation of land and scarce natural resources. This will impart immense cost in the form of lower yield outputs for future generations with ever-increasing threat from climate change. In this regard, sustainable agricultural practices are the dire need of the time, using modern technologies in incorporation with the latest efforts in genetic engineering to produce new cultivars will help in increasing yield outputs with a minimum resource penalty. In addition to this, it will also be beneficial in reducing greenhouse gas (GHG) emissions that ultimately further deteriorate climatic situations and have negative impact on food security. Sustainable practices will help in the adaptability of multiple crops, reduction in GHG emissions, food security, prevention of environmental deterioration, and economic sustainability.

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Chapter 11
Sustainable Rice Production Under Biotic and Abiotic Stress Challenges

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Introduction

Rice has importance as the major food crop as it is utilized by the 1/3rd population of the world as staple food. Rice is the member of Poaceae family and is the main source of daily calories as it provides 80% of them (Sahebi et al., 2018). The increasing human population in the world has increased the requirement for rice production (Liang et al., 2010), demanding enhancement of 50% by the year 2025 (Khush, 2001). China is ranked first, India second, Pakistan third, Japan fourth, Korea fifth, Southeast Asia is at the sixth number in production of Rice and these countries are responsible for 90%of world's rice production (USDA, 2014). Brazil and United states have immense production of rice Freestanding of Asia (Ansari et al., 2015). Rice is the chief food commodity of more than half of total population of the world (FAO, 2008). Rice adds 3.2% in value addition in agriculture and 0.7% in the Gross Domestic Product. Total area under the rice cultivation is approximately 2.891 million ha and the production was 7.005 million tons in 2014–2015 (Economic Survey of Pakistan, 2015). Rice has many areas of production in Pakistan that have versatile environmental conditions. Basmati rice is grown widely in the conventional rice belt of Punjab Province while the Japonica type is sown in high altitudes of alpines and temperate regions for example in Swat. As the rice has its distribution worldwide and has adaptation to many areas it has become an efficient system of study in plants such as evolutionary genomics with a great range of its physio-morphic and developmental diversity found in *Oryza sativa* and its wild ancestors, *Oryza rufi pogon* and *Oryza nivara* (Zhao et al., 2010). The major decline in the agricultural production in rice is caused by stresses. Biotic stresses are caused by the living organisms, such as sucking and chewing type of insects, bacteria, weeds and viruses. Those biotic studies involve elicitor (Wang et al., 2011; Chen et al., 2007; Lin et al., 2008; Liao et al., 2009) fungus (Kim et al., 2003, 2004, 2008a, b, 2009) bacterium (Chen et al., 2007), virus, herbivore, and mammalian. There are almost 50 major diseases that reduce the crop yield and very important of them are Rice-blast, Brown-spot, blight, leaf-streak, fusarium-wilt, stem rot and smut diseases (Khan et al., 2015; Ke et al., 2017). Many of rice biotic stresses have been reported throughout the world including Pakistan, for example Bacterial leaf blight, Paddy blast, Brown leaf spot, Stem rot, Foot rot/Bakanae and Rice yellow mottle disease (Sere et al., 2013). Abiotic stresses are those that are related to nonliving factors and these play a crucial role in damaging the crop and diminishing the production worldwide. So these are very important issues need to be dealt to fulfill the food requirements in the future climate change scenario (Wani & Sah, 2014). Among abiotic stresses salinity, drought, heat, submergence (the whole plant dipping) and cold are important factors that are risk in the food production (Wani & Sah, 2014; Sanghera et al., 2011).

Out of these stresses drought or water deficiency is foremost ecological stress that has crucial effect on the reduction in yield of rice and great efforts are made to control this (Cattivelli et al., 2008; Gosal et al., 2010). The areas dependent on rain for the water receive 25% of water requirement and there are more chances of rice to be attacked by drought and climatic threats. As rice is water loving plant so severely affected by water deficiency and its yield is reduced by 15–50%. It depends upon type cultivar and severity of stress (Kumar et al., 2008a, b; Srividhya et al., 2011). Water deficit has several negative impacts on plants in many ways like it affects plant growth, yield, amount of photosynthates, water pathways and content of different pigments formed (Benjamin & Nielsen, 2006). Salt stress is the second lethal factor in the rice yield in rice growing countries and rice is prone to get affected by this salt stress and this cause loss in production (Gosal et al., 2010; Mohammadi-Nejad et al., 2008; Lutts et al., 1995; Todaka et al., 2012; Wani & Gosal, 2011). Out of 130 million ha of rice area in the world, 30% have high salts in the soil and not letting the plants to grow and give good yield. The reduction in the rice production due to salt affected soil is guessed to be 68% (Naheed et al., 2007). Salinity reduces the growth and impedes the normal mechanisms and causes losses up to 50% (Molla et al., 2015). Resistance against salt stress is complicated because it is controlled by numerous physiological processes. A large area under rice cultivation including tropical and subtropical is affected by salt stress (Molla et al., 2015; Dissanayake & Wijeratne, 2006). As compared with other food cereals rice has greater sensitivity towards salt stress (Vu. et al., 2012) Thirdly heat or high temperature is third most important factor that causes reduction in yield of rice (Chebau et al., 2018). At present the major areas where rice is grown have a close temperature to that of optimum for rice cultivation, so if changes in these temperatures occur at the sensitive stages of rice development then it can cause drastic loss in yield. It has been observed that rice has reduction in yield to 41% by twenty-first century (Ceccarelli et al., 2010; Shah et al., 2011). High temperature affects all prospects of plant mechanisms such as germination, growth, Flowering and maturation (Mittler & Blumwald, 2010). The grain quality and fertility of the spikelet is greatly affected if there is heat stress at reproductive and grain formation stage. It is observed that heat stress at flowering stage is crucially damaging and an elevation of temperature from 32 to 36 °C causes high spikelet fertility (Jagadish et al., 2010). High temperature spikelet infertility is proportional to loss of grain yield. At temperature above 35 °C anthers are unable to dehisce and less pollen dispersion will be there and ultimately incomplete fertilization will lead to poor seed formation or reduced yield (Chapagain & Hoekstra, 2011). The following review is about the detailed description of Biotic and abiotic stresses related to the rice production in the world and in Pakistan along with future prospects in Rice production.

Biotic Stress in Rice

Rice (*Oryza sativa* L.) is profusely grown in the substantial rainfall areas, comprising tropics and subtropics of the globe (Chapagain & Hoekstra, 2011). Economy of Pakistan is highly reliant on the rice crop because the country is ranked second pertaining to rice export (Zahid et al., 2005; Islam & Ahmed, 2016). It is vulnerable to attack by 50 diseases, 6 of them are bacterial, and 4 nematode related, 21 fungal, 21 viral and 7 others. However, main diseases are rice blast, bacterial leaf blight

(BLB) and Bakanae are chief yield limiting factor for rice (Khan et al., 2015; Ke et al., 2017). This crop is affected by more than forty genera of fungi and bacteria at various growth stages. Numerous rice biotic stresses have been reported throughout the world including Pakistan, for example Bacterial leaf blight, Paddy blast, Brown leaf spot, Stem rot, Foot rot/Bakanae and Rice yellow mottle disease (Sere et al., 2013; Anonymous, 2011).

Climate Change and Disease Complexes in Rice
Climatic factors especially temperature and relative humidity are the key factors influencing development of any insect pest and disease of rice. Moreover, CO_2 is the key factor for global climate change, resulting elevated temperature. The intergovernmental panel on climate change predicted that with the current emission scenario, global mean temperature would rise between 0.9 and 3.5°°C by the year 2100. Under such condition frequency of precipitation, intensity of drought and UV-B radiation is predicted to increase, which might affect the structure of rice plant and intensity of insect pests and diseases. Climate change may change the pest–plant relationship resulting in positive or negative impact on incidence and severity of different diseases and insect pests. Sheath blight (*Rhizoctonia solani*), a minor disease in early 1970s, is now a most destructive disease of rice. Similar change has also been observed on the incidence and severity of some other diseases and insect pests. Ear-cutting caterpillar (*Mythimna separata*), a major pest of rice in Bangladesh in 1960s had only a few occurrences in the last few decades. Besides, leaf roller (*Cnaphalocrocis medinalis*, *Marasmia exigua*) that had lower ranks in the list of major pests has been coming at the top of the list since 1980s. In Bangladesh many interventions such as change in crop diversity, variety, cropping intensity, irrigation, fertilization, etc. along with climate change in the rice production system affected incidence and severity of insect pests and diseases. However, exact and individual contribution of such factors or interventions has not been worked out in Bangladesh or elsewhere. Probable change in the years to come on the status of insect pests, pathogens and natural enemies along with change in the host plants due to global climate change is discussed in this paper. Keywords Insects and pests-Climate change-Disease and pest problems-Integrated nutrient management-Cropping systems.

Bacterial Leaf Blight

BLB is caused by "*Xanthomonas oryzae pv.oryzae*". This disease is included in major destructive diseases causing substantial yield reductions, specifically in Asia (Islam & Ahmed, 2016; Sharma et al., 2017).

A. **History and distribution**

BLB disease was first spotted by the farmer of Japan in 1884 (Tagami & Mizukami, 1962). Worldwide, its occurrence has been found in various regions of

Asia, northern Australia, Africa and the United States. In Pakistan, this disease was recognized in 1977 (Mew & Majid, 1977). Formerly, 10–20% crop losses were observed under normal prevailing conditions, while under favorable conditions up to 50% crop losses were recorded in Asian countries (Mew et al., 1993). In recent years increased BLB attack was recorded in the rice growing areas of Pakistan (Ali et al., 2009; Akhtar et al., 2008; Bashir et al., 2010). BLB has high prevalent potential. It proves lethal for the high yielding cultivars in tropics and subtropics in Asia. Its incidence in the 70s in Africa and the Americas has led to concerns about its transmission and dissemination (Bashir et al., 2010).

B. **Economic importance**

BLB commonly invades the irrigated and barani areas (Rain dependent Areas) (Chu et al., 2006). Yield damage may reach 20–50% (Khan, 2014) which is reflected on the panicle appearance and grain development. The decrease in the production can reach 80% when severe, supported by favorable environment and susceptibility of cultivars (Mubassir et al., 2016; Noh et al., 2007). The losses in yield due to BLB, at the time of maturity, could be 4.5–29.1%. About 20–30% yield damage been reported in Pakistan by this disease. Absence of resistance in basmati varieties against bacterial leaf blight is one of the main causes of yield deterioration in Pakistan (Ashfaq et al., 2016).

C. **Causal organism**

Rice crop is attacked by numerous pathotypes (BLB). Disease of BLB is caused by Xanthomonas *oryzae* which has various virulent races (Ali et al., 2009). The causal organism is yellow colored and produces slime. It is mobile, gram negative shape similar to rod and a flagellum at the terminal. It invades the plant through wounds and natural openings. These different races have not been clearly identified and each genotype behaves differently (Niño-Liu et al., 2006; Anonymous, 2007).

D. **Pathogenic variation**

Two overwhelming pathogens of rice are *Xanthomonas oryzae pathovars (pv.) oryzae* and *oryzicola* (Long et al., 2018). Rice cultivars have differential response towards these patho types. A study conducted on varietal response to BLB races, prevalent in Pakistan, revealed that Super basmati, is more susceptible to Xoo pathogen with maximum syndrome incidence of 89.5% for PXO 340 and Basmati 2000 variety was highly resistant at both growth phases and exhibited susceptibility at the highest tiller formation phase for PXO 280, with highest disease frequency of 75.96% and PXO 340 with percentage incidence of 71.53% (Noor et al., 2006). It is very hard to control this disease due to extraordinary Xoo mutability. Resistance of the newly developed cultivars was easily broken down after three to four years (Shaheen et al., 2019). The limitations of this technology were increased with time and location, as onset of resistance against Xoo pathogen in rice and application on vegetative phase (Wang et al., 2006; Miah et al., 2017).

Rice Blast

Rice blast is the most harmful fungal disease among the biotic stresses which can cause losses 70–80% (Miah et al., 2017; Nasruddin & Amin, 2013). It is caused by *Magnaporthe oryzae*, its other names are rice blast fungus, rice seedling blight, rice rotten neck, oval leaf spot of graminae, blast of rice, pitting disease, Johnson's spot and rye grass blast. Rice blast disease has been known in 85 rice-growing countries (Wang et al., 2017).

A. **History and distribution**

Magnaporthe oryzae, a disease-causing pathogen of rice can be found worldwide and the diversity of pathogen population comprised 1700 isolates from 40 countries (Divya et al., 2014). Massive studies have been conducted using molecular markers and pathogenicity assessments on diverse rice varieties to identify the *M. oryzae* population structure all over the world. About 42 isolates were obtained from the infected USA commercial rice cultivars and confirmed the occurrence of eight lineages. This organism has been reported to form Simple population structure in Europe and Africa, and can be separated in to nine lineages (Takan et al., 2012).

B. **Economic importance**

Blast is caused by *Pyricularia oryzae.* It is lethal disease and results in crop losses of about $5 billion every year (Asghar et al., 2007). Blast is one of the most dreadful rice diseases globally which causes large yield losses each year and is a risk to world rice safety (Li et al., 2011). The disease destroys ample rice that can be fed to big population of 60 million in the world. It is also responsible for economic destruction of US$70 Billion (Scheuermann et al., 2012). The disease can appear at any stage and produce numerous symptoms. Attack of Blast has been reported in many countries causing losses 50–90%. Normally, the losses are between 1–50% but under favorable conditions, it can reach to 90%. That's why, it is considered as one of the deadly diseases of rice (Pathan & Mubeen, 2011).

C. **Causal organism**

Magnaporthe oryzae that is causal agent and filamentous ascomycetes that can reproduce by sexual and asexual methods of reproduction. The fungus produces conidia of size 20–22 × 10–12 µm. The conidia are translucent, two-septate, slightly darkened and are obclavate and pointed at the apex (Devi & Shama, 2010).

D. **Pathogenic variation**

The substitute of *Magnaporthe oryzae* is *Magnaporthe grisea* or *Pyricularia grisea* and*Magnaporthe* comprise of two divergent clades, one infecting Digitaria (crabgrass), is stated *M. grisea* and another is pathogenic in rice & millet denoted as *M. oryza. Magnaporthe oryzae* is the correct name for the isolates linked to the rice blast (Miah et al., 2017).

Bakanae Disease

Bakanae disease is due to *Fusarium fujikuroi Nirenberg* emerged as an important disease currently in Asia and other rice growing countries of the world (Singh & Sunder, 2012).

A. **History and distribution**

Bakanae was reported in 1828 in Japan. It is globally affecting the rice. From past decades its invasion in Asiatic countries has been increased dramatically. Pakistan has also severe attacks of this diseases from Past several decades (Zainudin et al., 2008; Haq et al., 2011; Gupta et al., 2014; Kim et al., 2015; Chen et al., 2016).

B. **Economic importance**

The fungus *F. fujikuroi* is found globally in temperate and tropical environments and found throughout the rice growing belt. It is claimed to be initial diseases that started destruction of rice production and quality deterioration and estimated yield losses ranging from 3.0% to 95.4% depending upon the area and type of cultivar grown (Singh & Sunder, 2012).

C. **Causal organism**

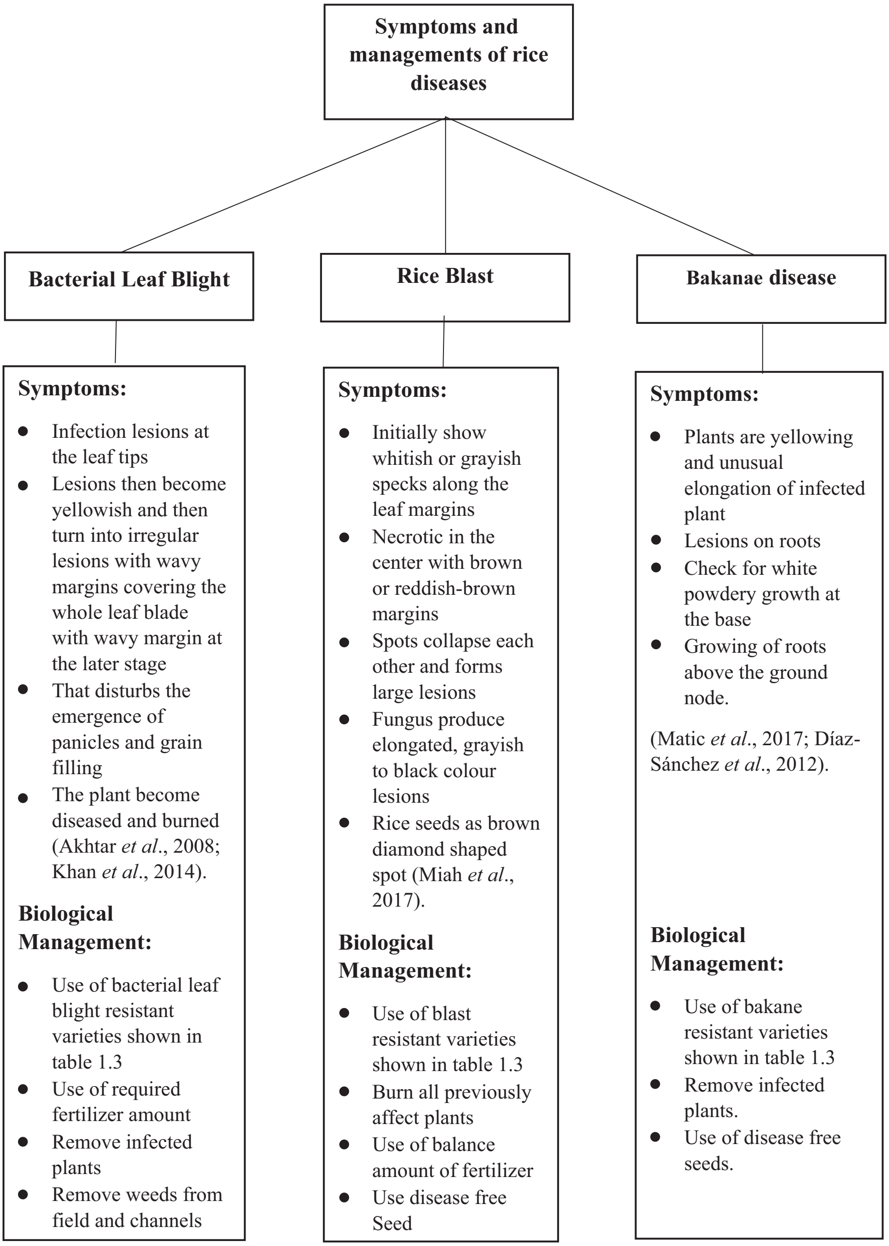
Fusarium fujikuroi Nirenberg, the causal organism of bakanae, it belongs to the *G. fujikuroi* polyphyletic taxon species complex (Carter et al., 2008; Karov et al., 2009).

D. **Pathogenic variation**

One hundred and forty-six strains of *Fusarium* spp. were separated from damaged rice plants and seeds from many different cultivated regions (Jeon et al., 2013). Different rice diseases and cycles are shown in Figs. 11.1 and 11.2.

Weed

Rice is cultivated over a wide range of environmental conditions, for example irrigated uplands, rain-fed upland and rain-fed lowlands ecosystems (Choudhary & Suri, 2014; Kaur et al., 2015). The biological constraints and challenges of rice production vary according to changing environmental conditions. In the biological constraints, weeds are main problems that affect yield productivity and quality as shown in Table 11.1 (Paul et al., 2014). In rice average yield losses are observed between 40–60% that may extent to 94–96% without controlling weeds (Chauhan et al., 2011). Weeds compete with rice crop for their growth in relation to space, nutrients, soil moisture and light. This competition causes serious yield loss to rice crop (Walia, 2006). Without controlling growth of weed, crop productivity decreased up to 57% in transplanted rice and up-to 82% in direct seeded rice (DSR) (Mahajan et al., 2009).



Symptoms and managements of rice diseases

Bacterial Leaf Blight

Symptoms:

- Infection lesions at the leaf tips
- Lesions then become yellowish and then turn into irregular lesions with wavy margins covering the whole leaf blade with wavy margin at the later stage
- That disturbs the emergence of panicles and grain filling
- The plant become diseased and burned (Akhtar *et al*., 2008; Khan *et al*., 2014).

Biological Management:

- Use of bacterial leaf blight resistant varieties shown in table 1.3
- Use of required fertilizer amount
- Remove infected plants
- Remove weeds from field and channels

Rice Blast

Symptoms:

- Initially show whitish or grayish specks along the leaf margins
- Necrotic in the center with brown or reddish-brown margins
- Spots collapse each other and forms large lesions
- Fungus produce elongated, grayish to black colour lesions
- Rice seeds as brown diamond shaped spot (Miah *et al*., 2017).

Biological Management:

- Use of blast resistant varieties shown in table 1.3
- Burn all previously affect plants
- Use of balance amount of fertilizer
- Use disease free Seed

Bakanae disease

Symptoms:

- Plants are yellowing and unusual elongation of infected plant
- Lesions on roots
- Check for white powdery growth at the base
- Growing of roots above the ground node.

(Matic *et al*., 2017; Díaz-Sánchez *et al*., 2012).

Biological Management:

- Use of bakane resistant varieties shown in table 1.3
- Remove infected plants.
- Use of disease free seeds.

Fig. 11.1 Symptoms and managements of rice diseases

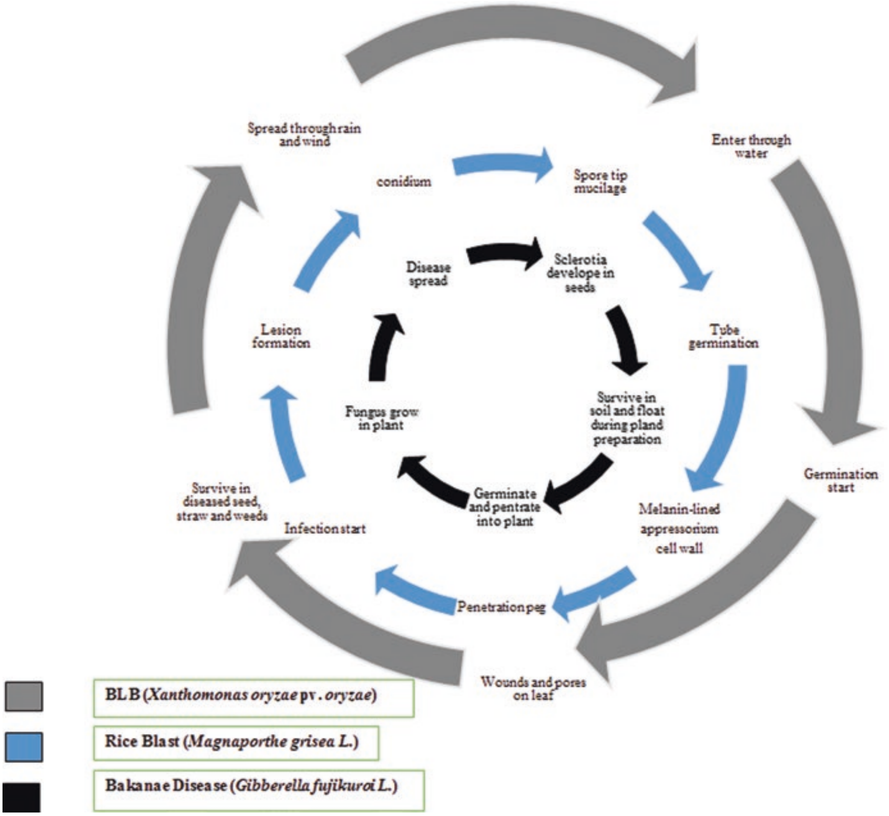


Fig. 11.2 Diseases cycle of rice

Table 11.1 Types of weeds in rice field

Broad leaf	*Eclipta prostrata*	Hossain et al. (2016)
	Ludwigia octovalvis	
	Monochoria	
	Commelina	
Grasses	*Leptochloa chinensi*	
	D. ciliaris	
	Eleusine indica	
Sedge	*C. rotundus*	
	Fimbristylis miliacea	
	C. difformis	

In Asia common method of rice crop establishment is transplanting of rice nursery plants in puddle soils with standing water which is costly time consuming and labor intensive (Matloob et al., 2015a). Other issue increase in water deficient condition all over the biosphere or in specific areas of rice farming in recent times and labor costs are increasing as compared to the increase in crop product prices (Saqib et al., 2012). So to secure food security in Asia there is a dire need to establish new technologies of rice production which can minimize our inputs cost i.e. irrigation water and traditional transplanting. New method is direct seedling of rice on dry lands which give good yield if weeds are properly managed which can reduce yield 30–80% or even all crop lose under favorable conditions (Matloob et al., 2015b).

Control

An appropriate weed management system required to keep weed population below threshold level by using all possible ways of weed control in a specific combination to have harmful effects at minimum level. By using weed control measure higher benefits could be achieved from applied inputs. Weeds have great diversity from season to season and location to location. There are different methods to control weeds include manual or physically, cultural or traditional, mechanical and chemicals (Chauhan et al., 2011).

For the establishment of rice crop most popular and oldest methods to reduce weed density are transplanting, puddling and submerged rice cultivation. Due to presence of anoxic soil environmental condition weed growth is usually subjected to suppression in flooded and puddle rice cultivation (Chauhan et al., 2011). However, it is a major problem in dry land rice production systems i.e. direct seeded rice due to less weed suppression (Ladha et al., 2007). In DSR crop yield loss due to more weeds than transplanted rice because rice and weeds grow together and farmers are unable to utilized flooded water to control weeds at initial growth phases (Chauhan & Johnson, 2010). If intense infestation of weeds occurs at the initial stage of crop growth and remains unchecked, then there will be no effective results of weed control on submergence at that time. Continuous submergence of the crop is required to suppress weed population and seed germination effectively for transplanted rice (Subramanyam et al., 2007). By increasing plant uniformity in combination with plant density in the field considerably control and reduced weed biomass automatically increasing the yield in many crops (Weiner et al., 2001; Olsen, 2012; Marin & Weiner, 2014). To control weeds, herbicides tolerance character has been used. To develop herbicides tolerant plant many genes are being used including bar gene and EPSPS gene. The *bar* and *EPSPS* genes are isolated from *Streptomyceshy groscopicus* and *Agrobacterium* strain CP4 which detoxifies herbicide glufosinate and detoxifies glyphosate herbicides (Kumar et al. 2008a, b).

A. **Manual weeding:**

Higher grain yield and yield attributes traits were observed with two-time manual weeding at 30 and 40 days later in transplanting rice (Lakshmi et al., 2006).

Another experiment is conducted at Kala Shah Kaku Research Institute, Pakistan to check the effect of four weedicides as compared to hand weeding to manage weeds in DSR. Although all weedicides gave significant results to control weed population but manual/hand weeding provided highest results 98.18% (Hussain et al., 2008). Another researcher reported the same finding in 2002 who stated that two-time hand weeding resulting in lower weed density than weedicides and untreated control. Manual weeding is expensive, laborious, tiring and time waste procedure so became difficult to apply at large scale (Rekha et al., 2002).

B. **Mechanical weeding:**

In-situ integration of weeds may help recycling of waste nutrients in an effective manner by mechanical weeding. To control weeds, present in inter row Rotating weeder was used but failed for weeds in intra row space. Randria mihariosa reported the benefit of soil aeration by using the rotating hoe. He performed experiments to compare hand weeding, mechanical and weedicides and noticed that four mechanical weeding give higher result by increasing yield (3.9 t/h) (Randriaharisoa, 2002). Rotating hoe improved the soil pores so that oxygen could be readily available to roots and microbes to increase tiller production. Use of cono weeder gave 10% increase in grain yield throughout the wet season and only 3% increase in yield throughout dry season as compared to conventional method of weeding (Thiyagarajan et al., 2002). Cono weeder helped to reduce labor, time up to 76% and optimize human work by improved posture by reducing muscles fatigue as compared to hand weeding. In paddy rice it was observed that cono weeder improved the speed of work and two times increase the yield (Mrunalini & Ganesh, 2008).

C. **Chemical control:**

Manual, mechanical and cultural systems of weed control are time consuming, difficult to practice, tedious and laborious apart from being less effective due to chances of weeds to regenerate from their remaining residues. At early stage of rice growth several weeds are similar to it in terms of morphology so it becomes quite difficult to uproot them by conventional weeding. To overcome this problem Weedicides, facilitate the most efficient, cost-effective, effective and applied ways of weed management (Hussain et al., 2008). Smartest and most viable option to manage weed are herbicides on a large scale rice farming (Anwar et al., 2012). In transplanting rice application of pyrazosulfuron ethyl at the rate of 20 or 25 g/ha considerably decrease the weed dry matter and density during khraif season (Chopra & chopra, 2003; Angiras & Kumar, 2005).

Application of pyrazosulfuran ethyl at the rate of 20 g/ha at 10 DAT increased the grain yield (4.45 t/ha) significantly (Shekhar et al., 2004). Saha reported most important class of herbicides is sulfonylurea group which represent higher activity level, excellent selectivity, flexibility of application and low mammalian toxicity. It has become popular all around the world to control broad spectrum weeds even at a very low dose (Saha, 2006) *E. colana, Cyperus defformis, Sphenchlea zelanica, Fimbristylis milliacea* and *Marsileaquadrifoila* were the major weed flora found in weedy plot. Saha and Rao reported that application of bensulfuran methyl 60 g/ha at 20 days afterward sowing maximum weed control efficiency (95.2%) was achieved (Saha & Rao, 2009). While with application of metsulfuron methyl 8 g/ha grain yield (4.79 t/ha) and higher number of panicle per m^2 (268) were recorded (Saha & Rao, 2010).

Several times due to various constraints at field it is impossible to apply herbicides in early growth stage and in other case weeds become resistant to herbicides due to continuous use of same herbicides. Under such condition post emergence herbicides may another choice (Puniya et al., 2007). By repeated use of same chemical and herbicides weeds become resistant to them to overcome that problem mixture of herbicides is used e.g. application of chlorimuron ethyl with Metsulfuron methyl 4 g/ha give excellent results to control broad spectrum weeds (Singh & Tewari, 2005).

Abiotic

Abiotic stress is main factors that adversely influence the yield worldwide. Later, these areas have main concern to achieve the essential food demand. Main abiotic stresses producing threats to food security are drought, high temperature, submergence and cold (Sanghera et al., 2011). In abiotic stresses, drought is responsible for reducing agricultural yields in the world and to achieve the demand, there is a need of efforts are being use to increase crop productivity (Gosal et al., 2010). High temperature is another abiotic stress in rice. If it goes above the critical temperature, it disturbs the normal physiological process. At the end, plant growth stunts or small plant, pollen sterility, less fertility occurs and severely yield decrease (Masuduzzaman et al., 2016).

Drought

Abiotic stresses that obviously decrease the crop yield, drought are one of the main stresses for rice production. In rain fed and upland environment, drought badly affects the grain quality (Masuduzzaman et al., 2016; Gosal et al., 2010; Bimpong et al., 2011). Drought retards growth of plant and yield loss in the rice. In the previous few years, experiment have been prepared to detect drought tolerance in modern rice varieties and consequently to make drought tolerant rice by different breeding methods (Kumar et al., 2012). In agriculture water is an essential feature and for food production it is an extremely limited source (Afiukwa et al., 2016). Climate changes harshly effects the water sources and in future, the occurrences of droughts and floods are probable to increase. Crop productivity determined by precise climatic circumstances and is extremely affected by climatic variability. Globally, rice yield changes day by day (Nahar et al., 2016). It was observed that rice yield significantly changes over the last three decades due to the climatic changes (Wang et al., 2014). A mainstream of the all tissues is formed by water which effectively helps as an important medium for movement of ingredients and nutrients inside the plant system. It is used in biochemical reactions and shows an essential character in regulatory soil plant water potentials or efficiency through enhancing turgor pressure (Pandey & Shukla, 2015). Drought stress affects fluctuation of the physiological processes of development and at the last the plant dies. The subsequent result of moisture because of stress inside the plant determined by the genotypes, span and period of duration to the stress effects and the growth stage of the rice plant (Ray et al., 2015; Farooq et al., 2008). Different morphological, physiological and molecular markers play vital role in drought tolerance as shown in Table 11.2.

A. Response of morphological markers:

Rice acclimatizes to drought stress by the selection of numerous physical or morphological, physiological and molecular markers. The severity and time duration of drought stress with responses which may arise at end, stress exclusion and relations among stress and additional factors are highly variant (Adejare & Unebesse, 2008).

In the vegetative phase, when rice was exposed to drought stress almost 80% of transpire able soil moisture shortage, leaf development rate decreased paralleled to well-watered plants (Abubakar, 2018). Drought stress at the tillering stage occurred to have some reverse consequence on the flag leaf length, area and number of actual tillers. There are some lines which are accomplished to stand with the drought stress when water needs almost normal (Farooq et al., 2009a). Yield under drought stress is also a main trait for identification of drought tolerant genotypes. Drought effects on yield because of the linkage with the irrigation time duration from flowering to physiological maturity. Drought at the time of vegetative growth and development, flowering, and terminal stages of rice farming can cause panicle sterility and empty grains (Farooq et al. 2009a, b, c). Long-lasting dry duration even for a less time period disturbs the crop. It changes to more severe when flowering period occurs

Table 11.2 Traits to improve drought tolerance

Sr. No.	Morphological traits	Physiological traits	Genes	References
1.	Plant height	Photosynthetic ability	OsNAC6	Riaz et al. (2017a, b)
2.	Leaf development rate	Osmotic potential	SNAC2	
3.	Flag leaf length	Changed metabolic functions	OsNAC5	
4.	Flag leaf area	Photosynthetic pigments	SNAC1	
5.	Number of tillers	Volume of rubisco activase	DREB	
6.	Grain filling	Proline	AREB1	
7.	Dry mass	Jasmonic acide	OsDREB2A	
8.	Number of panicles/plant	Glycinebetaine	AtDREB1A	
9.	Panicle length	Abscisic acid	TFHYR	
10.	Grain yield	Water use efficiency	SbDREB2	

with the drought stress (Venuprasad et al., 2009). In drought stress, roots respond by using of sending signals to the shoot as a result in generating many reactions like decrease in leaf expansion, gas exchange and stomata closure (Fatima et al., 2018). Root length and dry mass is useful indicator of rice yield in the drought stress (Ha et al., 2016). The significance role of flag leaf during grain filling can be used to develop drought tolerances genotypes. Grain filling in plant under drought, favorably indication for the role of flag leaf is essential to transport of photo assimilates and maintains synthesis. Therefore, numerous qualities of flag leaf have been projected for selection of drought tolerance i.e. leaf glaucousness, more flag leaf area, residual transpiration, canopy temperature depression, excised leaf weight loss, relative dry weight, higher carbon isotope discrimination (CID), late senescence and chlorophyll content. It was observed strong correlation in flag leaf and yield (Kumar et al. 2008a, b).

Lack of efficient screening methods against drought is a major limiting factor in the development of drought resistant cultivars.(Javed et al., 2012) used carbon Isotope Discrimination technique for indirect screening of rice against drought stress. In this technique variations in natural isotopes of carbon (^{13}C and ^{12}C) are measured and plants which show less depletion in ^{13}C as compare to susceptible plants will be resistant to drought. This technique can successfully be used to develop drought resistant varieties.

B. Response of physiological markers:

Photosynthesis is chief metabolic processes responsible of crop productivity. It is directly influenced by drought. When accessible water is reduced in drought stress then plants have a tendency to close the stomata (Feng et al., 2012; Biswal & Kohli, 2013). In drought situations, plants undergo several limitations as well as cell components injury through active oxygen and enhancing temperature (Farooq et al., 2009a). Rice under drought stress reduced its photosynthetic ability at the end, early stomatal closure and actual reaction to water stress (Feng et al., 2012). Physiological behavior of plants has capability to disturb the turgor pressure through increasing osmotic potential in drought (Iqbal, 2019). Drought produce several alterations associated to changed metabolic functions and one of them is also decreased the production of photosynthetic pigments. Major abnormalities in pigments quantities are significantly connected toward plant biomass and productivity (Farooq et al., 2008). Rubisco sites save from loss by volume of Rubisco activase and inhibition through promoting ATP dependent conformational modifications and increases during drought stress as a defensive tool. More concentration of this enzyme influences the improvement of rice by affect the Rubisco in drought (Maisura et al., 2014).

Chlorophyll is the most imperative pigment; take light and transmissions light energy to the photosystem reaction center. Chlorophyll-a and b are susceptible during soil dried. But additional carotenoids play extra role in photo protection, chloroplast photosystem and light collecting and moderately support the plants to survive challenges of drought. Drop in chlorophyll content and more quantum yield of PSII has been described in numerous studies (Jaleel et al., 2009). Abscisic acid (ABA) is a growth regulator and also shows an important role during stress. Many studies have described its part in plant reactions during drought by a most important of signal-transduction-pathways (Ji et al., 2012).

C. **Response of molecular markers:**

The drought tolerance is a multi-genic trait at the DNA level. Large amount of genes that show their behavior in drought stress at the transcriptional level have been observed by high quantitatively molecular research (Hu et al., 2006). The main methods used for recognizing to genes to play role in drought tolerance are transcript profiling through expressed RNA gel blot analysis, sequence tags profiling, massively parallel signature sequencing (MPSS), real time polymerase chain reaction (PCR) and microarrays, comparative proteome analysis a great amount of genes affected through drought tolerance have been observed by these methods. But, merely a minor role of genes has been functionally used for the improvement of drought tolerance genotypes in rice (Kumar et al., 2014).

In drought stress, stress responsive rice SNAC genes like, OsNAC6/SNAC2, OsNAC5 and SNAC1 improve the mechanism for drought tolerance after over translated (Nahar et al., 2016). Low regulation of XET and SAM synthetase proposes to disturbance of cell growth. ADFs perform a function in regulating the cellular actin cytoskeleton to alteration in cellular volume because of stomata regulation and also water loss during drought stress. Late Embryogenesis Abundant (LEA) protein is identified to be given protection to membranes (Kumar et al., 2014).

High Temperature

Global warming is becoming major complex problem which directly affects different crop yield and its quality. High temperature (HT) is also a major hurdle in rice production through the Asian tropical regions. Increase in critical temperature of rice that disturbs the standard physiological reaction of rice at different growth phases. At the end pollen sterility, low spikelet fertility and growth stunts occur and ultimately yield decreases severely (Sanghera et al., 2011).

Temperature deviations in top rice producing region are near to the optimal temperature 28 °C and 22 °C day and night (Tolleter et al., 2010). While rice can quiet continue with ideal growth at temperatures fluctuating from 27 to 32 °C without major decrease in yield (Das et al., 2014), temperatures more than 32 °C adversely disturb all growth and developmental stages. The extreme critical temperature was identified up to 33 °C during flowering stage (Aghamolki et al., 2014). High temperature is harmful to the physiological and chemical reactions including growth, photosynthesis, stomata opening and grain yield. High temperature tolerance studies have largely research on the flowering phase because of more sensitivity and its direct effect on yield (Kilasi et al., 2018). The increase in temperature cause permanent injury to plant growth and development. It has been observed each 1 °C rise in day time and rice yield decrease 7–8%.

To simplify breeding for heat tolerance genotypes, Different experiments were carried out in different temperature continues in field conditions:

- To observe high temperature effects on the anthesis, reproductive organs and to recognize the heat sensitive physiological methods.
- To observe association of high temperature responsive anther proteins in rice genotypes at anthesis.
- To evaluate genotypic variations in reproductive organs and physiological methods to panicle fertility (Sanghera et al., 2011).

Sexual reproduction usually needs in flowering plants which produce male and female gametes. The fertilization can be limited due to development of the male part (pollen) is more sensitive to the growing environment conditions (Hatfield & Prueger, 2015). Such as part of the reproductive organ, pistils and the maternal organ produce in the flower to provide nutrition and safety to the female gametophyte however also support the male gametophyte, While, this organ is observed more tolerant than the male gametophyte (Rieu et al., 2017). High temperature stress affects monocotyledonous and dicotyledonous plants (Hedhly, 2011). The seeds develop at early stages are sensitive against heat stress (Tayade et al., 2018). Due to the endosperm accounts for maximum of the rice caryopsis and defect in endosperm can affect the seed size. Development of the rice endosperm needs different developmental processes, i.e. nuclear cellularization, division, differentiation, maturation and syncytial (Folsom et al., 2014). Heat stress is related with various kinds of chalky rice grains like white-belly basal white, milky-white and white-black (Wu et al., 2016).

For stress tolerance, there are two biotechnological methods development of transgenic plants and advanced molecular breeding have been used for the increasing of crop yields. Transgenic approach is more effective for presenting new traits for biotic and abiotic stress tolerance. Transcriptional factors and Quantitative Trait Loci (QTLs) previously recognized in rice by different approaches. Genetic engineering can be used for transfer of genes from other species. There transgenic plants are present to tolerant severe temperature stress, i.e., 45–50 °C. Due to rapid global climate changes, a suitable approach is immediately required to decrease the damages due to high temperature. Production of useful and viable pollen used in pollination is an important character to confirm natural fertility and better crop yields (Hedhly, 2011). High temperature tolerance at various growth phases in rice is a complication occurrence in morphological and yield related components are shown in Table 11.3 (Wu et al., 2015).

Flood

Floods badly affect more than 20 million hectares in rainfed low land areas of the world each year. Rice is the main crop of these areas and provides food for millions of people. These areas have huge potential for more food production because of the

Table 11.3 Resistant varieties for biotic and abiotic stress

Countries names	Bacterial leaf blight	Blast disease	Bakanae disease	Varieties for DSR	Drought tolerant varieties	Heat tolerant varieties	Flood tolerant varieties
China					Mowanggunei, Qingsizhan1, Huhan 15, Handao3, Ganlangu,		
Philippines	NSIC Rc142, NSIC Rc154, PSB Rc82, IR-64, Macassane	Makassane, IR-64			IR6115-1-1-1, IR75942–9, IR45, IR53236–275-1, Nagina 22,	Tubigan 9, Nagina 22,	PSB Rc 18 Sub 1, IR 64 Sub 1
Brazil					Tre Smeses, IAC1, IAC1246,		
Bangladesh				BRRI Dhan-33, BRRI Dhan-39, BRRI Dhan-44	BRRI dhan 56		Samba Mahsuri, Panibhasha, Tilakkachari, Meghi, Jaldubi,
Nepal				Sona Masuli, Hardinath	Sookha dhan 3 Sookha dhan 1		
Indonesia					Inpago LIPI Go 1		Ciherang Sub 1
India	Improved samba Mahsuri' (ISM),		IR 20, IR 32, IR 38, IR 44, Punjab Mehak, Pusa Basmati no.1	HKR-120, Sarjoo-52, PD-4	Sahbhagi Dhan	Dular	Swarna Sub1, Samba mahsuri Sub 1, CR1009 Sub 1, IR40931, FR13A
Pakistan	Kashmir basmati		IR 6, KS 133	KSK-282, hybrid rice			Chenab basmati
Reference	International Rice Research Institute, Tabien et al. (2003)	International Rice research institute	Ghazanfar et al. (2013) and Fiyaz et al. (2016)	Tanno et al. (2007) and Tong (2008)	Luo (2010) and Dixit et al. (2014)	Poli et al. (2013) and Cantila et al. (2016)	Singh et al. (2009) and Goswami et al. (2017)

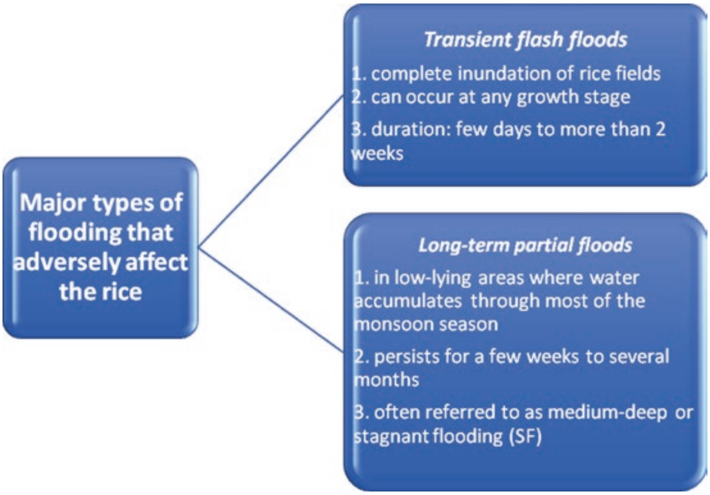


Fig. 11.3 Types of flood

availability of good textured soils and fresh water resources. However, rice production in these areas is becoming difficult due to adverse effects of climate changes and major type of flood shown in Fig. 11.3 (Mackill et al., 2010).

Rice cultivars having resistance to flood were identified 25 years ago. But these rice genotypes possessed undesirable agronomic characters and poor grain quality (Angaji et al., 2010). The trait is mainly controlled by a single locus carrying cluster of three genes, Sub1A, Sub1B and Sub1C but Sub1A is the major gene which is responsible for 70% of variation between tolerant and non-tolerant genotypes. It has been transferred successfully to develop the submergence tolerant versions of various locally adapted elite cultivars by back cross breeding followed by marker assisted selection (Collard et al., 2008). Fine mapping of single major quantitative trait locus (QTL) on chromosome has greatly facilitated this back cross breeding protocol to develop such cultivar which can tolerate complete submergence up to two weeks (Collard et al., 2008; Neeraja et al., 2007; Xu & Mackill, 1996). Other genes which are involved in submergence tolerance are shown in Fig. 11.4.

SUB1 genes have successfully been introduced in major cultivars of rice through marker assisted background selections. As a result of which tolerance of these cultivars to submergence has been improved without compromising on their yield and grain quality characters (Neeraja et al., 2007). These new *SUB1* varieties can tolerate submergence for 2–3 weeks when stress occurs after seedling stage before flowering stage. Due to the short stature, the vegetative growth in some of varieties is restricted when water level is above to 10–15 cm. These SUB1 introgressed lines provide huge yield advantage which in return will stabilize production in rainfed low land areas that experience flash flooding. Deep water rice escapes the flood stress by elongating its internodes (Hattori et al., 2009; Voesenek & Bailey-Serres, 2009).

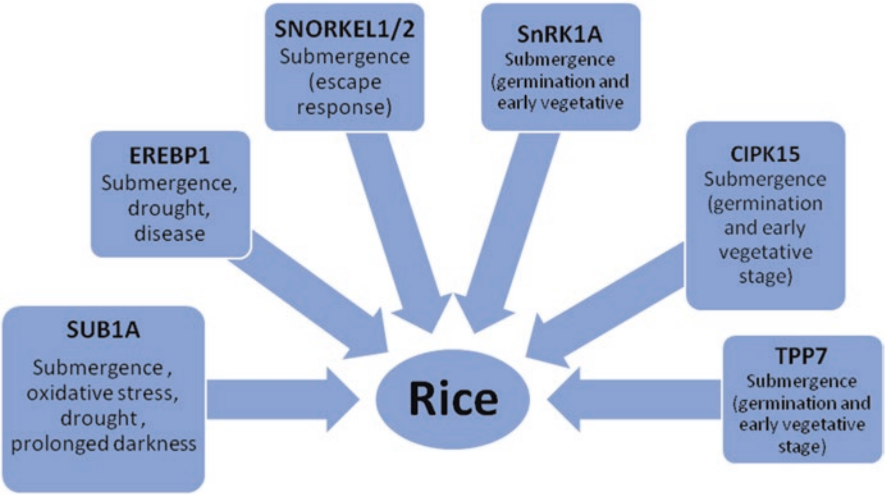


Fig. 11.4 Important genes involved in tolerance to submergence

For further improvements, proper understanding of agro-morphological, anatomical (structural changes in aerenchyma of root, shoot and leaf) and biochemical parameters (gibberellic acid, abscisic acid, ethylene, chlorophyll content and non-structural carbohydrate content of shoot) related to submergence stress will be very much useful. Suitable germplasm as well as management technologies are therefore needed to enhance and stabilize rice productivity in the submerged areas. Tolerance to flash floods and fast regeneration could contribute to better and stable productivity. Once we identify the tolerance and susceptible then we may create some variants for submergence tolerance through suitable hybridization program (Biswajit et al., 2017). Different varieties are resistant and tolerance against biotic and abiotic are shown in Table 11.3.

Conclusion

Biotic and abiotic stresses harshly disturb the crop yield and quality. Among the biotic stresses, it many diseases of rice like bacterial leaf blight, rice blast and bakane disease badly affect rice productivity. In Direct seeded rice, weeds compete for water, nutrients, light and air. Drought stress decreases the rice growth, affects pollination and grain formation with the stress timing, duration, severity and intensity. An integrated system is required to study the polygenic traits i.e. yield stability in drought condition in plants. We determined the destructive effects of high temperature on reproductive phase, flowering booting stages and yield components. By the use of morphological, physiological and molecular markers, we can better understand the genotypic and genotype x environmental interaction on the basis of plant response to diseases, weeds, drought, heat and flood for the improvement of rice crop.

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Chapter 12
Emerging Techniques to Develop Biotic Stress Resistance in Fruits and Vegetables

Afifa Younas, Madiha Rashid, Nadia Riaz, Muneeb Munawar, Sajid Fiaz, and Zahra Noreen

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Introduction

Fruits and vegetables have beneficial attributes; they provide the necessary calories for the human body, have a delicious taste, and meet the need for various nutrients (Ma et al., 2017). According to statistics, the annual production of fruits and vegetables in the world is about 3.7 billion tons (Barrett & Lloyd, 2012). However, fruits and vegetables are still living tissues after harvesting (Sanzani et al., 2016), and they continue their biological processes, such as dormancy, water transpiration, and respiration (Romanazzi et al., 2016).

In vegetables the factors for biotic stress are pests, diseases, and nematodes. The damages induced by these factors reflect highly on production, productivity, and quality. Crop cultivators suffer from high yield loss caused by various diseases. Biotic stress induced by microbes on crop plants reduces the crop yield and decreases the quality. Biotic stress causes disease in crops, which leads to the suffering of the plant. Diseases of the plant need to be controlled to maintain the abundance of food produced by farmers around the world. The management of crop diseases is very necessary to fulfill the food demand. Potato blight disease caused by plant pathogenic fungus *Phytophthora* caused more than one million deaths in Ireland (Yoshida et al., 2013). Around 20–40% of agricultural crop yield losses occur globally due to various diseases caused by phytopathogenic bacteria, fungi, pests, and weeds (Cabral-Pinto et al., 2020).

It is estimated that in 2050, the world's human population will reach around 10 billion, and around 800 million people in the world will be hungry, and around 653 million people in the world will be undernourished in 2030; thus, fulfilling the food demand will remain a huge challenge. The current research progress and disease management strategies are not enough to fulfill the food demand by 2050 (FAO, 2017). The first green revolution made a huge difference in yield and food production, but in the last few years, crop production has been stagnant, and food demand is increasing sharply, so now we need a second green revolution to fulfill the food demand of the population.

Different approaches are used by farmers to mitigate the impact of plant diseases. The agriculture system mainly relies on chemicals to manage crop diseases and inhibit the growth of phytopathogens, which cause diseases before and after crop harvesting. The excessive use of chemical pesticides, herbicides, and fungicides that are mainly used to control plant diseases causes harmful environmental and human health consequences. Tilman et al. 2002 observed that the high use of chemical pesticides increases resistance in pathogens and pests and reduces nitrogen fixation, and the bioaccumulation of toxic pesticides occurs.

Biotic stresses are the major factors limiting the crop productivity worldwide. Indiscriminate application of chemicals used for crop protection is a serious concern for health and environmental hazards. Moreover, such practices deteriorate the soil health and increase resistance in phytopathogens and pests. Nanotechnology, the novel interdisciplinary technique developed in the last decades, provides a sustainable solution. Nanotechnology has the potential to revolutionize agricultural practices. Nanoparticles (NPs), nanobiosensors, quantum dots (QDs), nanobarcodes, and microRNA (miRNA)-based approaches have a potential role in rapid diagnosis and combating insect pests and diseases in plants. This chapter deals with potential use of NMs in crop protection for better eco-friendly management against biotic stresses in plants (Hajong et al., 2019).

Biotic-Responsive Genes

Infecting plants and causing biotic stress is caused by a variety of pathogens, parasites, and pests. Fungal parasites could either be necrotrophic (damaging host cells by secreting toxin) or biotrophic (feeding on host cell). These might cause vascular wilts, leaf spots, and cankers in plants (Doughari, 2015 and Sobiczewski et al., 2017). Nematodes are parasitic worms that feed on plants and produce soilborne disorders such as nutrient deficiencies, stunting, and wilting (Bernard et al., 2017; Osman et al., 2020). Viruses, too, are capable of causing systemic and local damage, including as chlorosis and stunting. Mites and insects, on the other hand, harm plants by either eating (cutting and sucking) or laying eggs on them. Additionally, viruses and bacteria could also be transmitted by the insects (Schumann & D'Arcy, 2006).

Plants have adapted a complex immune system to fight such biotic stresses (Saijo & Loo, 2020). To keep viruses and insects at bay, plants have a passive first defense system that comprises physical barriers like cuticles, wax, and trichomes. Plants can also produce chemical substances to protect themselves against pathogen infection (Taiz & Zeiger, 2006). Plants also activate defense against biotic agents through two levels of pathogen identification (Dangl & McDowell, 2006). Pattern recognition receptors (PRRs) are the initial level of pathogen identification, and they recognize pathogen-associated molecular patterns (PAMPs). PAMP-triggered immunity (PTI) is the name given to this type of plant immunity (Monaghan & Zipfel, 2012). Herbivore-associated elicitors (HAEs), PRR herbivore effectors, and herbivore-associated molecular patterns (HAMPs) are used by phytophagous pests to respond to herbivores (Santamaria et al., 2013).

Plant resistance proteins (Avr proteins), which identify specific pathogen receptors, are the second level of pathogen identification (Abdul-Malik et al., 2020; Gouveia et al., 2017). It involves effector-triggered immunity (ETI) and is thought to be an effective mechanism of pest resistance in plants (Spoel & Dong, 2012). Infected and surrounding cells respond to ETI by triggering hypersensitivity responses (HRs) and programmed cell death (Mur et al., 2008). A majority of R genes encode proteins that have a particular domain with a conserved nucleotide binding site (NBS). The leucine-rich repeat (LRR) is the next most significant domain. NB-LRR receptors identify pathogen effectors either directly by the physical association or indirectly by associating with an accessory protein (Dodds & Rathjen, 2010).

Plant Defense Mechanisms to Biotic Stress

Systemic acquired resistance (SAR) occurs when a plant's R gene-mediated response results in a higher level of defense to an invading pathogen. SAR confers systemic resistance to a wide range of pathogens over the entire plant. Using intraplant communication, a local encounter in SAR may result in the induction of resistance in other plant parts (Fu & Dong, 2013). In general, both types of plant host defense elicit the same response, but ETI is thought to be more resistant to pathogen infection (Tao et al., 2003). The earliest signaling events that occur when plants are exposed to biotic stress are changes in cytosolic calcium ion concentrations. Plant immune signaling mechanisms are centered on Ca^{2+} signals (Aldon et al., 2018). Gene reprogramming is required to create an appropriate response, which in turn depends on rapid and transitory changes in Ca^{2+} concentrations (Reddy et al., 2011). The Ca^{2+} signals of plant immune respond differently. Calcium ion transients induced by PTI activation revert to basic levels within few minutes; however, ETI causes a substantial rise in cytosolic Ca^{2+} levels that may last for many hours (Lecourieux et al., 2005).

Lanthanum, a well-known calcium ion channel blocker, has been shown to restrict immunological responses linked to both PTI and ETI (Boudsocq et al., 2010). PTI and ETI trigger calcium ion channels in response to biotic invasion, causing cytoplasmic Ca^{2+} concentrations to rise. The four main plasma membrane Ca^{2+} permeable channels in *Arabidopsis thaliana* are stretch-activated Ca^{2+} channels (OSCAs), cyclic nucleotide-gated channels (CNGCs), MID1-complementing activity (MCA), and glutamate receptor-like channel (GLR) families (; Yuan et al., 2020). In *A. thaliana*, 20 different members of the CNGC family of plasma membrane Ca^{2+} permeable channels have been reported (DeFalco et al., 2016). Both plant development and biotic stress responses are heavily influenced by CNGCs (Breeze, 2019). The Ca^{2+} permeable channels including CNGC2, CNGC4, CNGC11, and CNGC12 have been reported to play key roles in the entry of Ca^{2+} ions into plant cells in response to fungal and bacterial infections (Ahn, 2007). The involvement of CNGC2, CNGC4, CNGC11, and CNGC12 in plant immune function is well known (Chin et al., 2013). The CNGC19 calcium ion channel's function has recently been broadened to herbivory-induced Ca^{2+} flow and plant defense responses against the parasite *Spodoptera litura* and basal defensive signaling to regulate *Piriformospora indica* colonization on *A. thaliana* roots (Jogawat et al., 2020).

CNGC was firstly discovered in barley plant, about 20 years ago, as a calmodulin binding protein (CaM) (Schuurink et al., 1998). The cytosolic N- and C-termini of CNGCs from plants and animals are reported to contain one or more CaM-binding domains, although the gating of CNGCs from plants is not well understood (DeFalco et al., 2016; James & Zagotta, 2018). Because of the difficulty in conducting electrophysiological investigations on CNGCs, plant CNGC research has slowly emerged. However, due to current technological advancements and the reliability of reverse genetics employing cngc mutants, only a few significant investigations have been conducted till now (Chiasson et al., 2017). CNGC7, CNGC8, and CNGC18 have all been proved to perform in conjunction with CaM2 as a signaling molecule that responds to cellular Ca^{2+} concentrations (Pan et al., 2019). Furthermore, CNGC18 is found to be co-expressed with CPK32, showing that phosphorylation regulates its activation (Zhou et al., 2014). Moreover, the GLRs are found to be associated with plant defense against *Botrytis cinerea* and *Hyaloperonospora arabidopsidis*, hence, categorized into three clades: clade I (GLRs 1.1–1.4), clade II (GLRs 2.1–2.9), and clade III (GLRs 3.1–3.7) (Manzoor et al., 2013).

The involvement of AtGLR3.3 and AtGLR3.6 in cytosolic Ca^{2+} increase induced by aphids is well understood (Vincent et al., 2017). The phosphorylation of AtGLR3.7 by CDPK3, CDPK16, and CDPK34 at serine 860 site has been confirmed by in vitro kinase test (Wang et al., 2019). Plant stress management and development have consistently been found associated to CDPKs (Singh et al., 2017). OSCAs (phosphorylation of OSCA1.3 by BIK1) and MCAs (MCA1 and MCA2), two additional plasma membrane-localized Ca^{2+} permeable channels, have been identified to control plant stomatal tolerance. Another plasma membrane-localized Ca^{2+} permeable channels, OSCAs (phosphorylation of OSCA1.3 by BIK1) and MCAs (MCA1 and MCA2), are found to regulate plant stomatal resistance and hyper-gravity in *A. thaliana* hypocotyls in dark, respectively (Hattori et al., 2020). Several more Ca^{2+} channels are thought to exist in the endoplasmic reticulum, mitochondria, Golgi bodies, and vacuole, in addition to the calcium ion channels found in the plasma membrane (Pandey & Sanyal, 2021). The organellar Ca^{2+} machinery of these channels include auto-inhibited Ca2+ ATPases (ACAs), ER-type Ca^{2+} ATPases (ECAs), P1-ATPases (e.g., HMA1), mitochondrial Ca^{2+} uniporter (MCU), Ca^{2+} exchangers (CAX), 1,4,5-trisphosphate receptor-like channel (InsP3R), two-pore channel (TPC), 1,4,5-trisphosphate (IP3), cyclic ADP-ribose (cADPR) activator ryanodine receptor-like channel (RyR), sodium-calcium exchanger (NCX), and slow-activating vacuolar channel (SV).

Many of these channels are considered to be crucial in plant immunity (Pandey & Sanyal, 2021; Taneja & Upadhyay, 2018). An array of Ca^{2+} binding proteins detects the calcium ion once it enters the cell. Ca^{2+} binding proteins function as sensors that decode complicated Ca^{2+} signals (Kudla et al., 2018). Ca^{2+} sensors are highly conserved proteins, classified into four categories (Ranty et al., 2016):

(a) CaM and CaM-like proteins (CMLs).
(b) Calcineurin-B-like proteins (CBLs).
(c) Ca^{2+}-dependent protein kinases (CPKs).
(d) Ca^{2+}- and Ca^{2+}/CaM-dependent protein kinases (CDPKs) (CCaMK).

The cross-talk of several biotic and abiotic stress signals is mediated by CaM, CMLs, CBLs, and CPKs (Aldon et al., 2018; Ranty et al., 2016). Stress signalling in plants is mediated by a number of Ca^{2+} and Ca^{2+} sensor-associated transcription factors (Chung et al., 2020; Ranty et al., 2016).

CAMTAs are the most numerous and well-studied Ca^{2+}/CaM-dependent transcription factors (Iqbal et al., 2020). CAMTA3 has been frequently reported as a repressor of biotic defense responses in plants (Jacob et al., 2018; Khan et al., 2020). It is directly phosphorylated and processed by flg22-responsive mitogen-activated protein kinases (MAPKs) and, therefore, functions downstream to MAP kinase (Bjornson et al., 2014). Furthermore, NAC TF modulates numerous biotic stress responses in *Solanum lycopersicum* when it interacts with Ca^{2+}/CaM (Wang et al., 2016). In wild strawberry, NAC is also found to be receptive towards *Colletotrichum gloeosporioides* and *Ralstonia solanacearum* contamination (Zhang et al., 2018).

Another Ca^{2+}/CaM-dependent TF involved in pathogen invasion is WRKY (Park et al., 2005; Bai et al., 2018). WRKY7, WRKY45, WRKY43, WRKY53, and WRKY50 bind to diverse CaM isoforms in a Ca^{2+}-dependent manner (Park et al., 2005). MYB TF is likewise a Ca^{2+}-dependent transcription factor. In the great majority of defense-responsive and abiotic stress-receptive genes, MYB plays an upstream role (Li et al., 2019). Considering CMLs into account, AtCML9 functions as a regulatory element of plant immune response. *Pseudomonas syringae*, as well as phytohormones including abscisic acid (ABA) and salicylic acid, were discovered to induce plant immune response (Leba et al., 2012). AtCML9 also interacts with the transcription factors WRKY53 and TGA3, which are also known to regulate biotic stress responses. AtCML37 and AtCML42 are both linked to the defenses against herbivorous insects (*Spodoptera littoralis*) (Scholz et al., 2014). Recently, the effect of 17 AcoCPK genes from *Ananas comosus* (pineapple) on biotic stress was investigated. In *A. thaliana*, AcoCPK1, AcoCPK3, and AcoCPK6 were found to confer disease resistance to *Sclerotinia sclerotiorum* (Zhang et al., 2020). CBLs, a different type of Ca^{2+} sensor, have been found to interact with a family of plant-specific CBL-interacting protein kinases (CIPKs). CBL reacts with Ca^{2+} and binds to CIPK, activating the kinase. Phosphorylation of downstream target proteins is actively regulated by the CBL-CIPK complex (Ma et al., 2020).

Basic/Molecular Mechanism of Host-Pathogen/Insect Interactions

The production of reactive oxygen species (ROS) and the activation of mitogen-activated protein kinases (MAPKs) are two other early responses to pathogen attack on plants (Muthamilarasan & Prasad, 2013). Other signaling pathways, such as light pathways, overlap with ROS and MAPKs (Foyer, 2018). Furthermore, pest infestation on plants triggers local or systemic defense responses via the signaling pathways of oligogalacturonoids, jasmonic acid, and hydrogen peroxide (Fürstenberg-Hägg et al., 2013). Plants are also able to produce volatile substances through lipoxygenase (LOX) and terpenoid signaling pathways that repel pests from attacking them (Dudareva et al., 2006; Pichersky & Gershenzon, 2002). Plants also produce defensive proteins and universal stress proteins, which is another important downstream defensive mechanism. Protein inhibitors like lectins, α-amylase, chitinases, and polyphenol oxidases are among these proteins (Lee et al., 2019). Furthermore, the role of pathogenesis-related (PR) genes in plant defensive responses has also received a lot of attention (Ali et al., 2018a, b).

Pathogenesis-related genes encode proteins that are activated in plants only under pathological or non-pathogenic conditions. They are involved in HR and SAR responses and are considered a significant component of plant innate immunity (Jain & Khurana, 2018). PR proteins have been classified into 17 different families based on their biochemical and molecular characteristics (van-Loon et al., 2006). Five PR genes, i.e., PR-1, PR-2, PR-3, PR-4, and PR-5, have widely been investigated in *A. thaliana* for their significance in plant biotic interactions (Hamamouch et al., 2011). SA-dependent SAR response was found to be mediated by PR-1, PR-2, and PR-5, whereas JA-dependent SAR response was mediated by PR-3 and PR-4 (Hamamouch et al., 2011; Thomma et al., 1998). The simultaneous involvement of PR proteins in biotic and abiotic stress is another significant characteristic (Ali et al., 2018a, b). The presence of distinct motifs linked with a variety of environmental conditions was determined bioinformatically in the 1000 bp upstream region among all five PR genes of *A. thaliana*. Interestingly, all of the PR genes had several light-responsive motifs including AE-box, GAP-box, GT-1 motif, G-box, GATA-motif, box-4, and chs-CMA2a. The occurrence of light-responsive motifs in PR gene promoters suggests the binding of light-dependent genes to these conserved regions. The notion of extensive cross-talks between biotic stress responses and light signaling pathways is also supported by this concept.

Therefore, role of plant hormones in controlling plant biotic defense response cannot be ignored. ETI and PTI activate particular downstream signaling pathways in which three plant hormones (SA, JA, and ethylene) play the key role. Among them, the biotrophic and hemi-biotrophic pathogenic agents activate SA regulatory mechanisms. Similarly, necrotrophic chemicals and chewing pests influence the JA and ET pathways (Bari & Jones, 2009). SA activates the SAR pathway, which promotes the expression of PR genes, resulting in resistance to a wide spectrum of diseases (Ádám et al., 2018). The regulatory mechanisms for plant defense (SA, JA, and ET) differ significantly, although they interconnect to provide protection against harmful pathogens (Ku et al., 2018). Furthermore, abscisic acid, auxin, brassinosteroids, cytokinin, gibberellic acid, and peptide hormones also help in controlling plant immunological responses (Chen et al., 2020; Ku et al., 2018). Among all plant hormones, jasmonic acid (JA) is the most important as it activates the plant defense system and interacts with other plant hormonal pathways to induce plant immunological responses (Yang et al., 2019).

Endophytes' Sustainable Plant Protection for Stress Management

Previously, much of the work has been reported about the role of endophytic plants' protection against different stresses. Applications of endophytes can help in the management of plants' tolerance regarding abiotic and biotic stresses (Wani et al., 2015). Plants regulate their defense system by releasing certain phytohormonal signals against different pathogens. Endophytes perform this task through three mechanisms, i.e., biofertilization, biocontrol, and phytostimulation under unfavorable conditions. Waqas et al. (2015) reported in his study that endophytic fungus *Penicillium citrinum* increases jasmonic acid levels and salicylic acid to combat against *Sclerotium rolfsii* (phytopathogen). Additionally, another endophytic fungus *P. formosus* increases plant growth by lowering abscisic acid and jasmonic acid concentration under heat stress. These two endophytic fungi regulate other phytohormones to produce secondary metabolites during heat stress. Similarly, *Aspergillus niger* regulate plant growth under stress by increasing auxin and gibberellins (Lubna et al., 2018). Under stress conditions, ethylene a plant ripening hormone inhibits growth parameters like root length, root hair, and lateral root development. In such situation endophytes produce an enzyme called 1-aminocyclopropane-1-carboxylate (ACC) deaminase to control ethylene concentration and promote plant growth (Santoyo et al., 2016). For further explanation, Sun et al. (2009) compared ACC deaminase production capacity in mutated and wild-type endophytic *Burkholderia phytofirmans* impacting canola. These scientists observed that the mutated strain did not promote the growth of canola seedlings, while the wild strain showed remarkable growth production. Hence, endophytes enzymatically affect plant growth and development.

Horizontal gene transfer and closed contact with plants have assisted the endophytes to produce effective bioactive compounds by mimicking plant host metabolism (Wang et al., 2012a, b, c). Endophytes can produce a vast range of secondary metabolites along with antibiotics, hydrolytic enzymes, toxins, and volatile organic compounds (VOCs) significantly increasing plant defense system performance (Afzal et al., 2019) considered as an emerging source of novel bioactive compounds (Singh & Pandey, 2021).

Streptomyces, an endophytic spp., can provide resistance in some plants by increasing defense-related compounds like phenols and flavonoids (Singh & Gaur, 2017). Similarly, Kang et al. (2018) observed in his findings that increased levels of nematicidal compounds such as 4-vinyl phenol, L-methionine, palmitic acid, and piperine in plants colonized by *Bacillus simplex* inhibited soybean cyst nematode. *Beauveria bassiana* coupled with mycorrhizae increased terpenoid concentration in tomato leaves, minimizing herbivory (Shrivastava & Kumar, 2015). Actinobacteria isolated from the wheat plant induced the SAR genes such as PR-1, PR-5, and pdf-1.2 and Hel genes regulating jasmonic acid and ethylene pathway making the plants resistant to several fungal phytopathogens in *A. thaliana* (Conn et al., 2008). Likewise, Gond et al. (2015) reported the *Bacillus amyloliquefaciens* activate jasmonic acid-dependent defense system by increasing PR-1 and PR-10 genes against fungal pathogens. They also protect plants from oxidation caused by excessive pesticidal applications (Jan et al., 2020).

Quorum sensing (QS) a density-dependent gene expression in bacteria is responsible for host and pathogenic communication via releasing signaling molecules like N-acyl-homoserine lactone (AHL). As this is a density-dependent bacteria, the signaling increases with density increase, and all cells act somewhat like multicellular organisms (Rosenblueth & Martínez-Romero, 2006). This regulation of gene expression in plants is required to make antibiotics, virulence factors, and coenzymes required for plant protection (Von Bodman et al., 2003). Under stress situation, plants produce signal molecules to copy bacterial QS to manipulate the QS-regulated behavior of phytopathogenic bacteria (Bauer & Mathesius, 2004). Endophytic bacteria isolated from *Cannabis sativa* were reported to work as biocontrol agents for bacterial phytopathogens (Kusari et al., 2014) as well as phylum *Actinobacteria* isolated from *Phaseolus vulgaris* provided resistance against gram-positive bacteria (Lopes et al., 2015).

Nanotechnology: An Emerging Technology for Biotic Stress in Fruits and Vegetables

By 2050, the world population is expected to rise 34-fold greater than the existing population (FAO, 2009), increasing the food demand with the same pace (Kumar et al., 2018). Nanotechnology is among the most potential technologies possessing advanced, progressive, and key technologies of this era (Ma et al., 2020). Nanomaterials are basically the small bodies embedded with nanoparticles, nanoclusters, and nanocrystallites developed under a specific nanometric range of 1–100 nm available in the form of aerosols, powder, suspension, emulsions, or dispersions (Lovestam et al., 2010). Nanoparticles have unique physical, biological, and chemical properties based on their atomic strength, high reactivity, surface area, and catalytic activities leading to uptake of nutrients from plants (Shang et al., 2019). Nanomaterials are very diverse in respect to their size and shape and therefore are applicable in a wide range of industries like ceramics, pharmaceuticals, agriculture etc. (Aitken et al., 2006).

Nanotechnology has the potential of revolutionizing the agriculture and food industry with its novel tools, techniques, and products to enhance the nutrient uptake by plants, molecular characterization, and management of insect-pest diseases (Fig. 12.1). Besides being potential, engineered nanoparticles are mostly used in food and other related areas working as biosensors, plant growth regulators, food additives, and genetic regulation for nano-fertilizers and nano-pesticides (Das et al., 2009). Moreover, it was also observed that these engineered nanoparticles could enter into the vegetable crops (tomato and cucumber) and eventually arrives at the food chain (Wang et al., 2012b).

Disease Detection and Management

Nanotechnology has upgraded the disease detection by fluorescent silica nanoprobes and nano-based diagnostic kits (Chitra & Annadurai, 2013). It can also be detected by using quantum dots, a closely packed semiconductor crystal consisting

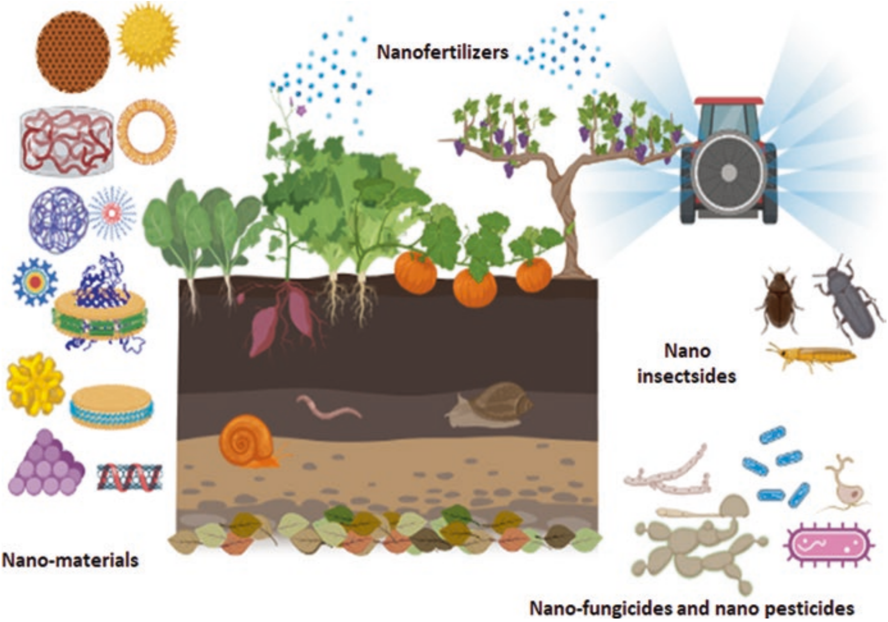

Fig. 12.1 Nanotechnology-based products for biotic stress

of multiple atoms, nano-biosensors used to detect crop health by detecting chemical or microbial contamination, and nanofabrication useful to study infection process and pathogen behavior inside the host plant (Alghuthaymi et al., 2015). Nano-biosensors are also used to detect and diagnose the disease caused by various micro-organisms in the infected soil (viruses, bacteria, and fungi) by the quantitative extent of differential oxygen consumption in the respiration of good and bad microbes in the soil (Rai et al., 2012). Nano-based products such as nano-fungicides and nano-bactericides have been utilized effectively for the management of diseases as it enables the smaller quantities of fungicides to be used effectively over a given period of time interval (Abd- Elsalam & Alghuthaymi, 2015). Silver nanoparticles extracted from $AgNO_2$ are reported to be the novel antimicrobial agents which can control diseases in a very effective way without any harmful effect (Franci et al., 2015).

Insect Pest Management

Nano-pesticide is a nanometric-size-ranged formulation with targeted elements successfully used for insect pest management in vegetable crops (Kookana et al., 2014). Nanomaterials present in nano-disks effectively strengthen the water solubility of the active ingredients associated with increased efficiency and stability and lower the pesticide use (Chhipa, 2017). There are various types of nano-formulation specifically used for insect pest management, namely, nano-emulsions, nano-encapsulation, and nanoparticles (Hayles et al., 2017).

Starting from the nano-emulsions, these are called mini emulsions or ultrafine emulsions size of a thermodynamically stable droplet ranged between 20 nm and 200 nm with improved active ingredient delivery and increased surface area (Jaiswal et al., 2015). Secondly, nano-encapsulation is packed with small−/nano-scale active ingredients in the form of an envelope or shell preferred over conventional pesticides in terms of slow and controlled delivery, effective pest control, and protection from photolysis and degradation (Urkude et al., 2019). Recently, nano-electronic nose, an artificial intelligence nose system, was introduced which make use of zinc oxide nanoparticles – a very quick and effective approach for the management of insect pests in vegetable crops. Nano e-nose operates like a human nose with the ability to identify various types of odors (distinguishing unpleasant smell) with respect to their concentrations (Cui et al., 2018).

Host Resistance Strengthening by Gene Cloning

Several pathogenic organisms like clubroot, fusarium wilt, black rot, sclerotinia stem rot, blackleg, white rust, downy mildew, white leaf spot, and turnip mosaic virus can infect *Brassicaceae* crop plants (Neik et al., 2017). Plants have a specific first stage immunity level called pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) (Jones & Dangl, 2006; Dodds & Rathjen, 2010). In the beginning, plants start to recognize pathogens through the PAMPs by pattern recognition receptors (PRRs) (Boller & Felix, 2009), and later this recognition takes the lead to the activation of (PAMP)-triggered immunity (PTI). PTI stimulates the expression of defense genes such as the mitogen-associated protein kinase (MAPK) cascade or WRKY transcription factors (Bigeard et al., 2015a, b). Interestingly in response, pathogens start delivering virulence molecules called as effectors to suppress PTI (Jones & Dangl, 2006). In result, the failure of PTI defense assists in activating an immune response called effector-triggered immunity (ETI). When plants recognize the effectors (Avr proteins) through disease resistance (R) proteins, an ETI is activated. This recognition between R and Avr proteins is termed as "gene-for-gene resistance" (Dangl & Jones, 2001). ETI has a stronger affinity than PTI against newly adapted pathogens in host plants (Noman et al., 2019a, b). R proteins consist of nucleotide-binding (NB) and leucine-rich repeat (LRR) domains, which are called NBS-LRR (nucleotide-binding site leucine-rich repeat) protein. NBS-LRR proteins are separated into two types by their N-terminus domain, having either a toll interleukin-1 receptor (TIR) domain (TIR-NBS-LRR protein) or coiled-coil (CC) domains (CC-NBS-LRR protein) (Marone et al., 2013). Generally, the LRR domain provides recognition specificity, the NB domain regulates activation, and the TIR domain regulates downstream signaling (Dodds & Rathjen, 2010). Moreover, few R genes also encode transmembrane receptor-like protein (RLPs), transmembrane receptor-like kinases (RLKs), cytoplasmic kinases (CKs), and proteins with atypical molecular motifs (Jones & Dangl, 2006). Hence, monocotyledon and dicotyledon genomes have different constitutions of R, where TIR-NBS-LRR genes are mostly absent in monocotyledons, while TIR-NBS-LRR genes are present in dicotyledons and found more abundantly than CC-NBS-LRR genes (Marone et al., 2013). The literature has reported comprehensive identification of R genes in several species of the genus *Brassica* (Dolatabadian et al., 2020).

Chickpea and pigeon pea are among the important pulses in South Asian countries. Many of the recent technologies and advancements on genetic tools have been reported related to genome assemblies, sequencing, pan-genomics, genotyping platforms, etc. (Roorkiwal et al., 2020). Moreover, the author focused on the use of a sequence-based holistic breeding approach, including the integration of functional omics, parental selection, forward breeding, and genome-wide selection in future chickpea improvement program.

Recombinant DNA Technologies

Malnutrition mostly occurs because of deficiency of vitamins and essential nutrients in food which eventually causes death (Akram et al., 2020a, b). Biotechnology integrates with using living organisms along with their systems and processes in manufacturing industries. Its applications have led to prolonged life expectancy and a quality and healthy lifestyle.

The transformation of plant cells consists of direct and indirect methods. For the indirect method, the agrobacteria (*Agrobacterium tumefaciens* or *A. rhizogenes*) or viruses are used as vectors, whereas the direct method which is also called biolistic includes cell bombardment with gold micro projectiles coated with DNA or direct insertion of DNA into protoplasts by means of liposomes or micro syringes or by electroporation. The most efficient vector for the transfer of exogenous DNA in fruit plants up till now is represented by the Ti plasmid (tumor-inducing plasmid) of *A. tumefaciens*, carrying the gene marker for the selection of gene of interest. The bacterium is engineered with one or more genes of interest and a marker gene needed for the selection of cells that host the genes. Alternatively, the biolistic transformation represents a direct method that allows shooting a spherical gold or tungsten bullets of 0.4–1.2 pm, covered with constructs of gene of interest at speeds of 300–600 m/sec. These constructs can reach the nucleus as well as chloroplasts and/or mitochondria, in which DNA will be integrated more decisively. For genome-editing technologies to escape from GMO regulations, the *Agrobacterium*-mediated transformation method is not reliable unless the first regenerated plants are submitted to self- or backcrossing with the wild type to eliminate the transgenic complex of modified plant (Rugini et al., 2020).

RNA Biology

RNA is just more than a messenger. RNA not only transfers genetic information between proteins and DNA but also plays a role in cell regulation because of its flexible structure and ability to interact with a wide range of inputs. And therefore, biologists take inspiration from diverse fields of engineering to develop frameworks for the design, construction, and characterization of new biological systems (Kim & Franco, 2020).

Effective pathogens have developed the advance strategies to overwhelm the defense responses of their plant hosts. For this purpose, many bacterial and fungal pathogens produce and release cell-wall-degrading enzymes (Kubicek et al., 2014). Pathogens can also transfer effectors into the host cytoplasm, resulting in the suppression of host defenses or stimulation of susceptibility (Franceschetti et al., 2017). To deal with such situations, almost all plants develop RNAi-based defenses against viral suppressors, whereas in contrast some viruses also hijack the host RNAi system to silence host genes to promote viral pathogenicity (Wang et al., 2012a, b, c).

Some other efforts have been made to provide genetic engineering for disease resistance to host-microbe interactions (Dangl et al., 2013). For such scenarios, proteins encoded genes capable of mycotoxin lysis (Karlovsky, 2011), or constraining the activity of cell-wall-degrading enzymes (Juge, 2006) can be introduced into plants. Another approach has led to the possibility of plant engineering to synthesize and secrete antimicrobial peptides or compounds that directly inhibit colonization (Osusky et al., 2000). Another possibility is the use of RNAi machinery to confer robust viral immunity by targeting viral RNA for degradation (Rosa et al., 2018). Some other possibilities are induction of natural or engineered immune receptors with the pathogen's recognition ability (Fuchs & Gonsalves, 2007), reprogramming of essential defense hub regulatory genes to fine-tune defense responses (Pieterse & Van Loon, 2004), and modification of susceptible host targets and host decoy proteins to recognize and manipulate pathogens. Similarly, host decoy proteins, which serve to trap pathogens, can be modified through genetic engineering for altered specificity in pathogen recognition (Kim et al., 2016).

Increased knowledge with respect to molecular mechanism underlying plant-pathogen interactions and advancements in biotechnology has provided new insights for engineering resistance to microbial pathogens in plants. The authors have observed through recent experiments that genetic engineering in plants for enhanced disease resistance along with highlight strategies has proven successful in field trials (Dong et al., 2019).

Plants expressing genes derived from viral pathogens often displayed immunity to the pathogen and its related strains (Lomonossoff, 1995). This finding has led to the hypothesis that ectopic expression of genes encoding wild-type or mutant viral proteins could interfere with the viral life cycle (Sanford & Johnston, 1985). Moreover, the recent studies have confirmed that this immunity is mediated by

RNAi, which plays a major role in antiviral defense in plants. The detailed molecular mechanism of RNAi in antiviral defense has been described in several excellent reviews (Galvez et al., 2014).

RNAi activation approach has proven to be effective for engineering resistance to viruses (Lindbo & Falk, 2017) as they rely on the host cellular machinery to complete their life cycle. Most of the plants have single-stranded RNA, while double-stranded RNA (dsRNA) replicative intermediates often form during the replication of the viral genome mediated by RNA-dependent RNA polymerase, triggering RNAi in the host. Transgenic overexpression of viral RNA often leads to the formation of dsRNA, which also triggers RNAi (Galvez et al., 2014). This process is often known as co-suppression. By far, several genetically engineered crops possessing resistance to viral pathogens have been approved for domestication. Especially, transgenic squash (*Cucurbita* sp.) and papaya (*Carica papaya*) varieties created using this technology have been put into cultivation in the United States for the past 20 years (Fuchs & Gonsalves, 2007).

The knowledge that dsRNA effectively induces RNAi (Lindbo & Dougherty, 2005) inspired the design of transgenes encoding inverted repeat sequences, the transcripts of which form dsRNA (Box 1; Waterhouse et al., 1998). This strategy was used to develop a transgenic common bean variety exhibiting resistance to the DNA virus bean golden mosaic virus (BGMV; Bonfim et al., 2007). BGMV contains single-stranded DNA as its genetic material, which is converted to dsDNA in the host, transcribed, and translated to produce the essential proteins required for its replication (Hanley-Bowdoin et al., 2013). To generate small interfering RNA targeting the viral transcripts, sense and antisense sequences of part of the BGMV replication gene AC1 were directionally cloned into an intron-spliced hairpin RNA expression cassette (Bonfim et al., 2007). The resulting genetically engineered bean exhibited a strong and robust resistance in greenhouse conditions (Bonfim et al., 2007) as well as field conditions (Aragão & Faria, 2009).

In 2011, Brazil deregulated the BGMV-resistant bean and encouraged farmers to cultivate this crop. To date, it is the only example of deregulation of a crop to induce resistance against a DNA virus by genetic engineering. When comparing with the co-suppression strategy, artificially designed inverted repeats transgenic expression allows concurrent generation of heterogeneous small interfering RNAs with multiple transcripts targeting in a relatively simple way. The microRNA (miRNA) discovery has led to the discovery of a class of endogenous noncoding regulatory RNAs (Reinhart et al., 2002) to further refinements of genetic engineering for viral resistance. The miRNA machinery has been exploited in engineering resistance to RNA viruses by replacing specific nucleotides in the miRNA-encoding genes to alter targeting specificity (Xie et al., 2015).

Such artificial miRNAs have been previously used for resistance engineering for a broad range of plant viral pathogens including turnip mosaic virus (Niu et al., 2006), cucumber mosaic virus (Hu et al., 2011), potato virus X, and potato virus Y (Ai et al., 2011). These findings have suggested that artificial-miRNA-based antiviral strategies are highly favorable, and disease resistance engineering by this strategy awaits future field tests.

Gene-Editing Technologies

Genome-editing technology has made it possible to manipulate DNA sequences in organisms' genomes to accurately remove or replace particular sequences, resulting in targeted alterations. Genome editing is an intriguing strategy for changing gene functions in plants to develop enhanced crop varieties. When compared to traditional mutant breeding, genome editing is expected to be easy to employ and having a lesser risk of off-target consequences. Additionally, genome-editing innovation techniques can be directly applied to crops with diverse genomes, which are difficult to breed using traditional approaches. Recently, very efficient genome-editing technologies result in accurate and predictable editing of nearly any locus in the plant genome, allowing functional genomics research and molecular crop breeding to be added to the list of its applications. Vegetables are important food sources for humans as they supply vitamins, fibers, and minerals to the meals, all of which contribute to the general health. This paper has presented a brief history of genome-editing technologies and the components of genome-editing tool boxes along with the basic modes of operation in representative systems. Moreover, the existing and potential applications of genome editing have been discussed, for the generation of more nutritious vegetables, as well as various case studies that demonstrate the technology's potential. Furthermore, the future prospects and problems in using genome-editing tools for research and commercial development in vegetable crops have also been discussed.

Traditional transgenic techniques or more contemporary genome-editing technologies have been used in the majority of cases of plant genetic engineering. In such techniques, genes encoding desired agronomic features are injected into the genome at random places by plant transformation (Lorence & Verpoorte, 2004a, b). Typically, these approaches produce cultivars with foreign DNA. Genome editing, on the other hand, allows for alterations to endogenous plant DNA, such as deletions, insertions, and substitutions of DNA of varying lengths at specific targets (Barrangou & Doudna, 2016). The product may or may not contain foreign DNA, depending on the sort of alterations used. Genome-edited plants that do not contain foreign DNA are not subjected to the additional regulatory restrictions that apply to transgenic plants in various parts of the world, including the United States, Argentina, and Brazil (CTNBio, Brazil, 2018; Orozco, 2018; USDA, 2018; Whelan & Lema, 2015). Despite changes in regulatory policies, both traditional transgenic methods and genome editing remain significant crop enhancement technologies.

Plants have developed complex defense systems against microbial diseases (Jones & Dangl, 2006). Prefabricated physical and physiological barriers, as well as their reinforcements, keep potential infectious agents out of the cell (Collinge, 2009). Upon detection of pathogens, plasma membrane-bound and intracellular immune receptors begin defense responses either directly by physically engaging with pathogen-derived immunogens or indirectly by monitoring changes in host targets caused by pathogens (Jones & Dangl, 2006). Antimicrobial peptides and other chemicals generated from plants can reduce pathogenicity either directly through detoxification or by inhibiting the function of virulence factors (Ahuja et al., 2012).

CRISPR-Cas9

Dietary fibers, vitamins, and minerals are abundant in fruit and vegetable crops, all of which are essential for human health. Many biotic stresses such as pests and diseases and abiotic stresses pose a threat to crop growth, quality, and productivity. Traditional crop breeding procedures entail a succession of backcrosses and selection to incorporate beneficial traits into fine germplasm, but this is a time-consuming and resource-intensive process. CRISPR-associated protein-9 (Cas9), a new breeding technique based on clustered regularly interspaced short palindromic repeats (CRISPR), has the potential to improve many traits in crops quickly and accurately, including yield, quality, disease resistance, abiotic stress tolerance, and nutritional aspects. This approach has been used to obtain novel germplasm resources via gene-directed mutation because of its ease of use and high mutation efficiency. CRISPR-Cas9 editing to precisely alter critical genes can swiftly develop new germplasm resources for the enhancement of significant agronomic characteristics, due to the availability of whole-genome sequencing data and information on gene function for important traits. This technology can also be used in fruit and vegetable crops. Here the obstacles, existing versions, and the regulatory structures that surround them have been discussed, along with their prospective uses.

CRISPR-Cas is an antiviral defense mechanism found in many bacterial species. An RNA-guided nuclease (typically a Cas protein) cleaves at precise target places on the viral DNA or RNA substrate, causing it to be degraded. Base complementarity between the CRISPR RNA and the target DNA or RNA molecules determines the specificity of the cleavage. A number of Cas proteins have been discovered to exhibit sequence-specific nuclease activity (Wu et al., 2018). For instance, the RNA-guided endonuclease Cas9 from *Streptococcus pyogenes* (SpCas9) generates double-stranded breaks in DNA, and RNA-guided RNases Cas13a from *Leptotrichia shahii* (LshCas13a) or *Leptotrichia wadei* (LwaCas13a) target RNA (Abudayyeh et al., 2017), while *Francisella novicida* Cas9 (FnCas9) cleaves both DNA and RNA (Price et al., 2015).

CRISPR-Cas' capacity to function like molecular scissors, breaking specific sequences in the substrate DNA or RNA molecules, makes it a great candidate tool for developing antiviral defense in plants (Fig. 12.2). In plants, CRISPR-Cas platforms based on Cas9 or Cas13a have been effectively used to develop resistance against DNA and RNA viruses. Tomato yellow leaf curl virus (TYLCV) is a member of the *Geminiviridae* family of plant viruses with circular single-stranded DNA genomes (Dry et al., 1993). During replication inside the nucleus of the host cell, the viral genome generates double-stranded intermediates. In *Nicotiana benthamiana* and *Solanum lycopersicum*, overexpression of SpCas9 and intentionally generated guide RNAs targeting specific areas of TYLCV induced virus resistance (Ali et al., 2018a, b). One possible drawback of this method is that DNA repair within the host cell may cause changes to the viral DNA sequence around the cleavage target. These mutations may prevent the guide RNA from recognizing the viral DNA. The ones targeting the stem-loop sequence inside the replication of origin

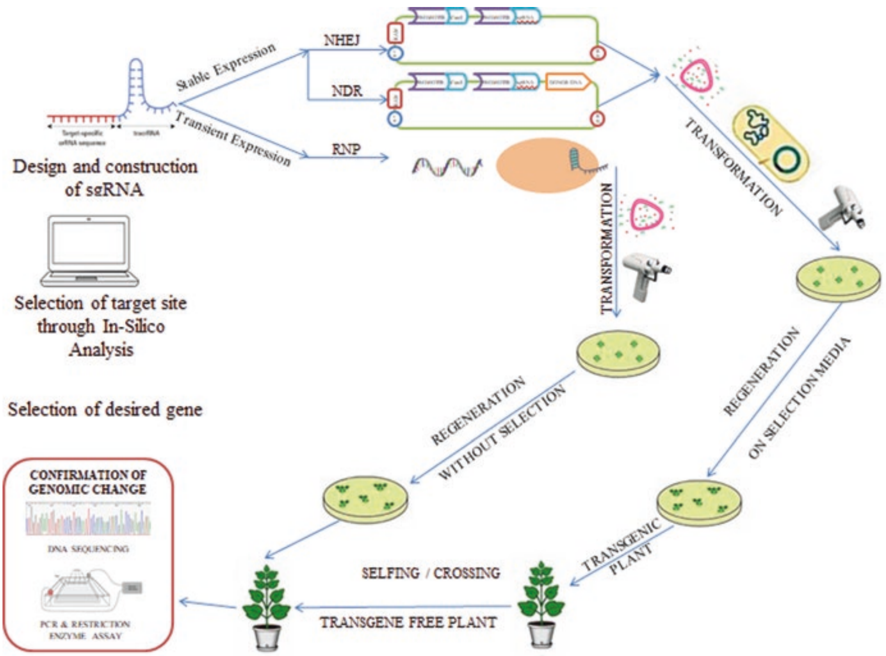

Fig. 12.2 CRISPR-Cas9-mediated genome editing

were the most successful of all the guide RNAs employed against TYLCV, probably because of the lower prevalence of viable escapee forms of the virus with mutations in this region (Ali et al., 2018a, b). According to a separate lab investigation, the overexpression of SpCas9 and guide RNAs in *N. benthamiana* and *A. thaliana* provided resistance to the geminivirus beet severe curly top virus. CRISPR-Cas has also been used to develop resistance against RNA viruses, which are responsible for the majority of known plant viral diseases (Mahas & Mahfouz, 2018). In *N. benthamiana*, for instance, the constant expression of the RNA-targeting nuclease Cas13a and the matching guide RNA conferred resistance to the RNA virus turnip mosaic virus (Aman et al., 2018). Cas13a targeting viral RNA substrates does not cause DNA breaking; hence, no unwanted off-target alterations are introduced into the host genome (Abudayyeh et al., 2017). In *N. benthamiana* and *Arabidopsis*, FnCas9 has been utilized to create resistance to the RNA viruses cucumber mosaic virus and tobacco mosaic virus (Zhang et al., 2018). Although there hasn't been a field test of CRISPR-Cas-based antiviral resistance on crop species, laboratory research has shown its potential as an antiviral agent. To ensure long-term resistance, potential viral evasions from the surveillance by the specific guide RNA utilized must be considered. Viral evasions are reduced by selecting genomic targets that are required for viral pathogen proliferation or movement. The robustness of the system can also be improved by multiplexing the guide RNAs. Furthermore, it has been suggested that using Cas12a (also known as Cpf1) may limit the likelihood of escapee viral variants because mutations induced by CRISPR-Cas12a are less likely to disrupt the original guide RNA's detection of the target (Ali et al., 2018a, b). The spacer acquisition mechanism in the CRISPR-Cas adaptive immune system in prokaryotes may be used in future attempts to improve CRISPR-Cas for antiviral resistance (McGinn & Marraffini, 2019).

Eco-Sustainable Approaches to Improve Vegetables Resilience and Quality

Extreme climatic occurrences are affecting organisms all around the world, not just in arid or semiarid regions. These events occur at such a fast rate that organisms with a high genetic diversity will not be able to tolerate harsh environments. Modern breeding techniques have enabled the development of more tolerant species in this situation. Their practical use, on the other hand, is time-demanding and always attempting to keep up with changing environmental conditions and abiotic and biotic stresses. In this context, some novel agricultural systems, such as the use of bio-stimulants, or well-established ones, such as grafting, can respond to enhanced agricultural demands and provide environmentally friendly techniques to boost plant tolerance to abiotic stress. Given that veggie crops are exposed to various and severe abiotic stresses in the real world, understanding plant defense mechanisms, together with the implementation of environmentally friendly agriculture practices, may help these vital crops to adapt to a swiftly changing climate. Furthermore, future research is needed to better understand plant defense systems in the face of numerous stressors, particularly in scenarios involving heat, drought, salinity, and increased CO2, as well as biotic stressors. Ultimately, local ecotypes and landraces should be rigorously screened in order to identify individuals who can survive environmental stressors, with the goal of understanding plant defense mechanisms (Giordano et al., 2021).

Conclusion and Future Prospect

Since the rate of spontaneous mutations is very slow, breeders are unable to use them in plant breeding programs. Therefore, the induced mutations are required to increase the rate of genetic variability. Induced mutations have the advantage of being able to isolate several trait mutants as opposed to transgenic approaches, which only allow for the introduction of a single characteristic into the crop, and there is also a lack of acceptability of genetically modified food. A reported outcome of mutation induction is the ability to create a variety of mutant lines and identify trait-specific genes in order to create a molecular gene database, study molecular functional genomics, and develop bioinformatics for future plant variety development to feed the rapid human population growth under climate change. Furthermore, mutagenesis has the advantage of allowing biologists to extract mutants with numerous features that would be excellent for growing in a changing climate.

In light of the above discussion, it is critical to introduce such resistance mechanisms to agricultural plants, aimed against a specific pathogen or insect component. A typical resistance method is the introduction of chitinase genes coding for chitinase enzymes in plants that destroy chitin in the cell wall of fungal infections. Bt toxin provides resistance to a wide spectrum of chewing insects. Plant breeders must extend their genetic base by including wild relatives, landraces, and exotic germplasms of crops into their hybridization programs, as these contain genes that confer resilience to biotic and abiotic challenges. Modern plant breeding techniques such as transgenic strategies, “TILLING,” gene silencing, and “VIGS” provide a lot of potential for developing crops that are resistant to biotic stressors in the future. Emerging countries with significant population growth cannot wait for genetic engineering to gain the benefits. Therefore, plant breeders have started using a combination of tissue culture and mutagenesis to develop genetic variability, which they could further use in their breeding operations.

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Chapter 13
Genome Editing in Crops to Control Insect Pests

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Introduction

Agricultural productivity is facing constant threats from various biotic and abiotic factors. Among them, insect pests are one of the most serious threats responsible for stagnation productivity due to a loss in potential yields. Despite operating control methods, most economically important crops experience various production losses. Different methods have been used (Ullah et al., 2022; Yan et al., 2022), but still chemical pesticides have been applied worldwide to control insect pests (Ullah et al., 2019a, b; Gul et al., 2021). However, the indiscriminate use of chemical pesticides affects the beneficial insects, induces hormesis effect resistance development, modulates both direct and indirect interactions among species within food webs, and leads to secondary pest outbreaks (Desneux et al., 2007; Ullah et al., 2019c, 2021; Wang et al., 2021). These issues have prompted scientists worldwide to design new, ecologically friendly insect control technologies. As a result, the main goal of contemporary agriculture has been to increase yields within standing land and resources to ensure agricultural sustainability and global food security (Bisht et al., 2019).

Pest management has expanded to new heights with plant biotechnology and genetic engineering. The ongoing use of Bt gene introgressed crops and multiple insect resistance genes (*Bacillus thuringiensis* Bt-ICPs) has been genetically modified into several crop plants, which have proven a reliable influence on the sustainability and production of technology. As a result, insect pests developing Cry toxin tolerance has become a big concern (Wang et al., 2016). Mutations in the genes encoding receptor molecules detract from the insect's interaction with the poison, leading to the establishment of a resistant insect-pest population. Chimeric toxins created through combinatorial ICPs and domain swapping toxins are used to give longer efficacy against insect pests and avoid resistance (Wang et al., 2016). Other techniques, such as RNA interference (RNAi), have been developed and tested for use in crop enhancement initiatives to silence specific genes involved in insect feeding and development.

In recent years, the advent of genomics and plant genome sequencing has opened up new paths for their use in crop development programs. As researchers probe deeper into plants' genomes and other levels of cellular activity. They have identified the importance of sequence variation in their ability to exhibit various traits (Rathinam et al., 2019). As a result, the next level of biotechnological intervention, known as "genome engineering" or "genome editing" was developed. This intriguing technology has emerged as a critical addition since it enables genome changes by adding, editing, or removing certain DNA sequences, allowing for applications in plants, animals, and humans. Genome editing will serve as a viable technique to combat insect pests in the current environment of constrained agricultural areas and growing insect pest load on crop plants and has thus been termed the "new breeding technology" (Razzaq et al., 2019; Vats et al., 2019). Here, we have discussed the novel opportunities of genome editing in crops to deter insect pests.

Genome-Editing Techniques

One of the most important tools in modern biology is genome editing, which allows scientists to edit an organism's DNA (Belfort & Bonocora, 2014). The concept of genome editing has grown in popularity over the previous two decades, with innovative and constant modifications for the advancement of technology. Targeted genome editing entails using various nucleases to precisely manipulate certain genomic sequences.

Transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR/Cas9), mega nucleases (MNs), and zinc-finger nucleases (ZFNs) are four primary groups of sequence-specific nucleases (SSNs) (Stella & Montoya, 2016). ZFNs were the first genome-editing strategy. CRISPR or TALENs methods are widely used in plants for genome-editing applications (Puchta, 2017) (Table 13.1).

CRISPR/Cas9-Mediated Genome Editing

CRISPR is a bacteria-specific adaptive immune system that protects bacteria from plasmids and bacteriophages (Jinek et al., 2012). As a spacer between crRNA repetitions, plasmids and invading phages from the DNA fragments are merged into the host CRISPR locus. Mature crRNA serves as a Cas9 protein guide. The mature crRNA-Cas9 combination directs Cas9 to the invading DNA's matching target region. Cas9 nuclease cleaves the target sequence upstream of the adjacent protospacer motif (PAM). Most often used, Cas9 is a *Streptococcus pyogenes* (SpCas9) which requires canonical PAM form 5′ NGG 3′ at the 3′ end of the target site to complete cleavage.

To synthesize chimeric single-guide RNA (sgRNA) for genome-editing applications, CrRNA is connected to tracrRNA. Cas9 protein may be guided to the target locus by this sgRNA, which introduces double-stranded breaks inside the base-pairing area (Fig. 13.1). Nonhomologous end-joining (NHEJ) and homology-directed repair (HDR) are used to mend these double-stranded breaks. NHEJ repairs the breaks without the template, which frequently results in inheritable mutations that change the encoded characteristic (Symington & Gautier, 2011). It can be simply programmed to mutate specific genetic targets as a two-component system. The modified gRNA-Cas9 combination has been selected in plants to evaluate economically significant traits (Yin et al., 2017) (Fig. 13.1).

Variants of Cas9 for Genome Editing

Several new cas9 variants with different PAM specificities have been discovered from various bacterial sources (Cebrian-Serrano & Davies, 2017) (Table 13.1). Furthermore, researchers have modified spCas9 to identify a wider range of PAM

Table 13.1 Modifications in genome-editing tool and their major features

Genome editing technique	Variants	Alteration	Effectiveness in plants	References
CRISPR/Cas	Lenti-multiguide	Combination of a lentiviral vector containing a doxycycline-inducible EGFP reporter gene (Lenti-iCas9-neo) and downstream SpCas9, as well as a lentiviral vector with several gRNAs targeting various genes	Lenti-multi-CRISPR is a commonly available vector for targeting multiple genes in plants at the same time	Vats et al. (2019)
	Dead Cas9	Cas9 has a mutation that prevents it from acting as an endonuclease	They have the ability to control the expression of transcript levels in plants, activate the *Arabidopsis* production of *anthocyanin Pigment1* (*AtPAP1*) gene, and suppress *Arabidopsis* cleavage stimulating *Factor64* in *Arabidopsis thaliana* (*AtCSTF64*)	Vats et al. (2019)
	Prime editing	Prime editor is a fusion protein that contains a CRISPR/Cas9 nickase and a reverse transcriptase that has been programmed with pegRNAs that encode the desired edit	The point mutations, insertions, and deletions have been documented in rice and wheat protoplasts using prime editors in the survival gene OsCDC48	Marzec et al. (2020) and Lin et al. (2020)
	Chimeric Cas9-VirD2	A fusion protein among Cas9 (which causes particular DNA DSBs) and *agrobacterium* VirD2 relaxase (which transports repair template near the DSBs)	Herbicide resistance (*ACETOLACTATE SYNTHASE*, *OsALS* allele), plant architecture (*CAROTENOID* CLEAVAGE DIOXYGENASE-7 allele), and in-frame fusions with the HA epitope at the *HISTONE DEACETYLASE* (*OsHDT*) locus were all achieved using wild-type rice (*Oryza sativa*)	Ali et al. (2020)

(continued)

Table 13.1 (continued)

Genome editing technique	Variants	Alteration	Effectiveness in plants	References
TALENs	Compact TALENs (cTALENs)	N-terminal wild-type I-TevI coupled to a TALE DNA binding scaffold at the N or C terminal. The catalytic domain of the N-terminus of I-TevI contributes to specificity via DNA cleavage selectivity, and it is a HE member of the GIYYIG protein family	Tobacco protoplasts were used to test the design. In the neomycin phosphotransferase II gene, a TALEN target site (TTS) was chosen. The cTALEN design revealed activity comparable to FokI TALEN	Kazama et al. (2019)
	mitoTALENs	Mitochondrial signal peptides are attached to TALENs	In rice and rapeseed, the genes that induce cytoplasmic male sterility (CMS) in the mitochondrial genome were knocked out using Mito-TALENs, restoring male sterility	Sauer et al. (2016)

sequences, including GAA, NG, and GAT (Hu et al., 2018). These modifications provide the capacity to edit different target areas in the genome that would otherwise be unavailable to normal spCas9. Cas9 is made up of four engineered variants: high-fidelity CRISPR-Cas9 (SpCas9-HF1), which has exceptional precision (Kleinstiver et al., 2016); enhanced specificity SpCas9 (eSCas9), which reduces the off-target effects (Slaymaker et al., 2016); CRISPR nickase, which generates single-stranded breaks at the targeted DNA sequence (Ran et al., 2013); and dead Cas9 or nuclease-deficient Cas9 (dCas9), which has no DNA cleavage activity (Hilton et al., 2015). By functionally modifying spCas9, nuclease-deficient Cas9 or dead Cas9 (dCas9) and Cas9 nickase have been derived. A double-strand break may be induced utilizing nickase and paired nickase, guided by sgRNA that matches the neighboring target site. This is especially useful when the objective is to reduce off-target activities.

A novel CRISP-Cpf1 has been reported in *Francisella novicida* U112 (Zetsche et al., 2015). Cpf1 has the same endonuclease domain RuvC as Cas9 protein. However, it lacks the HNH domain. Cpf1 recognized canonical PAM structure 5′TTTV-3′, and it also forms a staggered DNA double-stranded break upon cleavage of the target spot, making it an appealing option for certain goals such as genomic deletions and insertions (Fonfara et al., 2016). Cpf1 was found to have a much lower genome-wide off-target activity than spCas9 in a recent human genome investigation (Bin Moon et al., 2018). The CRISPR Cpf1 complex has recently been shown to be a good approach in soybean for DNA-free genome editing with no

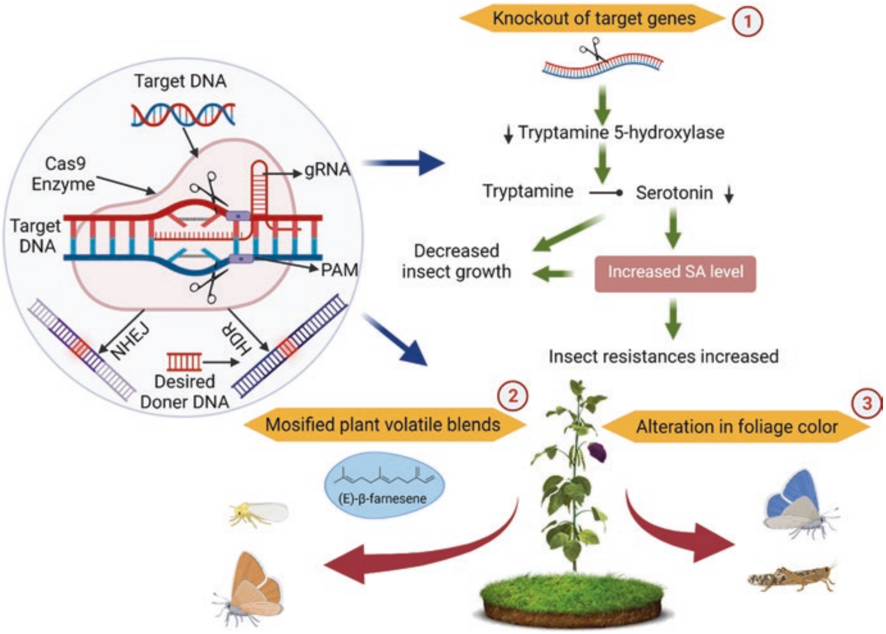

Fig. 13.1 CRISPR-based gene-editing strategies in plants to control insect pests. (1) CRISPR/Cas9-mediated knockout of the target gene that encodes tryptamine 5-hydroxylase, which catalyzes the conversion of tryptamine to serotonin, causes a reduction in insect pest development and increased insect resistance. (2) Insects recognize a few plant volatile mixtures as indicators of host selection and oviposition site detection. Changes in volatile combinations have been shown to deter insects away from host plants. Genome editing can change volatile plant mixtures, which might be an alternative technique to control the pest. (3) The visual appearance strongly influences the ability of insects to detect and attack host plants. Insect host choices have been discovered to be influenced by changes in plant pigmentation. Through genome editing, alteration in foliage color for biotic stress resistance is a key strategy to control insect pests

off-target activity (Kim et al., 2017). Due to the high frequency of genome duplication, the risks of off-target mutations are particularly high. Cas enzyme, PAM location, and gRNA backbone are all distinct in each of the novel CRISPR systems, which is comprised of RNA-guided endonucleases from *Prevotella* and *Francisella* 1 (Cpf1) (Zetsche et al., 2015), *Staphylococcus aureus* (Sa) (Ran et al., 2013), *Streptococcus thermophilus* (St) (Horvath et al., 2008), *Campylobacter jejuni* (Cj) (Dugar et al., 2018), *Treponema denticola* (Td) (Esvelt et al., 2013), and *Neisseria meningitides* (Nm) (Hou et al., 2013). CRISPR/Cas9 can cut single-stranded RNA if the PAM sequence is trans as a distinct oligonucleotide. Cas9-gRNA complex recognizes PAM-presenting oligonucleotide (PAMmers) and initiates site-targeted endonucleolytic cleavage of ssRNA. The capacity of spCas9 to recognize RNA has been exploited to investigate the live tracking of RNA molecules in living cells (Nelles et al., 2016). Cas9 orthologs and the generation of Cas9 variants utilizing recombinant DNA technology have moved this approach to the forefront of other genome-editing technologies.

CRISPR-Based Gene Editing Emerges over RNA Interference

Various research organizations worldwide demonstrate applying CRISPR-based genome-editing techniques instead of RNA interference (RNAi) in plants. The main distinction is that CRISPR permanently silences a gene at the DNA level (knockout), whereas RNAi lowers gene expression at the RNA level (knockdown) (Ullah et al., 2020). Due to poor consistency, RNAi's inefficient suppression of target genes results in leaky production of target mRNA, resulting in off-target effects where other genes are accidentally affected, while in the altered genotypes, CRISPR-based methods boosted sensitivity and reduced off-target effects (Fig. 13.1). CRISPR induces full loss-of-function consequences, whereas RNAi usually generates a hypomorphic impact, resulting in partial loss of gene function. As compared to RNAi, CRISPR creates stronger and more consistent phenotypes (Fig. 13.1).

Genome Editing in Plants

sgRNA and Cas9 genome-editing components were first adapted in mouse and human cell lines. They were gradually adopted by scientists, who tweaked the genome-editing components to make them plant-specific. A single-guide RNA (sgRNA) created by fusing two RNA moieties, CRISPR-RNA (crRNA) and trans-activating CRISPR-RNA (tracrRNA), as well as a designed nuclease Cas9, is now included in a genome-editing technique. Cas9 is directed to the PAM sequence NGG for selective cleavage (N is A, T, G, or C) by sgRNA, which binds to the PAM on the targeted region. Essential problems for obtaining desired genomic alterations are selecting good target locations and the accuracy of the CRISPR/Cas9 system. Several successful instances of agronomically significant features being engineered in crops such as wheat, rice, and maize are already available (Macovei et al., 2018; Ainley et al., 2013; Wang et al., 2014). For example, Lu et al. (2018) reported that CRISPR/Cas9-mediated knockout of the *CYP71A1* gene that encodes tryptamine 5-hydroxylase, catalyzing tryptamine conversion to serotonin, causes a reduction in insect pest development and increased insect resistance.

Genome editing in plants is effective against various bacterial, viral, and fungal diseases in various crops (Vats et al., 2019). For insect pest management, plant editing has received little attention (Larsson et al., 2004). To ensure the availability of preferred nourishment for its larvae, every insect likes to deposit eggs on a host plant. Insects recognize a few plant volatile mixtures as host selection and oviposition site detection indicators. Insects have been demonstrated to be repelled from host plants by changes in volatile combinations (Beale et al., 2006). Aphid infection promotes the synthesis of the sesquiterpene hydrocarbon (E)-β-farnesene (Eβf), stopping other host populations from feeding. *Diaeretiella rapae*, a parasitic wasp that controls the aphid population, is attracted to the sesquiterpene hydrocarbon (E)-β-farnesene (Eβf) (Beale et al., 2006). Genome editing can change volatile plant mixtures, an alternative technique to control the pest (Fig. 13.1).

The visual appearance strongly influences the ability of insects to detect and attack host plants (Tyagi et al., 2020). Insect host choices have been discovered to be influenced by changes in plant pigmentation. Malone et al. (2009) reported that overproduction of anthocyanin pigmentation in tobacco plants changes the leaf color, due to which *Spodoptera litura* and *Helicoverpa armigera* were deterred. These findings show the importance of leaf color and appearance in insect host recognition. Through genome editing, alteration in foliage color for biotic stress resistance is a key strategy to control insect pests (Fig. 13.1). Manipulating the anthocyanin pathway via CRISPR-based editing could be a potential pest management approach that must be investigated. However, caution should be exercised to ensure that a change has no negative consequences for the population of beneficial insects.

Genome Editing in Plants to Improve Resistance Against Insect Pests

Insect pests cause direct and indirect damage to plant growth and development by transmitting dangerous viruses, resulting in significant crop yield losses. The most common agrochemical-based management strategies are expensive and dangerous to the environment. It also harms beneficial insects, and it gradually loses efficacy as pesticide resistance evolves. In many situations, the lack of well-characterized resistance sources within the crossable gene pool restricts the area of breeding insect-resistant crops. Little progress was achieved due to a lack of understanding of the genetics of the resistance trait in uncharacterized accessions (Kolmer et al., 2018).

Transgenic approaches have introduced insect-resistant genes, such as Bt genes from bacteria, into crops. However, because of a lack of scientific information, such transgenic plant kinds faced significant political, ethical, and societal opposition. Environmentally friendly breeding tools for crops should be adopted that can achieve two goals: creating de novo resistance where adequate R-genes are absent and breaking pesticide resistance, killing or causing sterility in inflicting insects. Genome-editing systems offer the potential to create designer plants, particularly in circumstances where a targeted knockout is predicted to generate a gene drive for selectively propagating mutations causing mortality in female insect species.

Genome-Editing Targets

For many years, genome-editing technology has been effectively used by many taxa, including insects. However, recent advances in the precise use of this technique suggest that it might eventually replace procedures like radiation mutagenesis and random chemical. For insect control, genome editing through the transmission of a gene drive necessitates the elimination of potentially lethal target regions in the insect genome which affects the sex ratio and fecundity of the insects. Insect-pest host immunity may also be generated by editing important plant immunity genes such as susceptibility genes (S-genes), resistance genes (R-genes), and genes regulating the interaction between the target and the insect effector. Nonetheless, the findings from fundamental research on plant-insect interactions suggest that the CRISPR/Cas9-based genome-editing technique might be used to generate insect resistance.

Specificity, Efficiency, and Off-Targeting

In stable sgRNA integration, Cas9 and its tissue-specific expression may edit off-target regions. The interaction of PAM proximal region with Cas9 and hybridization of gRNA with target DNA play a big role in the specificity of designed nucleases (Lee et al., 2016). Target and nontarget cleavage efficacy is mostly dictated between the target sequence and guide RNA by the availability of the target site, nuclease activity, and binding affinity. When an essential gene is targeted, the editing efficiency can be lowered by using promoters (nanos and vasa) that express Cas9 in germ cells instead of somatic cells, avoiding biallelic mutations that cause embryonic mortality. On the other hand, less essential genes can be altered with the most efficient U6 promoter-driven method. Off-targeting refers to unexpected genomic alterations caused by designed nucleases. Beyond the guide sequence's first 8–12 nucleotides proximal to the PAM, mismatches are permitted in the PAM's distal region. Cas9's tolerance to these mismatches, on the other hand, is dependent on the level of its expression in the cell. High Cas9 enzyme concentrations boost off-site targeting, whereas low Cas9 enzyme concentrations increase specificity and reduce on-target cleavage activity (Hsu et al., 2013; Pattanayak et al., 2013). Non-tolerance of three mismatches was observed to generate DSBs in *Drosophila* (Ren et al., 2014). A prior bioinformatics study is required to avoid off-target impacts from genome-editing approaches. CRISPR MultiTargeter (Prykhozhij et al., 2015), CRISPR-P (Lei et al., 2014), and fly CRISPR, CRISPR fly design, and DESKGEN are the online platforms and tools for creating gRNAs in the model organism *Drosophila*.

Delivery Techniques and Efficiency

A safer and more effective DNA delivery technique is essential for genome editing. In this technique, there is the direct transmission of the Cas9 and RNA and indirectly new vectors designed to produce the transgenics. In insects, the sgRNA can be delivered as encoding with the genome, plasmid DNA and RNA. Construction of the plasmid contains three components like specific promoters for development/

tissue or ubiquitous, gRNA sequences with a terminator, and a Cas9 gene. Similarly, the NLS (nuclear localization signal) is necessary for the Cas9 expressions. The website https://www.addgene.org/ represents the construction of several plasmids. Under the control of the SP6 and T7 promoters, there is an in vitro transcripted gRNA and Cas9 mRNA. Mainly, early-stage insect embryos are injected with protein or RNA and DNA with the help of microinjections (Sebo et al., 2014). Insect cells are infected with the genetic material by the help of transfection (Bottcher et al., 2014). The microfluidic membrane deformation method was developed by Han et al. to transmit the Cas9-gRNA in cells that are hard to transfect (Han et al., 2015). In mice, two delivery systems, oviductal nucleic acid delivery system and electroporation, have been considered the best delivery methods (Hashimoto & Takemoto, 2015), though their viability in insects has also been discovered.

Moreover, a comprehensive checklist of nonviral and viral vectors has been introduced with the help of CRISPR/Cas9 technique that transports the Cas9 and RNA into tissues and in cells, thus producing the transgenic lines or strains (Gratz et al., 2013; Gokcezade et al., 2014). In the case of the *Drosophila* strain, the germline demonstrates the gRNA and Cas9 expressions (Kondo & Ueda, 2013). Mutagenesis effectiveness can be assessed with the help of endonuclease cleavage and can be evaluated by mismatched sites resultant after the process of gel electrophoresis. T7 endonuclease 1 (T7E1) assay and CEL1 nuclease family (surveyor assay or CEL1 assay) are included in endonucleases. HRMA (high-resolution melting analysis) showed dissimilar melting curves for fragments of concern at mismatched sites associated with wild-type major mutations detected with a higher sensitivity in less time (Bassett & Liu, 2014). Additionally, to discover accurate mutation, sequencing is the best method, whereas checking the effectiveness of mutant phenotype is considered the best for its effectiveness (Baena-Lopez et al., 2013).

Applications in Plant-Insect Resistance

The system of genome editing is helpful in various genome-related modifications containing incorporation of point mutation similar to single nucleotide polymorphism (SNPs), deletion of large chromosomal regions, the substitution of individual gene segments, and knockout (KO) and knock-in (KI) gene functions. Scientists are being facilitated to examine the function of a specific gene in organisms and different operative cells with the help of this type of modification. The CRISPR/Cas9 system is helpful for the modification of crops in case of high nutritive values and manufacturing of commercial products, comprising complex mutagenic trait incorporation, gene pyramiding, protein synthesis in humans, resistance by the use of herbicides (Svitashev et al., 2015), early yield (Soyk et al., 2017), resistance due to biotic stresses (Wang et al., 2014), extended shelf life (Xiong et al., 2015), biofuels (Jiang et al., 2017), and conferring resistance due to abiotic stress (Osakabe et al., 2016).

Assessment of Plants Developed Through Genome-Editing Technologies

Plant trait modification has been revolutionized by genetic engineering and transgenic technologies. The transfer of alien genes and T-DNA integration produce transgenics, resulting in positional or unintentional consequences and the insertion of a vector backbone. These issues are important concerns for living organisms and the environment. Before introduction on farmers' fields, transgenics must be closely monitored and subjected to rigorous risk assessments. Transgenics have received various degrees of approval and popularization despite comprehensive safety assessments across countries. As a result, the pace with which the technology may be applied in crop enhancement initiatives has been severely hampered. Over the last decade, very accurate and effective technologies for improving agricultural attributes through genome editing have been developed and propagated. The altered plants may be classified into Group I based on the degree of genome alteration, with single or few base modifications/deletions/insertions, resulting in less phenotypic/genotypic complexity. Group II modifies many base pairs, resulting in a higher phenotype/genotype complexity level. Inserting a foreign gene creates a new phenotypic characteristic, such as a novel metabolic pathway, protein, or RNA. GEd Group I products will be evaluated for phenotypic equivalence analysis, while Group II products will be evaluated for trait effectiveness using confined/contained field trials and phenotypic equivalence analysis. However, because Group III products have a whole gene introduced into the genome, they are subject to the same biosafety laws as GMOs. Furthermore, the designed nucleases, gRNAs, and other compounds integrated into the plant genome will only be retained in the T0 generation before being separated in subsequent generations. As a result, deliberate and thoughtful attempts to develop biosafety/risk evaluation criteria for genome-edited plants are necessary. Because of these facts, the United States has accepted genome-edited plants as nontransgenic, although the European Union still deems them to be transgenics (Dederer & Hamburger, 2019).

Challenges and Future Prospects

Like most biotechnological tools, genome-editing approaches exploit biological mechanisms in vitro to make targeted changes to the genome. We have no control over how the genome changes during evolution. However, when altering the genome in an experimental setting, it should be done primarily for the benefit of society. Its use in crop development should be limited to those breeding goals that are necessary and impossible to achieve within the constraints of current variability. Several ethical concerns have been raised about genome editing, which the scientific community must address as with any new technique. To harness the potential of this technology for the benefit of global agriculture while overcoming societal neophobia, a reasonable view with proper support from regulatory agencies based on scientific principles is required. The intentional dissemination of genetic elements in the wild using CRISPR/Cas9 that results in deadly mutations is a particularly nature-friendly technique for controlling insect pests. However, at the theoretical (Unckless et al., 2017) and experimental levels, insect resistance resulting from CRISPR-based gene drive is a genuine and pressing issue (Hammond et al., 2017). Multiplex genome editing can overcome resistance (Marshall et al., 2017).

Furthermore, suppose edited CRISPR/Cas9 insects carrying gene drives are released into the environment. In that case, rigorous risk assessment for nontarget outcomes is also required before deployment. The post-release repercussions to beneficial insects (pollinators) may harm food chains, resulting in a shift in community structure. The situation may worsen if there is any possibility of gene flow from the target species to its nontarget counterparts. In this regard, using two gRNAs in combination with a mutated nickase version of Cas9 (Ran et al., 2013), genetically engineered variants of Cas9 (Kleinstiver et al., 2016), orthologs of Cas9 with distinct DNA binding specificity, dimeric RNA-guided FokI nucleases (RFNs) (Tsai et al. 2014), short guide sequences (Fu et al., 2014), and guide sequences with no or tolerable nontarget activity (Bae et al., 2014) can significantly reduce off-target effects. Hence, targeted removal of insect pests, virus-carrying insect vectors, and alien species through gene drive technology might be beneficial. As for risk management, introducing terminator genes to allow transgenic insects to live a programmed life and tagged insects to monitor gene flow could be a vital step toward gene drives in biosafety. In conclusion, advances in the toolbox of genome editing and a better understanding of plant immune response are predicted to ensure future targeting of a larger number of immunity players with a high specificity, leading to a sustainable approach for global agriculture.

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Chapter 14
CRISPR Revolution in Gene Editing: Targeting Plant Stress Tolerance and Physiology

Asad Abbas, Anis Ali Shah, Adnan Noor Shah, Yasir Niaz, Waseem Ahmed, Habib Ali, Muhammad Nawaz, and Muhammad Umair Hassan

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Introduction

The twentieth century seems to be promising for genetics, as great interventions in genetic analysis and genetic manipulations have been done recently and their development is now at a rapid pace (Carroll, 2017). With advancements in DNA sequencing, genomes of more plants and organisms are now published and genetic manipulations extended to multiple organisms and plants which were restricted previously to model organisms due to limited genomic data available (Giani et al.,

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2020). Previously, inefficient and laborious reverse genetics techniques were used for functional analysis of genes in plants; however, these techniques are impractical and time-consuming (Ma et al., 2015). With the recent advancements, many gene editing techniques like TALENS, RNAi, and CRISPR Cas9 were invented and used for genetic manipulation in recent years in important agronomic and horticulture crops (Hung & Slotkin, 2021; Joung & Sander, 2013; Rasheed et al., 2022).

Clustered regularly interspaced short palindromic repeats (CRISPR) Cas9 system is the most robust and stable technique available for gene editing and functional analysis (Basso et al., 2019). CRISPR Cas9 has been efficiently and successfully used in numerous plant species such as *Solanum lycopersicum*, *Zea mays*, *Oryza sativa*, and *Arabidopsis thaliana* for fine-tuning transcription of miRNA coding genes (MIR genes) (Abbas et al., 2022; Bi et al., 2020; Damodharan et al., 2018; Jacobs et al., 2015; Zhao et al., 2016; Zhou et al., 2017). CRISPR Cas9 systems mainly include two components: one is guide sequence gRNA, and the other is Cas9 enzyme. gRNA is the guide RNA which guides the silencing complex to the targeted region of the selected gene; the silencing complex mainly consisted of Cas9 enzyme that is responsible for double-strand break within the selected gene (Wu et al., 2014). Double-strand break at the gene initiates gene repair mechanism which repairs the region with double-strand break; this repair causes addition or deletion in the targeted region. This mutation results in the knocking in or knocking out of the genes. The phenomena used in the CRISPR Cas9 technology are basically naturally present in bacteriophages which they use as defensive mechanism against viruses. Recently, it is identified and used as gene editing technology in plants and animals (Hsu et al., 2014). CRISPR systems were initially designed for single-base editing but on specific need were altered for multiple edits at a time, we will discuss this evolution in CRISPR systems in detail in the next portion of this chapter.

With the increase in population and changing environments, constant food supply is the biggest challenge for agriculturists nowadays; even the tolerant plants failed to survive in extreme conditions. To improve plant tolerance, recently many transgenic approaches are adopted. For achieving optimum plant tolerance, the primary step is the selection of genes regulating plant stress tolerance (Ahanger et al., 2017). In plant metabolism, plant physiology, disease resistance, and responses to stress environments are controlled by different genes (Ahanger et al., 2017; Andersen et al., 2018; Isah, 2019). So many genes are of high concerns, and their manipulation can be done using CRISPR Cas9 to produce plants with better resistance to disease and stress along with better physiology to improve plant stress tolerance and physiology. So far, CRISPR Cas9 has been adopted in more than 20 major crops for their traits that improve yields and biotic and abiotic stress tolerances. CRISPR has been used for improving crop physiology in rice. It is used to improve root structure to reduce lodging; likewise, it is used in tomato for early flowering and stem structural changes which had made it highly desirable for urban farming (Kwon et al., 2020; Romero & Gatica-Arias, 2019; Zafar et al., 2020a).

Evolution in CRISPR Systems

CRISPR Cas9 is the latest available gene editing technology. For many years, it was in use for plant genome editing; until then there is constant evolution in CRISPR system and its application. Initially used CRISPR system is a uni-plex system and is used for single gene editing; then, it evolved as multiplex gene editing system targeting multiple genes at a time (Bollier et al., 2021). Alike in start just CRISPR Cas9-derived nucleases were used, but now other genome editing agents like base editors, prime editors, and transposes are effectively used (Anzalone et al., 2020). Evolutionary studies revealed the presence of several other family members of Cas9 enzyme; a few of them are already in use (CRISPR Cas13a, CRISPR Cas13b, CRISPR Cas12b, and CRISPR SpCas9-NG), and a lot of them will be used in the future (Westra et al., 2016).

When it comes to the application of CRISPR Cas9 system, it was practically designed for targeting protein coding genes, but with time it is extended to noncoding regions and even to the small noncoding RNA (Bi et al., 2020; Li et al., 2019). It can be used for editing endogenous genes as well as for the targeted insertion of desired gene segments (Aglawe et al., 2018). Use of CRISPR Cas9 system mainly depends on the nature of the experiment. It is effectively used for point mutations with change in a single base, even extended to large segment deletions, which means practical applications of this system are quite versatile. CRISPR system can be redesigned with minor changes based on the requirements of the experiments (Song et al., 2017) (Table 14.1).

Table 14.1 Different CRISPR Cas systems and their member proteins

Type	Members	Processing protein	Target	References
Type one	Cas1	Cas3	DNA	Kumar and Jain (2014)
	Cas2			
	Cas3			
	Cas5			
	Cas6			
	Cas7			
Type two	Cas1	Cas9	DNA	Kumar and Jain (2014)
	Cas2			
	Cas9			
	Csn4			
	Csn2			
Type three	Cas1	Cas6	DNA & RNA	Kumar and Jain (2014)
	Cas2			
	Cas10			
	Cas6			

CRISPR Targeting Plant Stress Tolerance

With the changing environment, now plants are more exposed to stress conditions such as salinity, drought, and elevated temperatures. Plants are continuously on the verge of being attacked by different disease-causing agents including fungi, bacteria, and viruses which causes 20–40% loss in production globally. A lot of studies documented genes having a role in resistance (Jones-Rhoades et al., 2006; Jung et al., 2009; Nithin et al., 2015). CRISPR CAS9 can be used for targeting genes which are responsible for regulating disease resistance (R genes) and disease host susceptible genes (S genes) to efficiently manipulate disease resistance in plants (Das et al., 2018). CRISPR Cas9 technology has been used extensively for targeting protein-coding genes and genes in agronomic essential crops, fruits, and fungi (Curtin et al., 2018).

Previously, breeding of tolerant plants and selection are the only ways for crop improvement; nowadays, with the advancements in biotechnology, it's a bit revolutionized, but still the selection of genes is a challenge. Plant stress tolerance is regulated naturally by two types of genes; tolerance is positively regulated by (T) genes which are tolerance genes and negatively regulated by (S) genes that are susceptible genes which have roles in decreasing plant stress tolerance (Zafar et al., 2020b). Targeting these genes with CRISPR Cas9 will result in the effective manipulation of tolerance in plants. CRISPR Cas9 is the most robust and effective technology available in the hands of molecular biologists and is used for most important crop plants (Jaganathan et al., 2018). In *Brassica oleracea*, multiple genes (*BoiProH*, *BoiPlP2*, *BoiP1P2;3*) were noticed regulating salt tolerance; the expression of these genes is significantly changed by the silencing of *BoiCesA* cellulose synthetase gene (Li et al., 2017; Shah et al., 2021). In rice, *OsRR22* gene regulates salt tolerance when targeted by CRISPR Cas9 system improving plant tolerance to salinity; likewise, in another study NHX and SOS1 transcription factors when knocked out result in increased salt tolerance (Farhat et al., 2019; Zhang et al., 2019). Technical route of CRISPR-mediated gene editing in *Brassica* is shown in Fig. 14.1.

Likewise, with the changing environment and shrinking water resources, drought is also an emerging problem for today's modern agriculture. Drought stress is a significant constraint worldwide in sustainable agricultural production (El Sabagh et al., 2019a). Owing to water deficiency, osmotic stress affects gas exchange, water relationships, chlorophyll contents, and plant morphology (El Sabagh et al., 2019b; Fahad et al., 2019). Drought stress also causes the breakdown of membrane proteins caused by adverse cell growth (Ahmad et al., 2018). In addition, drought stress also controls composition and contents of oil in oilseed crops (El Sabagh et al., 2019a). Moradi Aghdam et al. (2019) estimated that the qualitative and quantitative traits of canola and its oil content decreased by 43% compared with usual watering in the silique setup process, subject to reduced irrigation.

It is reported that in comparison with canola, camelina showed more tolerance towards drought stress, though shoot-to-root ratio, stomatal conductance (g_s), transpiration rate (E), and photosynthesis rate (Pn) decreased significantly in both

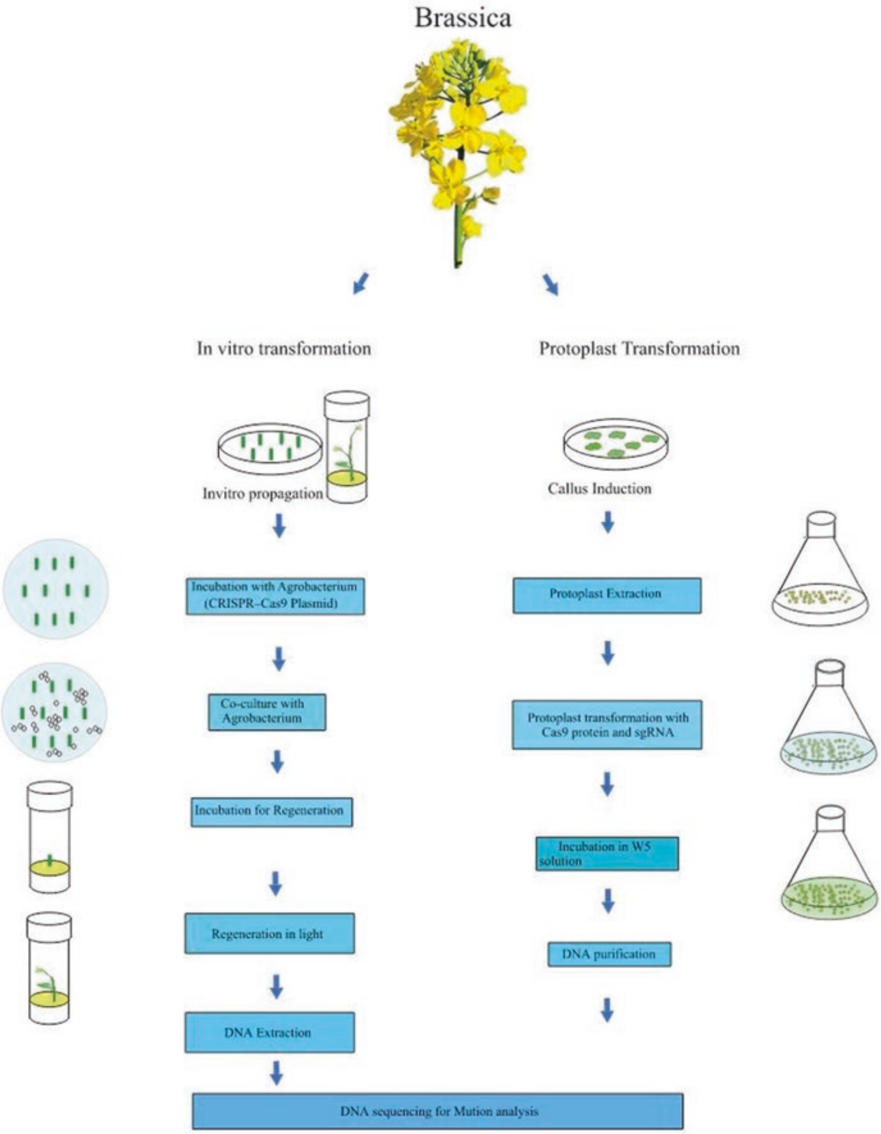

Fig. 14.1 Technical route of CRISPR Cas9-mediated genome editing in *Brassica*

crops. However, a relative decrease in g_s, E, and Pn was less in camelina than in canola due to water retention (Gao et al., 2018). Nevertheless, the available knowledge on canola and camelina tolerance is inconsistent and limited in drought stress. Using different fertigation techniques, drought stress is minimized.

Potassium (K) increases water stress tolerance in plants, ensuring optimal macronutrient production (Ahmad et al., 2018). K improves water relation, helps to open and close the stomata, improves various physiological plant processes in plants, and triggers enzymatic activity in plants under water-limited conditions (Ahmad et al., 2018; Waraich et al., 2011). K applications enhance the yield and physiological characteristics of oilseed crops under drought conditions. K enhances tolerance in plants against water stress by maintaining a lower water potential (Ahmad et al., 2018).

And this issue is also addressed by multiple scientific teams by improving root physiology in many crops as done in *Arabidopsis* (Martignago et al., 2020). Modified plants have comparatively better survival ability to withstand drought, making them resilient against drought conditions (Joshi et al., 2020). Similarly with other stresses, if stress responsive genes can be easily targeted by using CRISPR Cas9 and checked their roles in plant stress tolerance. This makes CRISPR a promising technology to be used in future agriculture.

CRISPR Targeting Plant Physiology

In modern agriculture, farming techniques and cultural practices are revolutionized. Due to the automation in agriculture and reduction in the cultivatable land, now urban cultivation is also in high consideration. Due to all these recent trends, sometimes plant physiology needs to be targeted (Kwon et al., 2020). This can be effectively done in tomato to restructure its vines to make it suitable for urban agriculture using CRISPR Cas9 (Kwon et al., 2020). Recently, in rice root physiology is also targeted using CRISPR Cas9 to improve drought tolerance and lodging resistance (Romero & Gatica-Arias, 2019; Zafar et al., 2020a). In addition to plant physiology, fruit physiology, shape, and physical appearance can also be manipulated by CRISPR-based gene editing which is very attractive for horticulturists; even grain length can also been increased (Liu et al., 2021). Some of the important miRNAs regulating plant physiology are listed in Table 14.2, which can potentially be targeted for physiological improvements in plants. In addition to physiology, the early flowering and early maturing varieties are also developed (Liu et al., 2021). Plant physiology is still a new avenue to be improved using CRISPR-mediated genome editing.

Challenges and Their Solutions

Along with the high efficiency and ease of use, CRISPR Cas9 system has some constraints which sometimes make its practical implication a bit tricky and complicated. There are three main limitations in the practical application of CRISPR Cas9 system. The first one is the selection of suitable gRNA, the second is the delivery of CRISPR Cas9 system, and the third is the off-target mutations. While designing CRISPR Cas9 system for knocking out a gene, there is a very short sequence that is

Table 14.2 MiRNAs regulating plant physiology

miRNA	Species	Role	References
miR156	*Brassica rapa*	Heading time	Ren et al. (2020)
miR319		Development	
miR156		Wrinkling of leaf	
miR166		Adaxial identity	
miR319		Leaf development, curling, and head formation	
miR156	*Zea mays*	Proliferation	Juárez-González et al. (2019)
miR164		Plant regeneration	
miR166		Plant regeneration	
miR393		Grain filling rate	
miR165	*Moringa oleifera*	Root development	Luo et al. (2015)
miR166		Leaf development	
miR156		Maintenance apical meristem, procambium identity, adaxial identity	
miR395		Plastochron length	Prakash et al. (2016)
miR164		Plant development, flowering	
miR393		Cell proliferation	
miR396		Leaf growth reduction development	
N-miR323		Flowering time	Pirrò et al. (2019)
miR159	*Arabidopsis thaliana*	ABA signalling silique development	Millar et al. (2019)
miR159	*Vitis vinifera*	Flower development	Jin et al. (2015)
miR167d	*Camellia sinensis*	Dedifferentiation of explants	Gao et al. (2019)
miR156		Leaf development	
miR166a-3p		Fruit development	
miR396		Stress responses	
miR157d-5p		Callus induction	
miR393c-3p		Shoot regeneration, development stage differentiation	
miR396	*Jatropha curcas*	Cell expansion	Yang et al. (2019)
miR164		Leaf growth	
miR319		Cell division	
N-miR005		Meristem initiation	
N-miR172		Cell wall organization	
		Ovule and seed development	
miR396	*Luffa aegyptiaca*	Browning	Xu et al. (2018)
miR399			
N-miR001	*Oryza sativa*	Developmental stage balancing	Mutum et al. (2016)

targeted, so it is quite difficult to design good-quality gRNA. As mentioned above, the problems in gRNA design can be minimized by "guide RNA design tool" which should be specially designed using improved logarithms; still, gRNA design tools for recently introduced CRISPR Systems (CRISPR Cas13a, CRISPR Cas13b, CRISPR Cas12b, and CRISPR SpCas9-NG) are not available for researchers (Aman et al., 2018; Ming et al., 2020; Qin et al., 2020). So efforts should be done to make them freely available for all. After being designed, the delivery of the CRISPR Cas9 system to the plant cell is quite challenging in a few plant species, and tissue culture is a bottleneck for CRISPR Cas9-based gene editing in most of the plant species which can be overcome by using de novo induction of meristems (Maher et al., 2020). After the transfer of CRISPR Cas9 to the plants, the main concern is the off-target mutations as the target recognition is done by Watson-Crick base pairing, so sometimes in addition to the primary target, some of the off-targets were also targeted which is discouraged. That can be minimized by using cytosine base editors (CBEs) (Bortesi & Fischer, 2015; Cho et al., 2014; Doman et al., 2020). Along with the many benefits of this system, off-targeting is the main and serious constraint in its acceptability by the scientific community which needs to be addressed by proper legislation and mutual understanding (Schiemann et al., 2020). CRISPR-based agriculture revolution is our necessity, as we are in need of improved crops to meet our increasing needs. But CRISPR systems should be in safe hands, and strict regulations are needed before introducing CRISPR products in world markets.

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Chapter 15
Genomics for Abiotic Stress Resistance in Legumes

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Abiotic Stresses and Importance of Legumes

Plants as being sessile grow under natural environmental conditions, and therefore they are under the influence of several harsh climatic conditions that negatively influence the growth of crops (Chaudhry et al., 2022). The major abiotic stresses include salinity (excessive salt contents in soil), drought stress (decreased water availability), and fluctuation in temperature (heat/cold stress) (Chaudhry et al., 2021a; Gökçe et al., 2021). Although individual stress conditions devastate the crop yield, however sometimes plants are under the influence of combined stress conditions that is even more lethal, and it is predicted to prevail more in drought-prone areas (Asim et al., 2021). It is essential to unravel genomic information to better adapt legume crops to cope with future environmental stresses (Rane et al., 2021).

Fabaceae is the third largest family after Poaceae family that is comprised of 650 genera and around 20,000 species (Koenen et al., 2019). The legumes solely account for approximately 27% of the agricultural crop production globally. It complements cereals that hold a significant importance to fulfil the requirement of carbohydrates for human diet; additionally, it also accounts for higher amino acids and dietary proteins. The major legume for human diet constitutes chickpea, cowpea, soybean, common bean, peanut, pigeon pea, pea, faba bean, lentil, lupin, and mung bean. Leguminous crops are famous and well studied for their nitrogen-fixing capacity that also prevents soil erosion, reduces weed competition, and enhances organic matter content of the soil. Although leguminous crops confer several benefits and have a major role in food security, climatic changes are influencing their growth and yield. A majority of the crops are reported to be sensitive to biotic (insects, viruses, nematodes, fungi, and bacteria) and abiotic (salinity, drought, high/low temperature, and waterlogging) stresses. Therefore, it is a need of the hour to adapt crops to cope with future harsh climatic conditions to ensure food security (Khan et al., 2012). Additionally, genomic approaches have been introduced and investigated to unravel the basis of plant stress responses and identify potential candidate genes/loci to confer stress resistance/tolerance. Subsequently these genes can be exploited in molecular breeding program for the development of stress-tolerant varieties (Fig. 15.1).

Gene Discovery through Sequencing in Legumes

It is the fundamental tool for the identification of gene and to understand the genomic composition of legumes in response to stress conditions. The earlier attempts were performed to sequence the genome of the model plants to unravel novel genomic information; later, crops whose genome size is small were sequenced. It is highly expensive, therefore alternative paved pathways to discover genes in legumes. The commonly employed ones were construction of expressed sequence tags (EST), in silico mining of genes, expression analysis of genes, and cDNA libraries (Kudapa et al., 2013). The EST proved to be a potent technique to discover

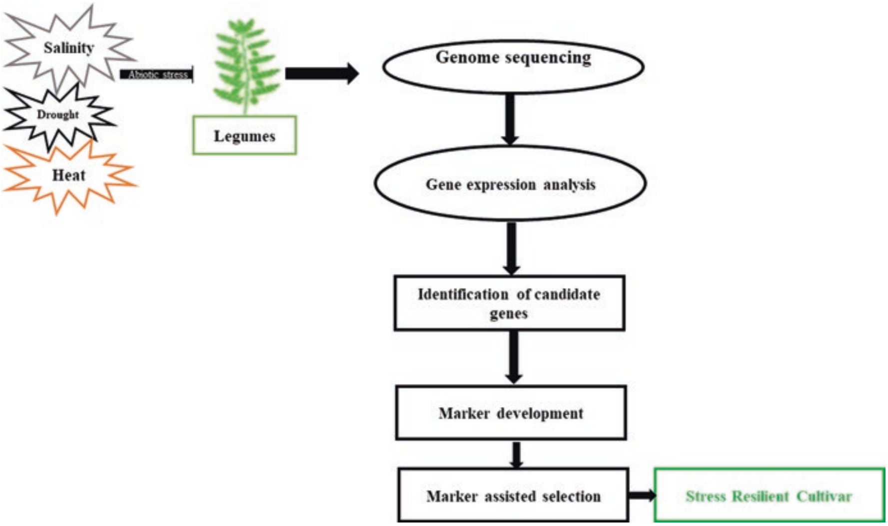

Fig. 15.1 Abiotic stress and genomic approaches to develop stress-resilient legume crops

Table 15.1 ESTs produced with NGS

Legume	Stress	Number of ESTs	References
Chickpea	Combined drought and salinity	103,215	Hiremath et al. (2011)
Chickpea	Drought	37,265	Jhanwar et al. (2012)
Common bean	Drought	59,295	Kalavacharla et al. (2011)
Mung bean	Drought	125,821	Moe et al. (2011)
Pea	Drought	324,428	Franssen et al. (2011)

novel genes, and it also assisted in mapping. Additionally, it also generated abundant ESTs from different tissues under abiotic stresses. Currently, approximately three million ESTs of legumes are available in the databases. The most predominant are from soybean (1.5 million) and cowpea (0.2 million) (Blair et al., 2011). The availability of modern cost-effective next-generation sequencing platforms, i.e., Illumina, GAIIx, MiSeq, and Roche, has sped up the sequencing of transcriptome/ genome economically and efficiently (Yousaf et al., 2021). Numerous NGS platforms have resulted in a huge set of transcript reads from several legume crops in response to stresses (Table 15.1).

Stress-Inducible Genes

The legume crops exhibit complex behavior in response to stress conditions that results in the activation or suppression of genes. It is also a good approach to determine the activation of positive gene and suppression of negative regulators. It provides insight into the gene responsible for conferring resistance in legumes against stress and controlling/suppressing the expression of negative regulators by functional genomics (Gupta et al., 2020). However, some of the genes are activated in response to every stress condition either heat stress or salt stress which suggested the similarity of genetic mechanisms to respond against abiotic stresses. Genes can be categorized into different groups: (i) genes responsible for the protection of plant cellular machinery, i.e., osmoprotectants, LEA proteins, chaperones, and antioxidant enzyme genes; (ii) signaling cascades and transcriptional control, i.e., transcription factors, mitogen-activated protein kinases (MAPKs), calcium-dependent protein kinase (CDPK), and phospholipases; (iii) aquaporins and ion transporters that help in water uptake and transport (Chaudhry et al., 2021b).

Alternative Approach to Identify Genes

Degenerate PCR is a technique used to identify a gene using the gene or amino acid sequence of a species in which there is no earlier genomic information reported or available. The degenerate approach paved the path to solve this issue to create knowledge or identify new genes. Generally, polymerase chain reaction with a set of primers is used to amplify the region in the genome, but specific primers are used. The degenerate primers differ in having wobble with possible options for binding to the base as compared to specific primers. For instance, the third nucleotide of the primer sequence harbors three possible nucleotide bases to anneal with template sequence. In this way, it provides flexibility for amplification. Moreover, it can also amplify the target sequence of all possible variants. It was exploited in cowpea in response to abiotic stress (Sadhukhan et al., 2014).

Transcriptomic Changes

Knowledge about gene expression provides imperative information of gene functions in plant's life at every growth stage. Especially during stress conditions, it regulates a different set of genes which upregulates and downregulates. Various methods can be employed to measure gene expression such as RNA sequencing, microarray, Northern blotting, semi-quantitative PCR, quantitative real-time PCR (qRT-PCR), and RNase protection analysis. Microarray paved a path to study several gene expressions simultaneously; however, contrarily it has several major limitations, including this technique being quite costly. The other major challenge for this approach is the quantity and quality of RNA. Moreover, its other weaknesses include a low precision, accuracy, and specificity and a high sensitivity to setup experiments for variations in hybridization temperature. The gene expression by exploiting this technique is also affected by the purity of genetic material, its degradation, and amplification process (Opitz et al., 2010). Alternatively, in the past three

Table 15.2 List of gene expression in response to abiotic stresses

Legumes	Stress	Gene	References
Soybean	Drought	*Gmdreb1a*	Stolf-Moreira et al. (2010)
Soybean	Drought	*GmNAC*	Le et al. (2012)
Cowpea	Drought	*P5CS*	Zegaoui et al. (2017)
Pea	Salinity	*AOX1a*	Analin et al. (2020)
Chickpea	Salinity	*DREB1E*	Kaashyap et al. (2018)
Faba bean	Salinity	*VfMYB*	Abid et al. (2021)

decades, qRT-PCR approach has been widely used for observing gene expression (Higuchi et al., 1992). It is utilized extensively even for low-expression-level studies because of its efficiency and precise quantification of target genes. It is a convenient and quick PCR approach that employs conventional RT-PCR with fluorescence resonance energy (Hu et al., 2014). Several gene expressions have been validated in different legume crops (Table 15.2).

Heat Shock Factors and Stress Tolerance in Legumes

Modern sequencing approaches have unravelled numerous candidate genes for stress tolerance in legumes. Transcriptomic analysis has also been conducted in several legumes for stress tolerance such as the expression of multifarious thermotolerant genes that have been analyzed by utilizing cDNA-AFLP. Additionally, efforts have been done for bridging the gap of genetic mechanisms underlying heat shock factors that play a vital role for the survival of plants in response to heat stress.

It is well known that HSPs are crucial for heat tolerance with the maintenance of protein functions and integrity of numerous biomembranes under heat stress conditions. They work as a molecular chaperone for the prevention of protein denaturation and aggregation. Several studies have reported that HSP has the potential to enhance heat tolerance in legumes. The genome-wide analysis and expression profiles indicated that HSP gene conferred heat and drought stress tolerance in soybean (Zhang et al., 2015). Heat shock factor-expressed sequence tags have been identified in soybean. Likewise, transcript expression of Hsp17.9CII gene elevated transcript levels with a 620-fold change in response to temperature stress (Kumar et al., 2015).

Transcription Factors

Legume plants face several abiotic stresses during their growth period. They have evolved several mechanisms to increase their tolerance by physical methods, molecular mechanisms, and cellular changes that start immediately with the onset of abiotic stress. The first step is switching on molecular responses to perceive stress and relay information via signal transduction pathways. It resultantly led to physio-biochemical changes, i.e., stomatal closure and enhanced activity of antioxidant enzymes to cope with oxidative stress. The WRKY TFs have been reported as the main family of transcriptional regulators that forms an entire part of cell signalling pathways that confer stress tolerance (Zhang et al., 2013).

The other gene families are AP2/ERF gene reported in chickpea. Moreover, TFs for heat tolerance have been reported in chickpea. Car-WRKY has been reported to multi-stress responsive TF and play essential role in signal transduction pathway (Konda et al., 2018).

MicroRNA

They are classified as small noncoding RNAs usually 20–24 nucleotides that negatively regulate gene expression by degrading mRNA or translation inhibition. It has been revealed that miRNAs play an essential role in plant responses to stress. In different legume crops, miRNA genes have been reported to increase agronomic traits as being tissue-specific, stress- or senescence-induced overexpression.

Transcriptome and genomes of soybean have been thoroughly investigated for stress-responsive miRNA-mediated regulation of genes. The earlier studies of miRNAs in soybean were performed by using approaches such as expressed sequence tags and genome survey sequence.

In drought-sensitive soybean, the miRNA was upregulated (miR397ab), whereas it was downregulated in the tolerant soybean. Its target was transcript encoding β-fructofuranosidase, which is a main enzyme for sucrose and starch metabolism. Therefore, it assisted in fixation of carbon and supply of energy to plants (Zhou et al., 2010).

Genome Editing for Stress Tolerance

As the name suggests, it is targeted mutagenesis of legumes’ genome. The invention of modern genomic editing tools allowed the introduction of site-specific modifications in the genome. Moreover, comprehensive genomic research has unveiled enormous information about stress tolerance in legumes. The genetic transformation of legumes to confer enhanced tolerance to abiotic stresses is limited to some extent (Mickelbart et al., 2015). Contrarily, targeted mutation has advantage for precise sequence modification that allows deletion and insertion by using contemporary CRISPR/Cas9. It is also considered to develop non-genetically modified crop plants with the desired trait to improve productivity of legumes against stress conditions (Huang et al., 2018).

Soybean is a diploid specie, and its genome is highly duplicated which poses challenges to conventional genetic approaches for gene functions. Other major challenge is the lower efficiency of agrobacterium-mediated transformation. Therefore soybean being the most important oil crop and enriched in protein contents was the first among other legumes for trait improvement by CRISPR/Cas9 technology to confer stress tolerance (Bao et al., 2020). Several successful gene-editing studies have been reported by using CRISPR/Cas9 (Cai et al., 2015; Sun et al., 2015). Cowpea is recalcitrant for transformation and hinders CRISPR/Cas9 practices. However, up to 37 successes have been achieved in cowpeas (Che et al., 2021).

Abdelrahman and co-workers reviewed the targeted mutagenesis in crop plants by CRISPR/Cas9. They report CRISPR/Cas9-mediated GE in many crops for yield improvements of crop plants grown under unfavorable conditions [**234**]. Mushtaq et al. [**235**] reviewed recent applications of the CRISPR/Cas9-mediated GE as a means to develop crop plants with increased tolerance to the abiotic stresses they encounter when grown under unfavorable conditions. CRISPR/Cas9 is a powerful tool in engineering for salt tolerance in legumes, but fewer investigations have been done previously in GE for SS tolerance in legumes. The use of targeted GE tools, especially CRISPR/Cas9, has a great potential to develop high-yielding legumes under saline conditions.

Breeding Approaches

The use of genetic and genomic analysis to help identify DNA regions tightly linked to complex traits in crops, known as molecular markers, can aid in crop improvement breeding strategies. They identify genomic regions (i.e., quantitative trait loci; QTLs) that control the phenotype of a complex trait and are distributed throughout the genome. Each QTL is made up of many genes that are investigated as potential candidate genes for a trait under investigation. In relation to abiotic stresses, a variety of molecular marker-related techniques have been used in legumes. Restriction fragment length polymorphism (RFLP), random amplified polymorphism (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), and derivatives have been reported for abiotic stresses. Furthermore, to map the QTLs/genes controlling a trait of interest, populations derived from two parents (i.e., biparental QTL mapping) and multi-parents/association panel (i.e., association mapping) are typically used (Samantara et al., 2021; Kitony et al., 2021). This resulted in the creation of high-resolution linkage maps (Jaganathan et al., 2015; Verma et al., 2015), which can be used in breeding programs to improve stress tolerance via marker-assisted selection (MAS). As a result, genetic maps for many species have been established, with potential resistance and/or tolerance loci or QTLs identified (Table 15.3). However, the use of molecular markers in breeding programs necessitates preliminary research to identify and validate potential markers. The following factors must be considered during this process: (a) level of polymorphism between parental lines, (b) unclear expression of some markers inherent to

Table 15.3 Some important QTLs associated with abiotic stress tolerance in legumes

Crop	Abiotic stress	QTL name	References
Chickpea	Heat stress	qfpod02_5 q%podset06_5	Paul et al. (2018) and Jha et al. (2021)
Lentil	Heat stress Winter hardiness	qHt_ss, qHt_ps *Frt* (OPS16750)	Singh et al. (2019) and Eujayl et al. (1999)
Pea	Heat stress	6	Tafesse et al. (2020)
Soybean	Manganese toxicity Salt stress Waterlogging P deficiency	BARC SATT318 Sat-091 Sat-064 *Fsw1* (L37 2I sat 36a)	Kassem et al. (2004), Lee et al. (2004), VanToai et al. (2001), and Li et al. (2005)
Alfalfa	Al toxicity	UGAc471 UGAc502	Sledge et al. (2002)

the marker class used, (c) false-positive markers, (d) discrepancy between the presence of the marker and the presence of the target gene which necessitates testing the gene with conventional screening, and (e) presence of multiple genes dispersed across several linkage groups (Yu et al., 2004).

Although the use of MAS in crop improvement may be beneficial, its practical application in legumes for the genetic improvement of resistance or tolerance to stress has been kept to a minimum, mainly hampered by genetic complexity and a lack of investment in most stress-related characteristics. With a few exceptions, MAS has already aided breeding efforts for several legume crops against significant abiotic stresses.

Chickpea and Common Beans

Schneider et al. (1997) demonstrated that MAS could be used to select drought-tolerant common beans. Varshney et al. (2013a) describe the first use of molecular breeding in chickpea for drought tolerance. Terminal drought is the most significant constraint to chickpea production, accounting for 40–50 percent of yield losses. Another study (Varshney et al., 2013b) identified a genomic region on linkage group 4 (CaLG04) that harbors several drought-related trait QTLs inclusive of root traits, contributing up to 58.20 percent, which was used for introgression from ICC 4958 into an Indian leading chickpea variety JG 11, using a marker-assisted backcrossing (MABC) approach. Based on foreground and background selection, 29 BC3F2 plants were selected and used for root trait screening after 3 backcrosses and selfing. A higher root length density (RLD) and root dry weight (RDW) were observed in all or most of the introgression lines when compared to the recurrent parent, JG 11, and donor parent, ICC 4958. The authors reported that after multi-location field trials, introgression lines developed in just three years could be released as an improved variety.

A 529.11 cM linkage map with 271 genotyping by sequencing (GBS)-based single nucleotide polymorphism (SNP) markers identified the major QTL in chickpea for the number of filled pods per plot, total number of seeds per plot, grain yield per plot, and percent pod setting under heat stress (Paul et al., 2018). Furthermore, genome-wide association studies (GWAS) allow for the identification of specific haplotypes in natural populations and even wild species (George and Cavanagh, 2015; Verdeprado et al., 2018). A recent GWAS in chickpea was carried out in a panel of 300 accessions to investigate the marker-trait association for heat tolerance (Thudi et al., 2014).

Cowpea, Lentils, and Soybean

Cowpea genomics is progressing, and several QTLs associated with seedling drought tolerance have been identified (Muchero et al., 2009). One of the most significant achievements of breeding efforts aimed at improving drought stress tolerance has been the development of productive early maturing cowpea cultivars. Early maturing cultivars can avoid the end-of-season drought that occurs in semiarid areas. Taking this into account, Hall (2004) proposed that breeding should concentrate on two types of cultivars, which should be grown concurrently to increase the likelihood of success that substantial grain production would be achieved each year. The first type would be early maturing with synchronous flower production, while the second type would begin flowering later, with more sequential rather than synchronous flowering. As a result, the first cultivar type, if subjected to intermittent drought, would have a negative impact on plant performance during the vegetative or reproductive stages, while the second type would be more resistant to drought; midseason drought survivors would survive but with a reduced ability to avoid late-season drought. In terms of heat stress, cowpea tolerant germplasm that is effective in both long-day and short-day conditions has been developed (Ehlers and Hall, 1997). Tolerance to heat-induced stress appears to confer suppression of floral buds. The ability to set pods under hot conditions is controlled by a single dominant gene.

Two dominant genes for heat stress tolerance and QTLs for pod set number per peduncle under HS have been identified in cowpea (*Vigna unguiculata* L.) (Marfo and Hall, 1992; Lucas et al., 2013; Pottorff et al., 2014). Further comparative genomic analysis identified HSPs and HSFs in these QTL regions in soybean (*Glycine max* L.) [3]. In azuki bean [*V. angularis* var. *angularis* (Willd.) Ohwi and Ohashi], QTL mapping was done for pollen viability trait under heat stress and identified two QTLs (i.e., HQTL1 and HQTL2) (Kaga et al., 2003; Vaughan et al., 2005).

Drought escape has been linked to lentil drought adaptation via early flowering, early growth vigor, and rapid root growth (Erskine et al., 2011). A useful genetic variation was discovered reported as a result of drought stress within the lentil crop variation and germplasm have also been observed in wild *Lens* species, particularly *L. culinaris* ssp. *orientalis*, which is frequently found in habitats with low average rainfall (Erskine et al., 2011). Drought-tolerant lentil cultivars, in particular, have a lower cell membrane injury and a higher seedling growth, osmotic regulation, and WUE (Stoddard et al., 2006). As previously stated, shifting sowing from spring to early spring or winter can significantly increase cowpea and lentil production (Ehlers and Hall, 1997; Erskine et al., 2011) but only if the cultivar is cold hardy. There was a significant variation in winter hardiness and cold tolerance in lentil cultivated germplasm as well as among wild relatives (Erskine et al., 2011). The winter hardiness of lentil was inherited polygenic and QTL mapped (Kahraman et al., 2004a and b). Molecular markers linked to these QTLs could be used in marker-assisted selection (MAS) programs after validation, which could include the use of ubc808–12, a "microsatellite marker" (Muehlbauer et al., 2006). Eujayl et al. (1999) discovered a RAPD marker (OPS-16750) as well; this could be useful for MAS because of its connection to a dominant seedling frost tolerance gene known as Frt.

Until recently, another factor limiting the improvement of abiotic stress resistance in some of these minor legumes was the scarcity of genomic resources (Varshney et al., 2012). Nonetheless, many minor legumes are currently becoming "genomic resources rich crops" (Varshney et al., 2009). For example, a genome sequence for pigeon pea is now available (Varshney et al., 2012).

Peas

Based on knowledge of the plant's response to stress, efforts have been made to produce pea stress-tolerant genotypes, and for that purpose molecular breeding is one of the best ways. For some traits, such as frost tolerance, QTL mapping is paving the way towards marker-assisted selection. Despite the complexity of most abiotic stresses, stress-tolerant cultivars have been bred on multiple occasions by introducing traits from stress-adopted wild relatives or landraces (Bartels and Sunkar, 2005). Landraces, in particular, have a high potential for improving abiotic stress tolerance because the transfer of beneficial traits from them is relatively simple due to the lack of a barrier to crossing. A deep rooting landrace from Ethiopia, for example, has been reported to be well adapted to a wide range of temperatures (JI1432). Furthermore, *P. fulvum* may be a source of stress tolerance for cultivated peas, particularly drought tolerance, because its main roots penetrate to greater depths at a rapid rate in the soil (Ali et al., 1994). *P. elatius* accessions adapted to freezing temperatures have also been reported (Ali et al., 1994).

So far, 76 QTLs for 11 traits in pea have been described, but none of them, with the exception of the recently reported freeze tolerance QTLs, have been linked to abiotic stress (Dita et al., 2006; Mc Phee, 2007). Using the Pop2 RIL population, which was derived from a cross between the freezing-tolerant line Champagne and the sensitive line Terese, Lejeune-Henaut et al. (2008) and Dumont et al. (2009) discovered four major QTL regions on multiple occasions for frost tolerance in the field or under controlled conditions. Coinciding QTLs for physiological traits were assumed in two of these regions. These included QTL for glucose and raffinose content in the leaves, as well as RuBisCO activity. Candidate genes implicated in freeze tolerance QTL in pea have been identified sometimes. It is possible that the flowering *Hr* locus co-localizes with the Pop2, which has the most explanatory freezing tolerance QTL. Winter frost tolerance is thought to be influenced by delaying floral initiation until the main winter freezing periods have passed (Lejeune-Henaut et al., 2008). Hr has been demonstrated to be an ortholog of the known EARLY FLOWERING 3 (ELF3) involved in the function of the circadian clock (Weller et al., 2012). Therefore, more efforts are required to use the available genomic resources for mapping/tagging the genes/QTLs controlling the traits of heat stress tolerance in cool-season grain legumes. Following the mapping of genes differentially expressed between Champagne and Terese under cold exposure, Legrand et al. proposed additional positional candidate genes related to freezing tolerance mechanisms. A genome-wide association (GWA) analysis using 16,877 known high-quality SNPs found correlations between genomic regions, chlorophyll concentration (6 QTLs), photochemical reflectance index, and canopy temperature (2 QTLs), reproductive stem length (7 QTLs), internode length (6 QTLs), and pod number (9 QTLs), as well as 48 candidate genes responsible for these traits under heat stress (Tafesse et al., 2020).

Forage Legumes

Alfalfa cultivars are artificial populations that are created primarily through phenotypic recurrent selection. In contrast to crop legumes that are inbred and homozygous, alfalfa cultivars are highly variable, heterozygous plants with complementary alleles. Long-term traditional breeding programs are underway to improve alfalfa yield under stress conditions (Vasconcelos et al., 2008; Li et al., 2010). Breeding for broad-spectrum disease and insect resistance should also remain a priority, as these are likely to be more prevalent in environments with high levels of abiotic stress. Moving forward, research focuses on identifying genes and tolerance mechanisms that are common both at the start and during the course of various abiotic stresses (Hirayama and Shinozaki, 2010) which may be beneficial to breeders who aimed at using genetic markers to target genes that allow a plant to increase (or at the very least maintain) biomass yields in a variety of environments. Understanding the mechanisms underlying the activation and regulation of specific stress-related genes, as well as the incorporation of molecular markers found in alfalfa (Han et al., 2011; Li et al., 2012), as well as genomic selection methodologies, has the potential to accelerate the rate of genetic gains during each selection cycle.

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Chapter 16
Genetic and Molecular Factors Modulating Phosphorus Use Efficiency in Plants

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Introduction

Phosphorus (P) is a crucial plant macronutrient for plant growth and development. It cannot be substituted by any other element, and provision of adequate amount is necessary for plant survival. Phosphorus fertilizers have increase crop production over the past 50 years and help in ensuring food security for increasing population (Pavinato et al., 2020). Phosphorus is an integral part of plant cells and performs key functions in plant metabolism such as photosynthesis, sugar metabolism and translocation, and transformation of metabolites. The normal phosphorus concentrations in plants are from 0.1% to 0.5% (Gagnon et al., 2020).

Phosphorus is an important phytonutrient complicated in several biochemical pathways and biosynthesis of biomolecules like lipids, nucleic acids, and cellular membranes. Phosphorus also promotes the signal cascade of cells by acting as a signal mediator and is an important for metabolism. Due to its extensive use in agriculture, phosphorus resources are limited. Therefore, it is vital to formulate scientific strategies to improve the efficiency of phosphorus utilization and recycle in the future. The soluble, bioavailable form of phosphorus (phosphate; Pi) used for absorption is readily removed from the topsoil, which can cause serious havoc. In addition to this, scouring Pi from the topsoil requires a constant supply of pi to preserve satisfactory fertilizer levels, which worsen the condition. To overcome phosphorus stress, plants are recognized to undergo fundamental cellular changes in physiology, metabolism, and hormone balance.

Better understanding to these govern changes can result in improving agronomic traits, resource conservation and step forward for sustainable agriculture as well as the use of biotechnology strategies to improve crop phosphorus utilization efficiency.

Phosphorus Uptake and Transport

As soil is the primary source, Pi enters through root hairs, root tips, or outermost layers of root cells (Zahra et al., 2020). The uptake can be increased by some soil microbes especially mycorrhizal fungi that grow as a symbiont with plants. Phosphorus is taken up by plant roots as the primary orthophosphate ion ($H_2PO_4^-$), which is the only possible available form, but in some cases, it is can also be absorbed as ($HPO4^{-2}$) secondary orthophosphate (Amin et al., 2017; Ceulemans et al., 2017). Once entered into the plant roots, P may be accumulated for the time in root tissues or transported to the aerial parts of the plant. Through several biochemical reactions, it is incorporated to organic compounds, like DNA and RNA (nucleic acids), phosphoproteins and lipids, various antioxidant enzymes, and energy-rich phosphate compound, that is, adenosine triphosphate (ATP). Phosphorus in inorganic as well as in organic form moved throughout the plant body and is available for different metabolic processes (Galatro et al., 2020).

Phosphorus Use Efficiency

There are numerous definitions for PUE (Brown et al., 2012). P absorption efficiency means the ability of plants to extract phosphorus from the soil, and PUE stands for biomass production capacity of the absorbed phosphorus (Wang et al., 2010). The PUE is improved either by improving intake or optimizing its internal usage (Reymond et al., 2006). Importance of each measure depends on the environment in which the crop grows. In agricultural ecosystems with high phosphorus consumption, the importance of phosphorus absorption is reduced because of the higher availability of phosphorus. Under these conditions, increasing the efficiency of phosphorus utilization and reducing the amount of phosphorus recovered during harvesting will bring agronomic benefits. In contrast, in environment with low P availability, both optimum acquisition and utilization are of benefit. Given the collective importance of this important macronutrient, we must deepen our understanding of the phosphorus-deficiency response due to cellular and physiological changes to ensure that plants with higher phosphorus efficiency are developed to increase agricultural productivity.

Plant Physiological Response to Phosphorus

When exposed to phosphorus-deficient conditions, plants exhibit several characteristics and physiological responses. Due to the lack of phosphorus, the development of buds and flowers is greatly slowed down, resulting in delayed plant growth and reduced bud branches. Maintaining root stem cells under phosphorus-deficient conditions requires (ER) endoplasmic reticulum to reside PDR2 (phosphate deficiency response 2, P5-like ATPase), which interacts genetically with low phosphate root 1, a multi-copper oxidase that is functionally important for phosphorus sensing at the root tip (Reymond et al., 2006: Wang et al., 2010). The accumulation of anthocyanin pigments usually occurs in leaf tissues to protect chloroplasts and nucleic acids from strong light or ultraviolet rays (Bustos et al., 2010). In addition, the growth of the taproot is strongly inhibited, while the development of lateral roots and hairy roots is strongly stimulated, which ultimately leads to a higher root/bud ratio (Brown et al., 2012).

Roots are an important entry point for phosphorus. In the case of phosphorus deficiency, changes in root structure are essential to maximize the effective nutrient absorption for plant survival (Niu et al., 2012). When phosphorus is lacking to utilize phosphorus in the form of phospholipids, organic Pi transporters are also induced (Ramaiah et al., 2011). Although plants have very limited ability to obtain phosphorus from other organic sources outside the roots, they can directly use phosphorus from phytate (Richardson et al., 2001). In addition, genes that respond to phosphorus deficiency, such as genes induced by phosphorus deficiency (IPS1), RNS1, phospholipase DZ2 (PLDZ2), Pht1;4, and At4, are strongly induced under phosphorus-free conditions (Jain et al., 2009). Due to the low availability of phosphorus in the soils, plants are often deficient in phosphorus. Plants that are prone to phosphorus (Pi) deficiency may suffer from inhibition of primary root growth, reduced phosphorus concentration in various tissues, and reduced photosynthesis rate, but the utilization of photosynthetic phosphorus is higher (López-Arredondo et al., 2014). The plant response to pi deficiency based mainly on experimental data obtained by many scientists on herb model plants like *Arabidopsis thaliana* has a different phosphorus feeding strategy than wood plants (Rennenberg & Herschbach, 2013) (Table 16.1).

Table 16.1 QTLs mapped in maize related to phosphorus use efficiency

Variety	Marker	Traits	Details	References
NY821/ H99	77RFLP	SDW, RDW, TDW	Six RFLP marker loci related to biomass under P deficiency were identified	Reiter et al. (1991)
Mo17/ B73	167 RFLP, SSR and isozyme markers	RDW, RV	Substantial variation between maize lines for growth with low P and response to mycorrhizal fungi	Kaeppler et al. (2000)
Mo17/ B73	196 RFLP, SSR, and isozyme markers	LRL, LRN	Eight QTL were identified for root-related traits	Zhu et al. (2005b)
Mo17/ B73	196 RFLP, SSR, and isozyme markers	RHL, TT, SDW, SPC	QTL located at npi409–nc007 on Chr5 related to root hair length plasticity were found with low and normal P	Zhu et al. (2005a)
Mo17/ B73	196 RFLP, SSR, and isozyme markers	SRL, SRN	Two coincident QTL flanked by umc34–bn112.09 on chromosome 2 and by bn112.09–umc131 on chromosome 2	Zhu et al. (2006)
082/ Ye107	275SSR+ 146AFLP	PH, SDW, RDW, TPC, APA, H^+, et al.	Five common regions for same QTL were found in the interval bnlg1556–bnlg1564, mmc0341–umc1101, mmc0282–phi333597, bnlg1346–bnlg1695, and bnlg118a–umc2136	Chen et al. (2008)
082/ Ye107	275SSR+ 146AFLP	SPUE, WPUE, RSR	SPUE and WPUE under LP were controlled by one QTL at interval of bnlg1518–bnlg1526 (bins 10.04)	Chen et al. (2009)
178/5003	207SSR	GY, HGW, EL, RN, KNPR, ED	Consistent QTL at umc2215–bnlg1429, umc1464–umc1829 and umc1645–bnlg1839 on chromosome 1, 5 and 10	Li et al. (2010)
082/ Ye107	275SSR+ 146AFLP	Biomass, the leaf age, PH	Two important QTL located at bnlg1832–P2M8-j in chromosome 1 and umc1102–P1M7-d in chromosome 3	Chen et al. (2011)

Molecular and Genetic Responses to Phosphorus Deficiency

In phosphorus-rich and low phosphorus conditions, different transcription factor groups perform regulatory functions to maintain phosphorus in plant, which is essential for normal plant metabolism. The first transcription factor described as related to phosphorus deficiency is phosphorus deficiency response 1 (PHR1), which is a transcription factor TF (MYB) that responds to phosphorus deficiency at posttranscriptional level (Rubio et al., 2001). PHR1 regulates genes that respond to phosphorus deficiency, including genes encoding AtIPS1 and ribonuclease. Responsive genes, such as AtIPS1, (AtPHT1) phosphate transporter1, (ribonuclease1) AtRNS1, (PHO1) phosphate 1, (SQD1) UDP-sulfoquinovose synthase 1, and (DGD2) digalactosyldiacylglycerol synthase 2, have common motifs (GNATATNC). It was also found that transcription factor responds to low phosphorus levels at the transcription level, which is the zinc finger C2H2 is in the nucleus (Devaiah et al., 2007). The main helix TF (bHLH) was found to be responsible for the resistance of rice to phosphorus stress (Yi et al., 2005). PHO1 gene family has been involved in phosphorus transportation from roots to shoots in Arabidopsis and rice (Secco et al., 2010). In phosphorus-rich conditions, the (AtIPS1) and GUS reporter line showed significant GUS activity, which was limited by the endoderm of the mutant pho1., while no GUS activity was perceived in wild plants, *PHO1* evolved as Pi exporter (Hamburger et al., 2002).

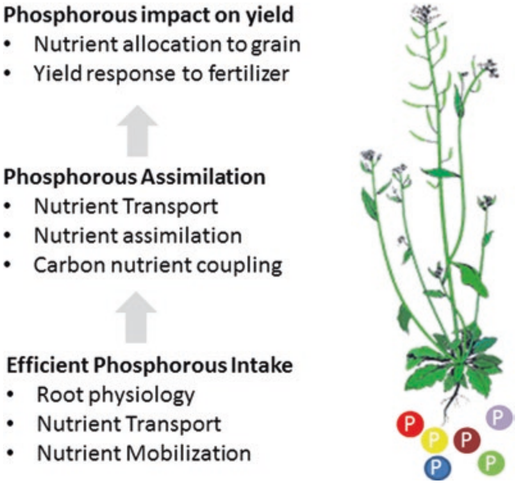
Phosphate monoesters are hydrolyzed by purple acid phosphatase (PAP) to Pi. The genes encoding PAP are strongly induced by phosphorus deficiency (Li et al., 2012). The resistance of plants to phosphorus deficiency is partly due to the release of acid phosphatase. In Arabidopsis, AtPAP26 is one of 29 acid phosphatases. It exhibits significant phosphatase activity during phosphorus deficiency and leaf aging and has dual functions of secretion or local vacuole form (Robinson et al., 2012). Posttranslational regulation clearly involves signal transduction for phosphorus deficiency. AtSIZ1 is a small plant, while ubiquitin-like modified E3 ligase is a regulator of plant stress response (Miura et al., 2011). When phosphorus is deficient, phosphorus can be recovered from the nucleic acid source. The lack of phosphorus strongly induces ribonuclease outside and inside the cell. Although AtRNS3 is expressed in different tissues and remains relatively stable in the case of phosphorus deficiency, AtRNS1 and AtRNS2 are strongly induced by phosphorus deficiency. AtRNS1 is highly expressed in flowers, and the protein it encodes is secreted outside the cell, while AtRNS2 remains inside the cell (Deal et al., 2007).

Noncoding RNA has become a key regulator of phosphorus deficiency signals. In Arabidopsis, selection of microRNAs (miRNAs) that specifically respond to phosphorus deficiency not to other nutrients has identified miR399 family as the mediator of the phosphorus deficiency response (Abbas et al., 2022; Fujii et al., 2005). There is increasing evidence that miR399s regulate signaling in response to phosphorus deficiency. The posttranscriptional closure of the AtPHO2 gene is precisely controlled and regulated by other noncoding RNAs of the AtIPS1 family, which are strongly induced in phosphorus-deficient conditions (Franco-Zorrilla et al., 2007). Cytokinins regulate the responses to environmental stress, including strong interactions and cross talks with ABA (Ha et al., 2012). More and more evidences show that cytokinins can act as negative mediators in response to phosphorus deficiency. Many independent studies have shown that the cytokinin signal pathway affects the phosphorus signal pathway. *P. vulgaris* response regulator 1 (PvRR1) transcript increases in the absence of P, N, and K (Camacho et al., 2008). Although treatment with exogenous cytokinin will increase the expression of PvRR1 under sufficient conditions, it will decrease the expression level of PvRR1 under conditions of phosphorus deficiency (Franco-Zorrilla et al., 2007). Ethylene also regulates the phosphorus deficiency responses, especially in the development of hair roots (Li et al., 2011). Overexpressed Pht1; Compared with wild plants under sufficient phosphorus conditions, 5 exhibits altered phosphorus redistribution, with lower phosphorus content in buds and higher phosphorus content in roots and pods, leading to premature senescence (Nagarajan et al., 2011). Numerous new hps mutants, namely, the hps3 and hps4, have been characterized. Hps3 is highly sensitive to phosphorus deficiency; however, the accumulation of anthocyanins is one of the typical reactions of phosphorus deficiency, but it accumulates less with phosphorus deficiency (Wang et al., 2012). The analysis of differential gene expression among the root tips of low-sensitivity mutants to phosphorus 4 (lpi4) and wild plant under low phosphorus conditions revealed that a large group of genes belonging to the family of jasmonic acid and ethylene synthesis and signal pathways have been changed (Chacon-Lopez et al., 2011).

A growing body of evidence also shows that gibberellin plays a role in regulating the phosphorus deficiency response. The proteins involved in gibberellin signal in the DELLA domain are composed of gibberellin-responsive repressor proteins ga13 (RGA), RGAlike (RGL) 1, RGL2, and RGL3, which are key mediators of gibberellin signaling in Arabidopsis (Sheerin et al., 2011). In the case of phosphorus deficiency, the lipid composition of the membrane will change dynamically. Phospholipids are the main component of membrane lipids and can also be regarded as storage of phosphorus. Genes such as MGD2 and MGD3 involved in membrane lipid remodeling are regulated by AUX and AUX signal. The expression of MGD2 and MGD3 is inhibited by AUX transport inhibitors (Kobayashi et al., 2009). Genes such as DGD2, SQD1, NPC4, and NPC5 are inhibited in the expression of slr and arf7 arf19 mutants, resulting in a significant decrease in DGDG and SQDG levels (Narise et al., 2010). Although the expression of these genes related to lipid metabolism is controlled by auxin signals and is mainly caused by phosphorus deficiency, this auxin-mediated regulation may be an indirect effect, because the elements that respond to auxin are not existing in their composition. Promoter sequences and genes that respond to phosphorus deficiency are not removed in transactivation systems. Various methods and strategies have been tried to produce effective transgenic plants, such as the high expression of ZmPTF1, resulting in better root development, increased ear yield, and the largest grain size in transgenic corn plants (Zhou et al., 2008). Phosphorus starvation tolerance gene 1 (PSTOL1) encodes a phosphorus-specific protein kinase, which has been shown to be important for increasing grain yield in low-phosphorus soils and promoting early root growth of various rice varieties, which is the tolerance to phosphorus deficiency (Gamuyao et al., 2012). This gene is constantly missing in the phosphorus-deficiency genome of modern rice varieties. Therefore, PSTOL1 is an important gene to be engineered to improve plant resistance to low-phosphorus conditions. Target genes include genes encoding transport proteins PAP, TF, Pi, protein kinase, oxidoreductase, and genes involved in the production of organic acids. Although the Pi transporter genes seem to be suitable and promising candidates for increasing phosphorus uptake, these genes can cause growth retardation due to excessive phosphorus levels (Li et al., 2011). Overexpression of PAP or excretion of malic acid improves Pi absorption and increases biomass growth and production (Lu et al., 2012).

In Arabidopsis, PHT1, PHT2, PHO1, and PHO2 are gene families encoding pi transporters, PHT1 and AtPHT1 that are transporters, which mainly work at low Pi concentrations (López-Arredondo et al., 2014). AtPHT1;5, it participates in the translocation of Pi from the source to the receiving organ according to the development signal and the level of phosphorus (Nagarajan et al., 2011). AtPHT1;9 is responsible for the absorption of Pi at the root-soil interface and the movement of Pi from root to shoot (Lapis-Gaza et al., 2014; Remy et al., 2012). AtPHT2;1 is situated in the membrane of the inner shell of the chloroplast and acts as a low-affinity Pi transporter to ensure the distribution of Pi in plants (Versaw & Harrison, 2002). Also it plays role in the transfer of Pi to root xylem vessels (Stefanovic et al., 2007). AtPHO2 encodes E2-bound ubiquitin and prevents excessive Pi accumulation in shoots (Kant et al., 2011). According to the internal and external states of phosphorus, these genes show different transcriptional expressions in Arabidopsis (López-Arredondo et al., 2014). Homologs of AtPHO2 have been identified in the genome of *Populus trichocarpa*, and PHT1 phosphate transporter gene family is identified and characterized in mycorrhiza *P. trichocarpa* (Loth-Pereda et al., 2011; Tuskan et al., 2006). However, little is known about their expression profile in response to phosphorus deficiency in poplar species (Fig. 16.1).

Fig. 16.1 Factors affecting phosphorus use efficiency



Role of Phosphorus in Plant Metabolism

Photosynthesis

Photosynthesis is an important biochemical reaction in nature. Plant converts light energy into sugars and oxygen with the energy being captured in ATP (Liang et al., 2020). This ATP is further available as a source of energy for many other metabolic processes that occur in plant. The carbohydrate produced is helpful in building other cell organelles and storge components. Most of the converted carbohydrate within plant body involve phosphorylated forms. Phosphorus is involved in the photosynthesis at a crucial point at which energy is transformed from light into sugar, so in order to achieve optimal growth, the phosphorus availability in soil solution should be ensure (Feder et al., 2020; Shah et al., 2021).

Plant Energy Reactions

P is critical in energy transfer mechanisms of plant. Phosphorus in the form of adenosine diphosphate (ADP) and ATP is a source of energy that drives various biochemical reactions within plant (Zhao & Yang, 2020). Phosphorus in plants transfers high energy phosphate molecules to the other molecules and provides the activation energy to photosynthesis, respiration, carbohydrates formation, and many other chemical reactions to occur (Fu & Zhang, 2020).

Genetic Transfer

P is a major component of various substances that constitute genes and chromosomes, and sugar phosphate is an important structural constituent of DNA and RNA (Cu et al., 2020). The biochemical structures of DNA and RNA are linked together with the help of phosphorus. An adequate phosphorus nutrition is essential for genetic transfer code from one generation to the next and is essential for the development of new cells. A significant concentration of P in the form of phytin is found in seeds of different agricultural and garden crops, and P is also believed to be essential for seed germination and development (Xu et al., 2018). About 60–70% of P in cereals grain and about 50% in legumes are stored as phytin or similar compounds. It is noted that an improper supply of P can retard seed number, size, and seed viability.

Nutrient Transport

Nutrient availability to crops depends on nutrient supply in the rhizosphere and root surface area. Roots are able to absorb nutrients when they come into contact with active cells, so P uptake is highly dependent on plant's root system (Suleman et al., 2018). Nutrients contact the root cells mainly by three mechanisms: diffusion, mass flow, and root interception. Root interception is a major pathway for P uptake, and better root growth provides additional root surface area for P uptake. Legume crops help in fixing nitrogen through their roots in the soil, but this fixation can only be possible with adequate phosphorous, which ensure root development (Ferrol et al., 2019). Movement of essential nutrients within the plant depends chiefly on transport through plasma membrane, which required energy to suppress the force of osmosis (Palmgren, 2001). High energy P compounds and ATP provides the needed energy for transport of macro- and micronutrients from root to aerial parts of plant (Bashir et al., 2019).

Transgenic Approaches to Improve Phosphorus Use Efficiency

For sustainable agriculture with the changing environment, it is very important to improve phosphorus use efficiency using latest available resources, and it can only be done by improving the intake capability and internal efficient use of phosphorous; this can be done by efficient breeding and proper selection as all the phosphorus sources are finite (Heuer et al., 2017; Thudi et al., 2021). Within this new era of modern agriculture, PUE is improved either by opting latest cultivation practices like hydroponic systems, using new fertigation systems like drip irrigation and fumigation, and the second way is to improve plant varieties (Lakhiar et al., 2018; Sambo et al., 2019). With the advancement in the field of plant genetics and plant biotechnology, it is possible now to engineer plants according to needs and according to immerging issues. With the reduction in sequencing costs, more and more genomes are available now, which is revolutionizing agriculture by revolution in fields of omics. New techniques in biotechnology like RNA-induced gene silencing, CRISPR-mediated gene editing, and TALENS-mediated gene editing equipped plant scientists with the enough capability to alter plant genetics and plant physiology (Mutezo et al., 2021; Steinwand & Ronald, 2020). Plant internal use efficiency can be improved by overexpressing phosphorus transporters and genes involved in phosphorus assimilation (Shen et al., 2011).

Future Prospects

Earth climate is changing rapidly, and element depletion is happening at the rapid pace, which was not noticed before, and this may also be due to consecutive cultivation of crops to secure food security. Alike other nutrients, soil is the only source of phosphorus, and supplies are shrinking. Phosphorus deficiency is very common in all our major crops, minimized by fertilizer supplement, which is not a permanent solution to this problem. This problem will increase with time and cause substantial decrease in yields, so it needs to be tackled in time. For sustainable agriculture and for the sake of future generations, we need to retain potential sources and develop varieties with increased phosphorus use efficiency.

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Chapter 17
Recent Trends in Genome Editing Technologies for Agricultural Crop Improvement

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Introduction

Plants are vital for the existence of living organisms since they are the primary source of food and energy. Increasing world population has threatened the food security as the current food production is not meeting the consumption demand. Over the last few decades, plant breeding efforts have made significant contributions to reduce the severe food insecurity (Javed et al., 2016). However, various plant biologists believed that traditional breeding alone would not be able to meet the food demand that is increasing due to increase in human population (T. Wang et al., 2019b). As a result, agricultural experts realized the significant need to develop new plant breeding strategies, specifically novel plant breeding techniques (NPBTs), that proved highly efficient, affordable, and adaptable in order to enhance production while eliminating negative environmental impacts (Chakraborty et al., 2020). These techniques have resulted in improvements of crop productivity, including enhanced nutrient value and resistance to abiotic and biotic stressors.

Sequence specific nuclease genome editing techniques, such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein9 (Cas9), are important tools for incorporation of new gene functions and plant characteristics. Zinc finger nuclease (ZFN) was the first ever genome editing technique used in genetic engineering in plants (Lloyd et al., 2005). While TALENs have been used to edit the genome of yeast, drosophila, rice, and somatic cells of human being (Joung & Sander, 2013). ZFNs and TALENs, both genome editing techniques, are dependent on protein-DNA-binding specificity, while CRISPR/Cas9 system relies on RNA-guided approach, which proved as the most improved genome editing technique (Ming et al., 2020). Further, CRISPR/Cas9 system is easy to design and is currently inexpensive genome editing technique (H. Kaur et al., 2021). The genome editing procedure of TALENs and ZFNs is more complicated because protein engineering is needed for their construction, and these constraints have made their applications limited in plants (Y. Yu et al., 2022).

There are three basic steps in genome editing procedure:

Firstly, the recognition of target DNA sequence by modified exogenous nuclease that is comprised of nuclease domain and recognition module.

Secondly, the binding of target DNA sequence and modified nuclease creates double-strand breaks (DSB) at or somewhere near the target site.

Lastly, the DSB is repaired by endogenous nonhomologous end joining (NHEJ) or homology-directed repair (HDR) pathways.

CRISPR/Cas9 was emerged in 2012 as a tool of genetic engineering and after that progressively used for bacteria, fungi, plants, and mammalian cell editing (Jinek et al., 2012). CRISPR/Cas9 system has become increasingly popular because this technique entirely focuses on an RNA-guided nuclease system. Basic CRISPR/Cas9 system is comprised of two components, such as RNA-dependent DNA endonuclease, Cas9 protein, and modified single-guided RNA (sgRNA) (Jinek et al., 2012). This means that nuclease activity of a single protein is combined with guided RNA binding and target recognition. CRISPR/Cas9 system can edit multiple target genes simultaneously, which could be significantly helpful for disintegration of specific sequences and formation of knockout mutants where frameshift mutation could not interrupt the normal functioning by deletion of a certain region (Bate et al., 2021). Furthermore, CRISPR/Cas system is a site-directed nuclease or oligonucleotide-directed mutagenesis editing method such as zinc finger nucleases (ZFNs) and TALENs (transcription activator-like effector nucleases) editing methods (X. Huang et al., 2020).

The CRISPR/Cas9 system has been successfully utilized for editing the genome of different plant species like cabbage, brassica, Arabidopsis, rice, wheat, tomato, potato, and banana (Hashimoto et al., 2018; Nakayasu et al., 2018). It has significant applications in plants like enhancement of resistance against biotic and abiotic stress, modification of metabolic pathways, and enhancement of the overall crop production (Wu et al., 2020). The modifications generated by using genome editing technique could be inherited to next generation, which proved that it can be employed for plant research purposes and increased productivity of valuable plants (Ma et al., 2019; Wu et al., 2020). So far, many CRISPR/Cas9 toolboxes have been advanced and generated targeted mutagenesis at distinct genome loci, epigenome editing, transcriptome regulation, and explicit targeted gene/allele replacement of tagging in plants (T. Li et al., 2020a).

This book chapter focused on recent techniques to alter the genome of various crop plants. Though most emphases have been given to improve the cereals, vegetables, and fruits, however some researchers are also focusing on ornamental plants.

Mechanism and Framework of the CRISPR/Cas9 System in Plants

Advances in CRISPR/Cas9 technology have massive potential for precise and convenient genome editing tool for editing the plant genome (Dong et al., 2021). More specifically, the CRISPER/Cas9 as means for genome editing has appeared as an efficient focused alteration in various cereal crops, vegetables, fruits, and ornamental plants. Moreover, the agility of CRISPR/Cas9 genome editing is influenced by a variety of factors, including promoter sequence, RNA polymerase uniqueness, vector types, Cas9 expression, and guide RNA expression (H. Kaur et al., 2021). On the basis of artificially increased genetic diversity or directed evolution, the CRISPR/

Cas9 system can develop substantial genetic variants (Dong et al., 2021). It has many advantages over TALENs and ZFNs regarding design, easiness, and cost. For instance, CRISPR/Cas9 and Cas12a, which were adapted from various bacterial immune systems, are the CRISPR–Cas system's groundbreaking RNA-directed DNA endonuclease (Ahn et al., 2020; Dong et al., 2021).

CRISPR/Cas9-based genome editing in plants generally involves four stages:

- First, a gene-specific sgRNA is designed and constructed.
- Second, the sgRNA activity is primarily validated in protoplasts prior to application.
- Third, CRISPR/Cas9 system components are conveyed into plant cells (using Agrobacterium-mediated transformation or particle bombardment), followed by integration and expression of sgRNA into the plant genome.
- Finally, targeted modifications in the newly transformed or regenerated plants are assessed via polymerase chain reaction (PCR) genotyping and sequencing.

The benefit of this system is that functional products (both Cas protein and the gRNAs and) can be delivered directly into the plant cells. This implies that both sgRNAs and Cas proteins can be transcribed and synthesized in vitro before being delivered directly via protoplast transformation and particle bombardment -mediated transformation (T. Wang et al., 2019b).

Recent Advances in Genome Editing Technologies

Multiplex Genome Editing

The CRISPR/Cas9 system has been successfully used in all kinds of plant species, enabling for the application of the technology's multiple benefits to molecular genetic investigations in a variety of plant species (Cheng et al., 2013). Using the CRISPR/Cas9 system, many loci can be modified all at once by multiple or single target-specific gRNA(s) (Cheng et al., 2013). Multiplexed CRISPR technologies enable for the simultaneous expression of several gRNAs or Cas enzymes that enabled strong biological engineering applications and greatly extending the breadth and efficiency of genetic editing and transcriptional control (R. Li et al., 2018a). Thousands of researchers have used CRISPR/Cas9 technologies in recent years to alter, or transcriptionally control, specific genomic loci based on sequence complementarity with specified guide RNAs (gRNAs). As a result, there is an increasing trend in research that employ multiplexed methods for transcriptional regulation or multi locus editing rather than mono-guide approaches (X. Huang et al., 2020). Researchers may construct multilayer genetic trails that regulate cellular activity or modify metabolic pathways by simultaneously editing, activating, and downregulating numerous target genes in vivo by generating multiple gRNAs and a Cas protein (Gander et al., 2017; Lian et al., 2017). A change in one or more amino acids can remove DNA cleavage activity in the Cas9 and Cas12a enzymes, resulting in nuclease-null mutants known as the dCas9 and the dCas12 (Qi et al., 2013). The fusion of dCas enzymes with effector domains allows for transcriptional regulation, such as CRISPR-mediated activation (CRISPRa) and CRISPR-mediated inhibition (CRISPRi). Multi-targeted gRNAs for CRISPRa and CRISPRi increase the effectiveness of DNA editing and transcriptional control in gene editing (McCarty et al., 2020). For multiplexed CRISPR–Cas technology to be used, it is necessary to express several gRNAs at the same time concurrently (McCarty et al., 2020). Genome editing, transcriptional repression, and transcriptional activation may be conducted in parallel at multiple sites in the genome by including orthologous Cas enzymes (CRISPRa and CRISPRi). In the future, rather than the typical upregulation and downregulation of gene expression, a better, mechanistic knowledge of gRNA:DNA binding could permit extremely accurate control of gene expression. Multiplexed gRNAs in combination with "noncanonical" Cas enzymes, particularly Cas13a, might lead to complement diagnostics for specific bacterial and viral infections (McCarty et al., 2020).

Although with the development of numerous gRNA expression systems for multiplex genome editing in the context of the CRISPR/Cas9 system, optimization of the promoter utilized to produce Cas9 has not yet been extensively tried in various site-directed mutagenesis (Hashimoto et al., 2018). Research on CRISPR/Cas9-mediated genome editing utilizing tissue-specific promoters for Cas9 expression in egg cells, germ cells, or meristematic cells was published, and it was shown that optimizing CRISPR/Cas9 expression in the Arabidopsis genome increased mutagenesis effectiveness (Hashimoto et al., 2018). As a result, using tissue-specific Cas9 promoters might enhance plant cell multiple mutagenesis. We may soon have the unparalleled capacity to regulate, rewrite, and program genomes in a completely predictable fashion, with the help of biologists, chemists, and engineers, as well as quantitative measures (Jakočiūnas et al., 2015) (Fig. 17.1).

Modulation of Metabolite Production

Plants are primely cultivated for food, fodder, flavoring, cosmetics, and pharmaceutical products (Ahmad et al., 2021). Recently, the use of plant secondary metabolites has intensively increased due to their widespread use in drugs and pharmaceutical industry (Ahmad et al., 2021). The modern genome editing approaches in agricultural biotechnology include modifications in the metabolic pathways that are responsible to control the gene of interest (Schröpfer & Flachowsky, 2021). The use of plant secondary metabolites has been preference due to their multi-targeted spectrum and safety profiles. However, due to complexity in bioreactor system, metabolite pathways, and bioprocess optimization, it is still challenging to enhance the production of plant secondary metabolites (Mamun et al., 2015; Marchev et al., 2020). Metabolite engineering has made it possible to transfer genes in plant cells to obtain novel compounds with enhanced production of plant secondary

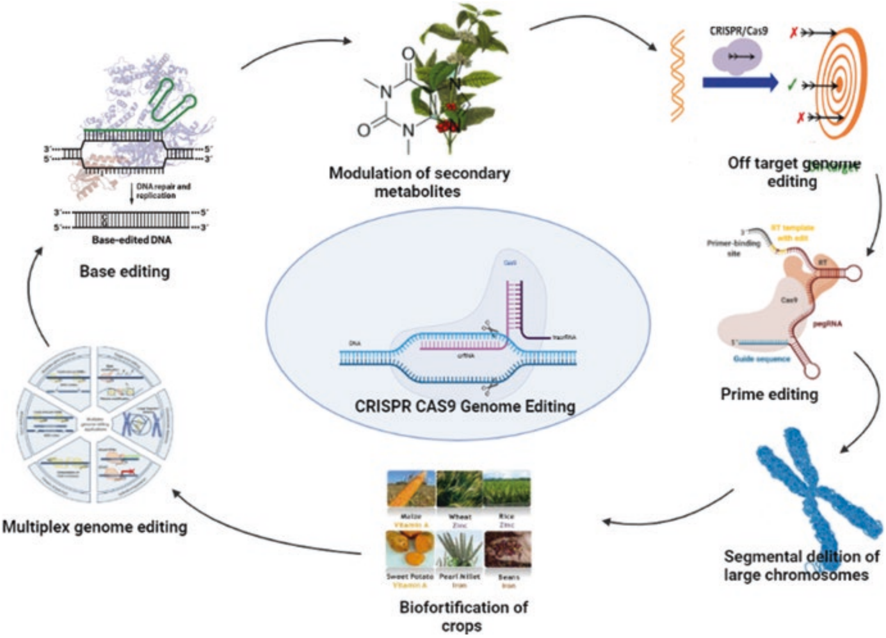

Fig. 17.1 Trending techniques for genome editing of important crops

metabolites (Cloutier et al., 2021). Nowadays, CRISPR/Cas9 technology has been used for production of recombinant proteins and manipulating of plant secondary metabolites (Cloutier et al., 2021) through modification of genes and targeted mutations to regulate the biosynthesis pathways of plant secondary metabolites (Mercx et al., 2017). Plant secondary metabolites (PSM) such as B-carotene have been successfully enhanced in tomato, cassava, and soybean by implementing the CRISPR/Cas9 technology (Jha & Warkentin, 2020; Lian et al., 2017). Further, it was observed that production of human toxic compound has been successfully regulated in tobacco plants. A gene *SIMYB12* affects the flavonoid accumulation and color in tomato fruit. *Pectate lyase (PL)* synthesis genes manipulated through CRISPR/Cas9 have enhanced the shelf life and fruit firmness of tomato fruit without alteration of other ripening traits (Uluisik et al., 2016).

Biofortification of Crops

Globally, more than two billion people are affected by hidden hunger (micronutrient malnutrition). To ensure human good health, biofortification is considered as the one of the mechanisms to enrich crops with key nutrients. It is contrasted to adding nutrients to food during processing (Elbasiouny et al., 2022). Biofortified crops have been created mainly through conventional breeding as well as genetic engineering or through mineral fertilizer application (nano-biofortification), although that has yet to be fully approved and released to farmers. Biofortification through the use of genetic engineering can facilitate high levels of micronutrient accumulation and is not limited by variation in currently offered germplasm (El-Ramady et al., 2021). Biofortified crops via conventionally breeding can contribute from 20% to ≥100% of additional estimated average requirement (EAR), and simultaneous increase of various micronutrients is also possible by combining conventional breeding with genetic engineering (El-Ramady et al., 2021). Genetic engineering, particularly the use of CRISPR based genome-editing technique, is regarded as a promising tool for disseminating biofortified crop production. Moreover, CRISPR technology can target multiplex genetic alteration while also being fast and DNA/transgene-free (McCarty et al., 2020). CRISPR/Cas9 has been used in biofortification of field crops such as rice and wheat, and more recently, the technology is being used in legumes such as cowpeas (*Vigna unguiculata*) (Jha & Warkentin, 2020). In cowpea, CRISPR/Cas9 technology was effectively used to suppress the activation of symbiotic nitrogen fixation (*SNF*) gene (Ji et al., 2019). Thus, tailored guide RNAs (gRNAs) intended to target symbiosis receptor-like kinase (SYMRK) had a mutagenesis effectiveness of roughly 67% in the hairy root of transformed cowpeas (Ji et al., 2019). Consequently, nodule development was totally suppressed in mutants with both alleles impacted. In short, Agrobacterium rhizo-genes K599-mediated hairy root transformation inactivated the symbiosis receptor-like kinase gene. Similar to this, a CRISPR/Cas9-based approach was used to modify the lycopene epsilon-cyclase gene to produce a provitamin-A (β-carotene) to improve nutritional quality of banana (*Musa acuminata*) (H. Kaur et al., 2021). Because of its parthenocarpy nature and triploid genome, genetic improvement in banana has always been a tough task. Thus, metabolic analysis of modified lines' fruit pulp indicated a sixfold increase in B-carotene concentration (24 g/g) as compared to unedited banana cultivars. Similar technique was applied to mutate the *CCD8* gene in tomato (Bari et al., 2019). The carotenoid cleavage dioxygenase 8 (*CCD8*) gene is often involved in the synthesis of strigolactones (SLs). SLs are plant hormones that render tomatoes more sensitive to parasitic weeds such as broomrapes (*Phelipanche aegyptiaca* and *Orobanche* spp.), which are notorious agricultural pests (Bari et al., 2019). This study found that CRISPR/Cas9-mediated mutagenesis or *CCD8* gene deletion may be exploited to generate host tolerance to parasite weeds (Bari et al., 2019). CRISPR-based technologies provide a novel approach to reducing the most common cause of nutritional insufficiency. The robustness of CRISPR-based genome editing has demonstrated remarkable promise for biofertilizers in agricultural crops, and other crops should be exploited and explored using the same technique soon.

Off-Target Effects of CRISPR

CRISPR-based gene editing technology has enable the plant biologist to insert or delete the gene of interest; however, this technique has disadvantage due to its off-target effects (Naeem et al., 2020). Mismatches between targeted sequences and 20 nucleotides of guided RNA may lead to off-target effect. Off-target effects commonly occur due to homology among the targeted loci and in genome other than target loci (Naeem et al., 2020). By incorporating short repeats of viral DNA into the bacterial genome, the natural CRISPR system provides virus defense (Jinek et al., 2012). Off-target effects usually lead to modify genetic functions and lethal mutations in organisms. Off-target effect of gene editing can be reduced by using in silico approaches. Various tools such as CasOT, CasOFFinder, Crisflash, CRISTA, and deepCRISPR have been developed to detect and evaluate the efficiency of gRNA (Naeem et al., 2020). Off-target effect can be minimized by optimizing GC contents such as GC content between 40% and 60%, which enhances the on-target activities. Length of gRNA is also an important tool to reduce the off-target effect (Ren et al., 2014). Various researchers reported that gRNA length up to 17 bp improves the gene editing efficiency, whereas it decreases as the length increases (18–20 bp). Further chemical modification of gRNA by incorporating 2′-O-methyl-3′-phosphonoacetate leads to improve the on-target efficiency (Ryan et al., 2018). Selecting the appropriate Cas9 variants also improve the on-target efficiency of gRNA (Ren et al., 2014). Few selective variants such as eSpCas9, HypaCas9, SpCas9-HF1, and CjCas9 showed high on target efficiency with minimum off-target effects (Karlson et al., 2021). Further off-target effects of CRISPR-based gene editing may be diminished by applying appropriate methods for rapid degradation of CRISPR components in plant cells. Research proved that extended incubation period of CRISPR/Cas proteins in cells increases the chances of off-target binding. Direct delivery of Cas9 and sgRNA through ribonucleoproteins (RNPs) reduces the off-targets in plant cells (Karlson et al., 2021). Another study revealed that RNPs were instantly degraded by targeting the *CCR5* genes and showed lower off-target effects as compared to plasmid transformation (Bae et al., 2014). In *Hardium vulgar* and *Brassica oleracea*, two genes *HvPM19* and *BolC.GA4.a* were edited by targeted mutation to study their off-target effects, and in both plant species, off-target effects were reported (Lawrenson et al., 2015).

Production of Transgene Free Genome-Edited Crops

Genetic engineering is commonly utilized in plant species to produce and analyze mutations in plant species. However, genetic modification in plant genomes presents serious legal problems about genetically engineered species. Several ways for removing or preventing the integration of gene editor constructions have been devised, which may be grouped into three primary categories: (a) genetic segregation to eliminate transgenic sequences, (b) temporary editor expression from DNA vectors, and (c) DNA-independent editor delivery, incorporating RNA or pre-assembled Cas9 protein-gRNA ribonucleoproteins (RNPs). The most extensively utilized method for delivering CRISPR/Cas9 components into dicotyledonous plant cells is stable genomic integration of expression units via Agrobacterium-mediated transformation (Bánfalvi et al., 2020). When working with sexually propagated plants, such as tomatoes, the transfer DNA (T-DNA) can be deleted in the following generations due to Mendelian segregation, resulting in altered but transgene-free plants (Bánfalvi et al., 2020). This method, however, cannot be applied to vegetatively propagated and/or highly heterozygous plants, such as potatoes, since sexual reproduction would result in the loss of favorable features (Bánfalvi et al., 2020). To get around, these constraints recently devised a labor-intensive technique based on Agrobacterium-mediated transformation of CRISPR/Cas9 reagents followed by a high-throughput screening strategy in tetraploid tobacco, resulting in transgene-free mutants without selective pressure (Chen et al., 2018). Regeneration from protoplasts has lately been demonstrated to cause significant genomic instability in the tetraploid potato, aching the possibilities for using protoplasts in genetic modification in this species (Malnoy et al., 2016). It is critical to determine whether this condition can be alleviated by employing regeneration from Agrobacterium-transformed explants (H. Kim et al., 2017). Recent techniques used for transgene-free genome editing include geminivirus-mediated gene editing, CRISPR/Cas9 ribonucleoprotein delivery, delivery of CRISPR/Cas9 protein through agrobacterium, and PEG-mediated protoplast transformation (Veillet et al., 2019b). Further transgene-free genome editing has been achieved through virally delivered CRISPR Cas9 proteins (Yu Zhang et al., 2020c). In a recent study in tomato and potato plants, acetolactate synthase (ALS) gene was transformed by Agrobacterium-mediated transformation through cytidine base editors (CBE) by Veillet et al. (2019b). In melon, agrobacterium and PEG-mediated transformation of *phytoene desaturase* (*CmPDS*) gene was successfully achieved using CRISPR/Cas9 system to produce the albino phenotypes (Hooghvorst et al., 2019). Agrobacterium transformation technique was used to insert the *acetolactate synthase1* (*ALS1*) gene in potato using geminivirus and developed the reduced herbicide susceptible species (Butler et al., 2016). *Phytoene desaturase* (*PDS*) gene that is responsible for carotenoid biosynthesis showed its expression in tobacco plants when transformed through agrobacterium (Chen et al., 2018). Transformation of PEG-mediated CRISPR/Cas9 nucleoprotein system in banana was achieved by N. Kaur et al. (2018). CRISPR-based transgene-free edited *DsRED* genes have been identified in rice, tomato, and Arabidopsis (Aliaga-Franco et al., 2019). Maize agrobacterium delivered the CRISPR/Cas9 system with high efficiency to create the targeted mutagenesis in *ZmAgo18a* and *ZmAgo18b* genes (Char et al., 2017).

Another method to edit the genome without double-strand break (DSB) is prime editing, which uses prime editing guide RNA (pegRNA) and reverse transcriptase fused to nCas9 (Tang et al., 2020). pegRNA contains guide sequence that recognizes the target cites and can reverse the transcriptase template (Karlson et al., 2021). Prime editing technique was successfully adopted to induce point mutation in rice plants (Lin et al., 2020; Tang et al., 2020).

Discovery of New Cas Proteins in Addition to Cas9

The current CRISPR/Cas9 systems were discovered in archaea, and bacteria have some drawbacks such as mismatches of gRNA, which reduce the editing efficiency and off-targets. So, researchers discovered the other Cas proteins to improve the editing efficiency with minimum off-target nicking. Refined Cas9 proteins include *Streptococcus pyogenes CAS9* (SpCas9), Dead Cas9, *Staphylococcus aureus* Cas9 (SaCas9), FokI Cas9, plant codon-optimized Cas9 (pcoCas9), *Brevibacillus laterosporus* Cas9 (BlatCas9), *Francisella novicida* Cas12a (FnCas12a), and *Leptotrichia shahii* Cas13a (LshCas13a) (Tussipkan & Manabayeva, 2021; Veillet et al., 2019b). Cas9 proteins require transactivating crRNA to deliver the targeted genes. Hence, further refinement was achieved by discovering the class two CRISPR/Cas12 system, which was comprised of Cas12a-Cas12b. This CRISPR system has advantage on Cas9 because it requires only CRISPR RNA (crRNA) instead of transactivating crRNA (Lee et al., 2019). Further Cas12a-Cas12b have tendency to process multiple crRNAs and ease the multiple genome editing (Makarova et al., 2018). Other advantage of Cas12a-Cas12b on cas9 is its widespread dsDNA nicking and off-target effect as well as complementary genome editing tool (Ming et al., 2020; Murugan et al., 2020). CRISPR/LbCas12a-based genome editing has been successfully achieved for temperature-tolerant plants (Schindele & Puchta, 2020). In rice, *Phytoene desaturase* (*PDS*) gene was edited, and compared with the editing efficiency of Cas9 with Ca12a, it was observed that Cas12a showed higher targeted mutagenesis frequency than Cas9 (Banakar et al., 2020). Research proved that genome editing in plants is temperature sensitive and affects the editing efficiency of Cas proteins. For detecting the editing efficiency, three Cas12a proteins such as AsCAS12a, LbCas12a, and FnCas12a in rice were noted, and that editing efficiency of these Cas proteins was high at 28 °C and reduced with the increase in temperature (Malzahn et al., 2019). Despite DNA cleavage now, another CRISPR protein called Cas13 has been discovered that target and cleavage the RNA of eukaryotes (Aman et al., 2018). Cas13 can identify pathogens and mutations, repair them, edit full-length transcripts, and delete the nucleotides (Granados-Riveron & Aquino-Jarquin, 2018). Another advancement in discovery of Cas enzyme is CRISPR/Cas14a, which is used for single-stranded DNA (ssDNA) cleavage, deletion of nucleic acid, and impart plant defense. CRISPR/cas14a is used for editing and cleavage the ssDNA; thus this Cas enzyme is useful to generate resistant crops against ssDNA viruses (Khan et al., 2019). Cas14a has been discovered and is used to optimize the nucleic acid deletion and impart microbial immunity and plant defense mechanism (He et al., 2021; Makarova et al., 2018).

Base Editing

Base editing is a recent technique that allows the conversion of one DNA base to another DNA base without breaking the DNA double strand. The interaction of a single-guide RNA molecule (sgRNA) with the nCas9/deaminase fusion drives the complex to the target locus and allows deamination on the noncomplementary strand (Kumlehn et al., 2018). There are two basic types of base editors, namely, adenine base editor and cytosine base editors. Adenine base editor allows the conversion of A-T to G-C by fusion of nickase (nCas9), whereas cytosine base editor with nCas9 can covert the A-T base pair to C-C base pair (Shimatani et al., 2017). But the drawback of these base editors was that it can only have one transition. So advance base editors have been discovered that are categorized according to their uses, such as dual base editors, multiplex base editors, and transversion base editors (Azameti & Dauda, 2021). Dual base editors have the tendency to convert the G:C to A:T and A:T to G:C simultaneously (Azameti & Dauda, 2021; Qin et al., 2020). In cotton, base editor was used to convert C.G to T.A through modify CRISPR/Cas9 system (Qin et al., 2020). Scientist have discovered a system "simultaneous and wide editing induced by a single system," which can simultaneously edit multiplex bases in crop plants. Multiplex base editing has been successfully adopted in rice improvement (C. Li et al., 2020b). Another type of base editors known as transversion base editors can convert C to A and C to G (Zhao et al., 2021). Transversion base editors have been successfully used in human cell impairment; however, there is no evident of these base editors in plants yet (Zhao et al., 2021). Base editors significantly reduce the off-target effects and can be used to develop the superior agriculture crops (Molla & Yang, 2019). Cytidine base editors have been employed effectively in rice, tomato, and watermelon (Shimatani et al., 2017). In potato, cytosine base editor was used to engineer the granule-bound starch synthase genes *StGBSSI and StDMR6-1* by conversion of C to T and transversion of C to G in potato (Veillet et al., 2019a). Herbicide-resistant gene *OsALS1* was engineered in rice through base editing to develop a herbicide-resistant cultivars (Kuang et al., 2020).

Chromosomal Engineering

CRISPR/Cas9-based gene editing technology enables the plant biologist to delete the large intervening chromosomal sequences. Deletion of large segmental chromosomal part is helpful for the study of noncoding RNAs and gene clusters. Chromosomal deletion or insertion is also useful for improving the crop traits. Recently, two techniques, that is, CRISPR-mediated chromosomal deletion (CRISPR-PCD) and CRISPR-mediated chromosomal replacement (CRISPR-PCRep), were used for chromosomal deletion and replacement in *Saccharomyces cerevisiae* (Easmin et al., 2020). Further, it is was reported that CRISPR-PCD has the tendency to delete the two internal or terminal chromosomal regions simultaneously during a single transformation whereas CRISPR-PCRep can repair three chromosomal regions at the same time (Easmin et al., 2020). CRISPR/Cas9 technology has implemented successfully for chromosomal deletion in yeast, apple, and tobacco (Easmin et al., 2020; Schröpfer & Flachowsky, 2021). In another study, large fragmental deletion was achieved for *CBF1*, *CBF2*, and *CBF3* genes in Arabidopsis (Zhou et al., 2014). *Sugar efflux transporter (SET)* genes were engineered by deletion of 115–245 kb chromosomal regions in rice (Zhou et al., 2014). Replacement and insertion of intron-targeted gene rice was achieved through CRISPR/cas9. In oil palm *(Elaeis guineensis)*, *brassinosteroid-insensitive 1* (*EgBRI1*) and *phytoene desaturase* (*EgPDS*) genes were edited through nucleotide substitution to produce the stunted phenotypes (Yeap et al., 2021). In carrot, anthocyanin biosynthesis gene *flavanone-3-hydroxylase (F3H)* was inserted by deletion of large chromosomal fragment 1,160,119 kb mediated by gRNA (Klimek-Chodacka et al., 2018).

Role of Gene Editing for Crop Improvements

Genetic variation is being used to study the metabolic pathways, physiology, development, and plant interactions with pathogens, and most GM varieties have been obtained through Agrobacterium tumefaciens and biolistic methods (Yeap et al., 2021). Various studies have been reported regarding the use of CRISPR/Cas9 system in genome editing of important agriculture crops such as barley, rice, maize, sorghum, cotton, and wheat (Jiang et al., 2013; Tussipkan & Manabayeva, 2021). In maize, four genes, acetolactate genes (*ALS1 and ALS2*), male fertility genes (*MS26* and *MS45*), and liguleless-1 gene (*LIG*) were successfully edited to create the target specific mutation using CRISPR/Cas9 (Svitashev et al., 2015). In wheat, powdery mildew and drought stress resistance were achieved by editing the *TaEDR1-A, TaEDR1-B, TaEDR1-C, and TaDREB2* genes, respectively, through CRISPR/Cas9 (D. Kim et al., 2018; Yunwei Zhang et al., 2017). In rice, *OsABA8ox2, OsSRL1, ABA catabolic, and OsSRL2* genes were engineered through gene editing technology to achieve the drought tolerance cultivars (Liao et al., 2019; Yan Zhang et al., 2020b).

CRISPR-based gene editing has been successfully adopted in fruits and plants like citrus (Jia et al., 2019), banana (N. Kaur et al., 2018), apple (Charrier et al., 2019; Malnoy et al., 2016; Schröpfer & Flachowsky, 2021), grapes (Nakajima et al., 2017), kiwifruit (Tello-Ruiz et al., 2018), coffee (Breitler et al., 2018), cacao (Fister et al., 2018), pomegranate (Chang et al., 2019), walnuts (Walawage et al., 2019), oil palm (Yeap et al., 2021), and pear (Charrier et al., 2019). CRISPR/Cas9-based genome editing has successfully used to devolve disease-resistant horticulture crops. This technology was used to develop canker-resistant citrus cultivar by targeted disruption of *CsLOB1* gene (Jia et al., 2019). Powdery mildew-resistant tomato cultivars were developed by targeted modification of tomato *SlMLO1* gene

(Nekrasov et al., 2017). CRISPR/Cas9 technology can be adopted to control the viral diseases and development of herbicide-resistant plants. CRISPER/Cas9-based virus resistance tomato and tobacco pants have been developed by targeting the viral genomic sequences (Baltes et al., 2015). ZFN-based genome editing technology was used to induce mutation in tobacco *ALS* genes to develop the herbicide-resistant plants (Townsend et al., 2009). In potato, CRISPR/Cas9-mediated technology was utilized to engineer potato leaf blight genes *StDMR6–1 StDND1, and StCHL1* (Kieu et al., 2021).

In addition to cereals, fruits, and vegetables, some ornamental plants such as rose (*Rosa hybrida*), carnation (*Dianthus caryophyllus*), petunia (*Petunia hybrida*), and chrysanthemum (*Chrysanthemum morifolium*) were used in genetic engineering (Boutigny et al., 2020). *Petunia hybrida* was the early ornamental plant to be genetically edited using the CRISPR/Cas9 system, according to Ahn et al. (2020). Currently, several genes in *Petunia* have been targeted, including phytoene desaturase (*PhPDS*), 1-aminocyclopropane-1-carboxylic acid (*PhACOs*) (*PhACO1, PhACO2,* and *PhACO3*), and nitrate reductase (*PhNR*), which are responsible for the following gene functions: albino phenotype, petal senescence, and nitrate assimilation deficiency respectfully. S-locus F-box-interacting SKP1-like 1 (*PiSSK1*), the gene responsible for self-incompatibility in *Petunia inflate*, was aimed by researchers (Boutigny et al., 2020). Another popular ornamental plant in the scientific community is *Ipomoea nil*, which is primarily targeted for the following genes: dihydroflavonol-4-reductase (*InDFR*), carotenoid cleavage dioxygenase 4 (*InCCD4*), and EPHEMERAL1 (*EPH1*), which are responsible for anthocyanin biosynthesis and white flowers, altered petal color, and petal senescence respectfully (Watanabe et al., 2018). The genes, cinnamate 4-hydroxylase (*C4H*), coumarate 3-hydroxylase (*C3H*), cinnamoyl coenzyme A reductase (*CCR*), 4-coumarate/coenzyme A ligase (*4CL*), and irregular xylem 5 (*IRX*) involved in lignocellulose biosynthesis were targeted in *Dendrobium officinale* (Ding et al., 2013; Sawettalake et al., 2017). The phytoene desaturase (*LpPDS*) gene, which is responsible for the albino phenotype in *Lilium longiflorum* and *Lilium pumilum*, was purposed (Ahn et al., 2020). The *MADS* gene, which is responsible for floral initiation and development in *Phalaenopsis equestris*, was also aimed using the CRISPR/Cas9 editing tool. Ultimately, in *Torenia fournieri*, the flavanone 3-hydroxylase (*F3H*) gene, which is responsible for flavonoid biosynthesis, was also targeted by researchers (Ahn et al., 2020). Furthermore, a deletion mutant of the red clover (*Trifolium pratense*) is isoflavone synthase (*IFS1*) gene, which plays a defense role in the rhizosphere, by using CRISPR/Cas9 (Dinkins et al., 2021). Flower color modification was achieved in *Petunia* by performing site-specific mutagenesis targeting both *F3HA* and *F3HB* genes simultaneously via Cas9-ribonucleoproteins delivery (J. Yu et al., 2021) (Table 17.1).

Table 17.1 Traits improved through genome editing in important agricultural crops

Plant name	Trait improved	Gene involved	Gene editing technology	Reference
Rice	Carotin biosynthesis	*Phytoene desaturase (PDS)*	CRISPR/Cas9 CRISPR/Cas12a	Banakar et al. (2020)
Rice	Rice blast resistance	*OsERF922*	CRISPR/Cas9	F. Wang et al. (2016)
Rice	Abiotic stress tolerance	*OsNCED3*	CRISPR/Cas9	Y. Huang et al. (2018)
Rice	Drought tolerance by rolling leaves	*OsSRL1, OsSRL2*	CRISPR/Cas9	Liao et al. (2019)
Rice	Vertical root growth and reduce ABA degradation	*OsABA8ox2*	CRISPR/Cas9	Yan Zhang et al. (2020b)
Wheat	Drought tolerance resistance	*TaERF3, TaDREB2*	CRISPR/Cas9	D. Kim et al. (2018)
Wheat	Powdery mildew resistance	*TaEDR1-A, TaEDR1-B, TaEDR1-C*	CRISPR/Cas9, TALEN	Yunwei Zhang et al. (2017)
Chickpea	Drought stress tolerance	*Ca4CL, CaRVE1*	CRISPR/Cas9	Badhan et al. (2021)
Soyabean	Delay flowering time	*GmFT2a*	CRISPR/Cas9	Cai et al. (2018)
Cabbage	Male-sterile cabbage mutant	*BoMS1*	CRISPR/Cas9	Ma et al. (2019)
Cabbage	Allen oxide/jasmonic acid biosynthesis gene	*BoAOS*	CRISPR/Cas9	Ma et al. (2019)
Cabbage	Jasmonic acid regulated cabbage male-sterile line	*BoSRK3*	CRISPR/Cas9	Ma et al. (2019)
Cabbage	System verification	*BnALS, BnPDS*	CRISPR/Cas9	Kang et al. (2018)
Cabbage	Herbicide resistance	*BnALS1, BnALS3*	CRISPR/Cas9	Wu et al. (2020)
Tomato	Generating photobleached phenotype	*slyPDS*	CRISPR/Cas9	R. Li et al. (2018a)
Tomato	Manipulation of the gamma-aminobutyric acid (GABA)	*GABA-TP1, GABA-TP2, GABA-TP3, CAT9, SSADH*	CRISPR/Cas9	R. Li et al. (2018a)
Tomato	Enriched lycopene tomato fruit	*SGR1, LCY-E, Blc, LCY-B1, LCY-B2*	CRISPR/Cas9	X. Li et al. (2018b)
Tomato	Fruit shape	*Lycopene beta-cyclase (LBC)*	CRISPR/Cas9	Zsögön et al. (2018)
Banana	Phytoene desaturase (PDS)	*RAS-PDS1, RAS-PDS2*	CRISPR/Cas9	N. Kaur et al. (2018)
Apple	Albino shoots	*MdPDS*	Cas12a	Schröpfer and Flachowsky (2021)

(continued)

Table 17.1 (continued)

Plant name	Trait improved	Gene involved	Gene editing technology	Reference
Apple	Disease resistance	*DIPM-1, DIPM-2, DIPM-4*	CRISPR/Cas9	Malnoy et al. (2016)
Oil palm	Chimeric albino phenotypes	*EgPDS*	CRISPR/Cas9	Yeap et al. (2021)
Oil palm	Chimeric albino phenotypes	*EgBRI1*	CRISPR/Cas9	Yeap et al. (2021)
Melon	Albino phenotypes	*CmPDS*	CRISPR/Cas9	Hooghvorst et al. (2019)
Watermelon	*Fusarium oxysporum* resistance	*ClPSK1*	CRISPR/Cas9	M. Zhang et al. (2020a)
Turnip	Turnip mosaic virus resistance	*TuMV-GFP*	LwaCas13a	Y. Yu et al. (2022)
Cucumber	Cucumber mosaic virus resistance	*CMV-RNase3*	LwaCas13a	Y. Yu et al. (2022)
Cucumber	Virus resistance	*eIF4E*	CRISPR/Cas9	Chandrasekaran et al. (2016)
Citrus	Canker susceptibility gene	*CsLOB1*	CRISPR-LbCas12a	Jia et al. (2019)
Potato	Carotenoid biosynthesis	*Phytoene desaturase (PDS)*	CRISPR/Cas9	Bánfalvi et al. (2020)
Potato	Cytidine base editor	*Acetolactate synthase (ALS)*	CRISPR/Cas9	Veillet et al. (2019b)
Potato	Sweet potato virus disease	*SPCSV-RNase3*	RfxCas13d	Y. Yu et al. (2022)
Potato	Reduce steroidal glycoalkaloids	*St16DOX*	CRISPR/Cas9	Nakayasu et al. (2018)
Potato	Polyphenol oxidases (PPOs)	*StPPO2*	CRISPR/Cas9	González et al. (2020)
Potato	Control γ-aminobutyric acid levels	*slyPDS*	CRISPR/Cas9	R. Li et al. (2018a)
Potato	Impaired amylose biosynthesis in potato	*StGBSSI*	CRISPR/Cas9	Veillet et al. (2019a)
Potato	Reduced sugar contents	*VINV*	TALEN	Clasen et al. (2016)
Potato	Synthesis of α-solanine-free hairy roots	*St16DOX*	CRISPR/Cas9	Nakayasu et al. (2018)
Potato	Resistance to potto late blight	*StDMR6-1 StDND1, StCHL1*	CRISPR/Cas9	Kieu et al. (2021)
Sweet potato	Starch biosynthesis genes	*IbGBSSI, IbSBEII*	CRISPR/Cas9	H. Wang et al. (2019a)
Tobacco	Acetolactate synthase genes	*ALS SuRA, ALS SuRB*	ZFN	Townsend et al. (2009)
Carrot	Anthocyanin biosynthesis	*F3H*	CRISPR/Cas9	Klimek-Chodacka et al. (2018)

Conclusion and Outcomes

Agricultural crops are the major source of food, feed, minerals, vitamins, and fiber throughout the world. However, increasing world population and changing climatic conditions desire resistant cultivars with improved production. Existing plant breeding techniques such as mass selection, pedigree method, and backcross method are unable to obtain the desired results as they are totally dependent on existing allelic variations. CRISPR/Cas9 system allows the plant biologists to engineer the desired genes for generation of stress tolerance crops with improved nutritional quality. Chromosomal engineering allows the segmental deletion, insertion, and replacement of desired nucleotides to generate the crop plants with desirable traits. Base editors have prolonged the possibilities of genome editing as they do not require donor DNA or DSB to generate base substitutions. Recent advancements in CRISPR-based genome editing technology for agricultural crops not only allow the manipulation of single genes in the genome, but it can also target the multiple genes simultaneously. Genetic engineering techniques offer a wide range of applications in crops such as biotic and abiotic stress tolerance, improved plant secondary metabolites, and nutritional quality. Several crop plants have been transformed using gene editing technology over the last two decades. In contrast to traditional breeding, gene editing technology enables the transfer of desired traits from plants, or microorganisms into agricultural crops, thereby expanding the crop production. Despite lot of advantages, CRISPR/Cas9 system has some drawbacks such as off-target effects, gene delivery, and designing of appropriate Cas protein, which can be reduced by applying appropriate techniques.

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Chapter 18
Recent Trends and Applications of Omics-Based Knowledge to End Global Food Hunger

Muhammad Tanveer Altaf, Waqas Liaqat, Muhammad Azhar Nadeem, and Faheem Shehzad Baloch

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Introduction

The human population is expected to rise from 6.8 billion to 9.1 billion by 2050. Rapid urbanization and decreasing cultivated lands is forwarding agriculture production system to produce more food to meet the needs of a growing population (Joshi et al., 2016; Kumar et al., 2020). Furthermore, global warming and the unpredictable meteorological fluctuations posed by numerous biotic and abiotic factors have hampered crop development and productivity around the world, resulting in

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billions of dollars in crop losses (Ramegowda & Senthil-Kumar, 2015; Pereira, 2016; Pandey et al., 2017; Dhankher & Foyer, 2018). Plants cannot move like animals and are unable to survive adverse climatic circumstances such as drought, salt, or cold stress. These challenges will rise in the future as the global climate changes, substantially altering the genetic association and leading to alterations in the plant's basic metabolism, which represents its morphology (Gupta et al., 2015). Crop production and other agricultural technologies have made significant contributions to the alleviation of hunger in recent decades.

Fundamentally, we require a food supply system that can meet social demands while also supporting long-term development. As a result, a modern breeding strategy is needed to encourage sustainable development in agriculture, along with new ways of promoting various new varieties and crops with high yield potential, high yield consistency, and remarkable seed quality; however, for environmental reasons, less water, fertilizer, and chemicals should also be considered. Crop development by traditional breeding methods is time-consuming and insufficient to meet global food demands. Therefore, current crop improvement research focuses on OMIC technologies (a branch of biology that ends in omics, such as genomics, phenomics, proteomics, and so on) to maximize the productivity and accuracy of crossbreeding. In recent years, the number of crop improvement programs that use OMIC-assisted breeding approaches has expanded dramatically.

Current Statistics of Major Food Crops and Their Future Demands to Increasing Population

An increase of almost 50% in primary crop production has been recorded between 2000 and 2018 with 9.1 billion tons in 2018, which is 2.9 billion tons more in comparison to 2000. Cereals were the major crops produced in 2018, which contributed almost one-third of the total primary crop production, with sugar crops and vegetables as a second and third group with 24% and 12% contribution, respectively (FAO, 2020). The individual share of fruits, oil crops, tubers, and roots was 9–11% of the total production. Though a huge number of crops are grown worldwide, just four crops (sugarcane, maize, rice, and wheat with 21%, 13%, 9%, and 8%, respectively, of the total production) accounted for almost half of the total production of primary crops globally in 2018 (FAO, 2020). According to FAO estimates, the population of the world is expected to rise by 9 billion by 2050, and to feed such a huge population, 50% more food than the present product will be needed (Cervantes-Godoy et al., 2014). The wide genetic diversity present in plants is needed to be utilized for crop improvement under the shadow of changing climatic events to feed the growing population and ensure food security (Ahmar et al., 2020).

Major Stresses Induced Due to Global Climate Change in Achieving Food Security Globally

Climate change will significantly affect the food system and agriculture sector of the economy more than other sectors (Rosenberg, N., & Lehner, P., 2022). The unprecedented climate change scenerios resulted in droughts, floods, wildfires, heatwaves, pests and diseases, and more, which are threatening heavily agriculture production and resulting in food shortage for the rapidly growing population (Rosenberg, N., & Lehner, P., 2022). Moreover, climate change is also a threat to the food system because it can reduce protein content in staple crops and decrease micronutrients in vegetables (Rosenberg, N., & Lehner, P., 2022). Climate change is a great challenge for the global food security of the increasing population (Rathore et al., 2022). The extreme transitions in climate will reasonably affect the world food production, but a sustainable approach in agriculture can help reduce the drastic role of changing climate in agriculture (Rathore et al., 2022).

Drought and Temperature

Drought is the major abiotic stress contributing a major share in negatively affecting global agriculture and food production (Farooq et al., 2009). Climate change brings extreme weather conditions, which in turn will jeopardize agricultural production (Troy et al., 2015). Drought has almost a share of one-fifth of the losses caused by natural hazards worldwide (Wilhite, 2000). The occurrence of drought is more likely to rise in the future, specifically in semiarid regions (Achour et al., 2020; Zeroual et al., 2020). The developmental needs are increasing with increasing population resulting in deforestation and more usage of fossil fuels producing more greenhouse gases, thus inducing global warming (D. Pimentel, 1991). The increase in temperature due to global warming considerably intensifies the intensity, frequency, and duration of drought (Bouras et al., 2019; Dai & Zhao, 2017). Global warming will more affect the temperature and rainfall pattern (Rathore et al., 2022). Evapotranspiration increases with an increase in temperature (W. R. Cline, 2008), and rises in temperature accelerate plant growth and development but negatively affect the crop yield (Rathore et al., 2022). Globally, the extreme climate changes have induced fluctuation in temperature, which has negatively affected the growth, distribution, and yield of the crops (Raza et al., 2020). Climate change affects agriculture directly and indirectly by changing rainfall patterns, producing floods and drought, and increasing temperature (FAO, 2022). Climate change is limiting the agriculture yield and also deteriorating the quality of major cereals. Extremes in weather conditions are expected to increase with changing climate and will ultimately disturb crop production (Troy et al., 2015). In the last few decades, modern biotechnological approaches like omics (genomics, proteomics, transcriptomics, and metabolomics) have shown to be the most promising and advanced tools to play a key role in crop improvement and global food security (Raza et al., 2021). These promising tools are used to enhance low- and high-temperature stress resistance in various plant species (Tai et al., 2020; Wu et al., 2020).

Omics Introduction

Remarkable progress in the last few decades has been made in the field of genomic and genetic technologies helping us to get more details of the difficult biological system in a very comprehensive and integrative manner (Filipp, 2013; Mewalal et al., 2014) for detail cellular level study. In biotechnology, omics is the advanced procedure having various tools like genomics, proteomics, phenomics, transcriptomics, metabolomics, and physiognomics, and the application of integrated omics-based tools helps in recognizing the cause and effect relationships between phenotype and genotype (Cowie et al., 2013; Kumar et al., 2015). The main components of "omics" technology are outlined in Fig. 18.1.

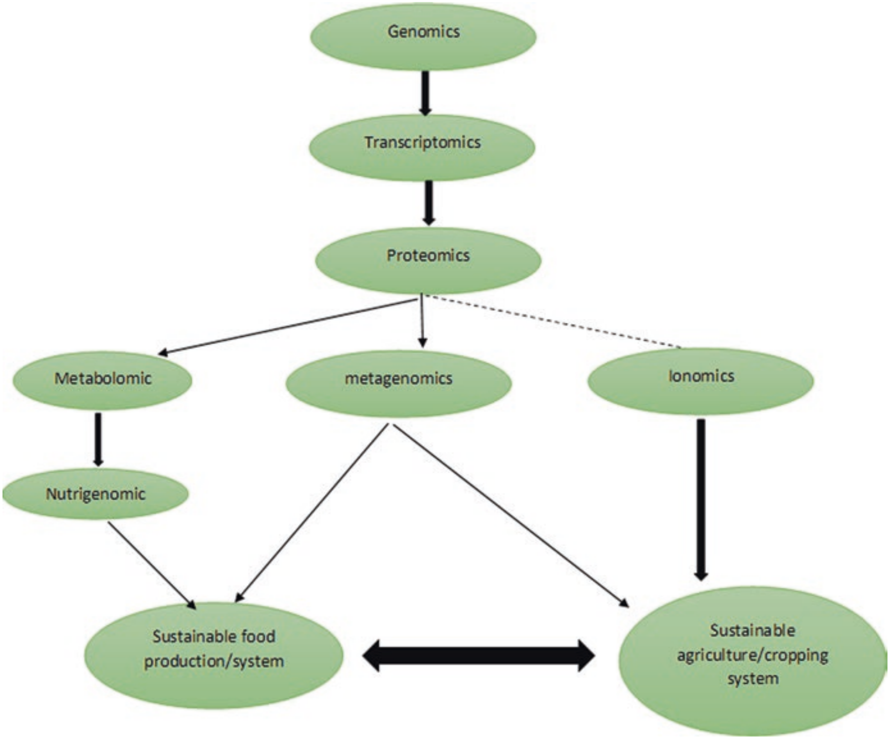

Fig. 18.1 Omics technology and its applicability in food production systems and sustainable agriculture. (Adopted from Benkeblia, N. 2016)

Omics tools are a powerful approach when combined with advanced breeding and molecular techniques (Li & Wen, 2021). Genomics has the potential to improve the efficiency and precision of traditional and advanced breeding procedures. Moreover, genomics and other omics tools like proteomics and epigenomics provide critical information for understanding genes and how they work (Zhang et al., 2008). Omics technologies are a collection of powerful tools having the ability to boost yields in general via increasing net volume production and also through enhancing resilience to biotic and abiotic stresses (Kaur et al., 2021). The combination of proteomics, genomics, and metabolomics helps in selecting the desirable phenotypes. Marker-assisted breeding is more efficient than classical breeding and also reduces the duration of classical breeding by more than half (Sinha et al., 2021). The predictability and consistency in plant breeding have been efficiently improved by using genomics, metabolomics, transcriptomics, and proteomics, and also these tools help in reducing the expense and time to produce good quality crops having resistance to stress without compromising on their nutritional value (Kaur et al., 2021).

Foodomics is an omics terminology that deals with food and nutrition via integrated omics procedures (Ibáñez et al., 2013). It helps in advancing the trend to link food with health and will ultimately play a big role in nutrition and preventing diseases and might help in producing food having medicinal value (García-Cañas et al., 2012). The importance of omics technologies is continuously increasing and will play a tremendous role in all the sectors of food production (Balkir et al., 2021).

In the future, omics-based technology will not only be used for traditional crops but will also be applied to new food resources (Balkir et al., 2021) (Fig. 18.2 and Table 18.1).

Agricultural Limitations and Their Alleviation via Omics Technologies

Overexploitation causes an increase in demand for grain and decreases in prime farmland supply worldwide (Viinanen, 2016). Hence, there is an urgent need of developing such new crops that are optimized for alternative sustainable farming (Viinanen, 2016). Important agronomical traits controlled by genetic loci can be identified with the advent of genome-wide molecular markers, which can be generated by using second-generation sequencing technology for any species. Phenotypic characterization combined with genomic data will allow direct selection for yield traits on a genetic base also linking the genome revolution to the new green revolution (Viinanen, 2016). Latest biotechnologies help the breeders in using natural variations in imperative traits by giving better understandings of physiological restrictions and genetic trade-offs and also inform the possibilities and limitations of plant designing according to the twenty-first century to create a more sustainable cropping system (Viinanen, 2016).

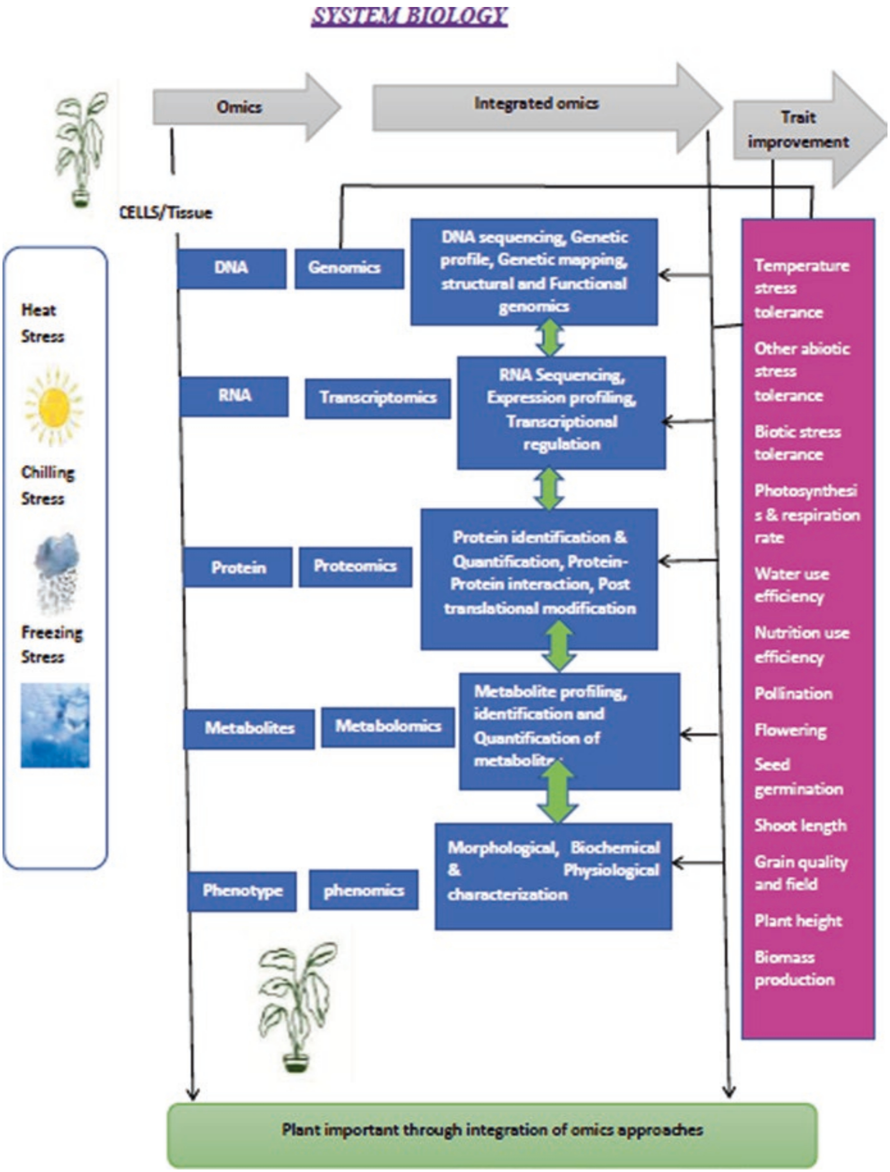

Fig. 18.2 Summary of omics approaches in the biological system contest. (Adopted from Raza et al. 2021)

Rapidly increasing population results in water scarcity, increase in energy demand, environmental destruction, and climate change. All of these factors will impose a tremendous challenge to the future production of agricultural crops (Lamberth et al., 2013; Agrawal et al., 2013; Wortman & Lovell, 2013; Nelson et al., 2014; Haddeland et al., 2014). Food availability is an essential element for food security that is also a key point to eradicating poverty. It is necessary to use

Table 18.1 A brief overview of omics technology for crop enhancement to alleviate food scarcity

Genomics	1. Genomics is a set of disciplines that study the structure, operation, development, characterization, and modification of genomics 2. DNA sequencing-based markers, genetic mapping, and genome-wide selection are all examples of genomics' broad prospects (GWS). Plant biologists and molecular breeders develop varieties and hybrids that are resistant to biotic and abiotic challenges as well as the whims of a fast-changing climate 3. Furthermore, these technologies and strategies can be utilized to increase reliability traits and yield to assure long-term national food security	1. Di-deoxy or chain termination method 2. Shotgun sequencing method 3. Illumina 4. Roche/454 5. SoLid 6. Polonator 7. Helicos 8. SMRT
Transcriptomics	1. The study of transcribed genes, their expression, and functions is known as transcriptomics 2. This method is more flexible and reliable and offers a more accurate measurement of total expression patterns (Fu et al., 2009; Nagalakshmi et al., 2008)	1. DNA microarrays 2. Est 3. Sage 4. Long SAGE 5. MPSS
Proteomics	1. A large-scale description of an organism, cells, or tissues is defined. The proteome survey includes sub-proteomics, total protein extraction methods, and posttranslational modifications (PTMs) 2. Plant proteomics' key goals are to understand more about the physiology and biochemical mechanisms in different plant and crop varieties, as well as how they respond to biotic stress, to generate better and safer crops to achieve food security goals. This strategy encourages the use of sustainable agricultural methods	1. 2D-PAGE (2-D polyacrylamide gel electrophoresis) 2. MS (mass spectrometry) Electron spray ionization
Metabolomics	1. Metabolomics is the examination of an organism's metabolite profile, both qualitative as well as quantitative (Hall, 2006) 2. These approaches, in combination with other technologies such as transcriptomics and proteomics, attempt to provide precise representations of the entire biological process 3. Using metabolomics to identify metabolic markers to study plant metabolism and the nature and evolution of abiotic/biotic stress tolerance could be a future use of the technology (Sharma et al., 2021) 4. Metabolomics-assisted breeding will be utilized to generate high-yielding crop varieties and climate change-adaptable crops (Sharma et al., 2018, 2021; Kumar et al., 2017; Fernie & Schauer, 2009)	1. MS (mass spectrum) 2. Nuclear magnetic resonance (NMR) 3. Fourier-transform infrared spectroscopy (FTIR)

(continued)

Table 18.1 (continued)

Phenomics and physionomics	1. This one is defined as a set of all organisms with multidimensional genomic information (David et al., 2010) 2. It's a fast-growing area of study that aims to characterize phenotype meticulously and accurately, revealing information of concerning genes linked to specific characteristics (Close et al., 2014) 3. Various phenomics approaches, such as short-wavelength imaging, x-ray, hyperspectral imaging, and others, are used to speed up phenotyping and are utilized at numerous steps of the growth and development of plants 4. Physionomics is the application of several omics approaches to functional investigations and plant breeding in various crops. "Phenomics" refers to a close approach to physionomics (Tisné et al., 2013; Furbank & Tester, 2011; Berger et al., 2010)	1. LEAF-E 2. Phenocart 3. Phenovator

SAGE Serial Analysis of Gene Expression, *SMRT* Single Molecule Real-Time, *MPSS* Massive Parallel Signature Sequencing, *EST* Expressed Sequence Tag

omics by identifying QTLs and other key genes controlling grain yield to enhance crop production to fulfill the increasing demand for food through adopting omics-based techniques in the future (Curtis & Halford, 2014; Kumar et al., 2015; Nadolska-Orczyk et al., 2017; Varshney et al., 2018; Wambugu et al., 2018; Xie & Wang, 2018). Through genomics-assisted crop breeding methodologies and genome engineering, genomics-based sciences have the potential to alleviate malnutrition and hunger by developing superior ecologically friendly crops for society (Kumar et al., 2015).

Omics Role in the Present Agriculture

With the latest changes and innovations in omics technologies, organisms are investigated at gene, genome, metabolome, ionome as well as proteome levels on a large scale in a short period at a faster rate (Mosa et al., 2017). According to various studies, many traditional plants are rich in nutrition exhibiting varying degrees of stress tolerance that is why the extension of integrative omics tools is not only important but also urgent to decipher the stress-related traits in traditional plants on a genetic and molecular basis (Muthamilarasan & Prasad, 2021). Recently, genomic sequencing technologies have been applied to some traditional pants giving incredible results that are important for improvement programs in the future (Muthamilarasan et al., 2019; Jamnadass et al., 2020; Li & Yan, 2020; Singh et al., 2020). Omics tools give information to the non-model traditional food crops, which is important, and provide unique opportunities to improve crops in a much shorter time (Hack et al., 2020; Li & Yan, 2020; Singh et al., 2020). Omics technologies are also beneficial in diagnosing and understanding the resistance mechanism of diverse crops against abiotic and biotic stress. These mechanisms rely on the association of molecular dynamics with anatomy, development, biochemistry, evolution, physiology, and genetics.

Genomics covering the genome of an organism provides enough information about physiological, chemical, and biological processes as well as structure, sequence, and functional annotation of the gene (Gilliham et al., 2017; Varshney et al., 2018). Functional genomics aids in identifying genes and their functions involved in stress stimuli (Wang et al., 2018). The gene expression and regulation information along with complex stress-responsive traits contribute to generating climate-resilient crops at a genome-wide level (Gilliham et al., 2017). Online genome data and genomics give a platform for further research by using different approaches such as proteomics, transcriptomics, genome engineering (CRISPR/Cas) system, and metabolomics (Zhang et al., 2018; Shen et al., 2019).

Omics Intervention to Improve Quality of Crops

Omics methods are essential for finding the genes that regulate a desired feature in a crop plant (Van Emon, 2016) and also transformed for crop improvement programs (Benkeblia, 2014). The taste, quality, nutritional composition, and stress resistance of food crops can be enhanced by using information obtained by omics tools. It also enhances the agricultural production for food, feeds energy, and also minimizes the expense and time of production (Van Emon, 2016). These technologies just focus on the specific traits of interest with accuracy. Omics can improve the nutritional aspects of food for the advantage of consumers, such as a tomato high in lycopene, fruit with delayed ripening characteristics, and products with strong antioxidant properties (Ahmad et al., 2012). Omics can improve protein quality, increase mineral and vitamin levels to minimize nutrient deficiency, and reduce allergens and antinutritional substances that diminish the quality of food. Integrating multi-omics technologies can aid in understanding the structural organization, function, and metabolic and biological pathways of genes. It can also help in understanding the regulatory network that governs growth, nutrients, stress response, and other quality traits of plants more effectively that ultimately help in crop improvement programs (Benkelia, 2014) (Fig. 18.3). Some of these crop improvement techniques are mentioned below:

1. Marker-assisted breeding
2. DNA sequencing and genomics-assisted breeding
3. Genome editing
    - (i) DNA-free genome editing (DFGE)
    - (ii) Base editing
    - (iii) Prime editing
4. Phenomics and artificial intelligence (AI)
5. Speed breeding

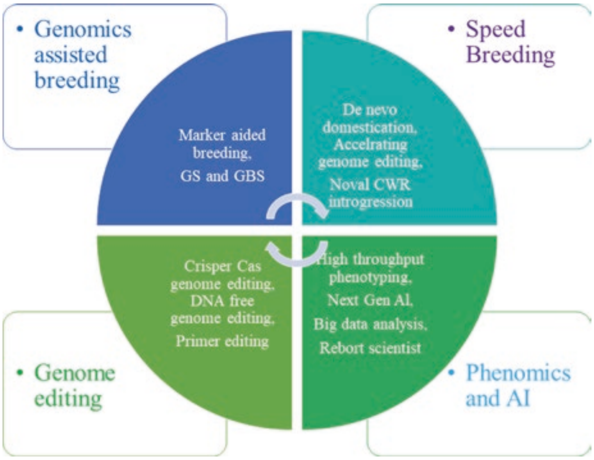

Fig. 18.3 Smart breeding approach for climate resilience in agriculture, combining state-of-the-art genomics, phenomics, and computational techniques. (Adopted from Bakala et al. 2020)

Applications of Omics

The use of omics techniques in plant breeding saves the time, effort, and expense of growing more food crops while also making them more stress-resistant and ensuring that they have higher nutritional benefits. Omics has helped researchers identify the molecular causes of resistance and response to biotic and abiotic stress conditions. It also enables the use of systemic biology approaches to better comprehend the complicated connections among metabolites, genes, and proteins. Omics is dependent on computer analysis, bioinformatics, molecular biology, and other life science disciplines. It is now possible to produce extensive omics datasets for many agronomic traits owing to the rapid improvements in omics technologies. Table 18.2 shows the current state of omics technology for the ‘Top 20’ crop species in worldwide production.

Challenges to Food Security

In 2020, almost 720–811 million people around the world faced hunger. Globally, food insecurity is slowly increasing from 22.6% in 2014 to 26.6% in 2019 (UNICEF, 2021) (Fig. 18.4). Climate extremes and variability possess multiple effects on the global food system by negatively affecting agricultural production. Moreover, climatic variations disturb food imports because every country tries to compensate for the local production losses (UNICEF, 2021). The disasters created due to changing climate can significantly affect the global food system and will have to threaten both the food and nonfood industries (UNICEF, 2021). The Earth is warming, and all the simulation models forecasted an increasing trend in the global temperature (Lean & Rind, 2009). The yield of wheat, maize, and rice might reduce by up to 2% per

Table 18.2 Overview of omics technology's application to the top 20 crop species in worldwide production

Genotype	Scientific name	Genome size and polidy level	EST	Transcriptomics	Proteomics	Metabolomics	References
Sugarcane	*Saccharum Officinarum*	Polyploid, 10 Gbp	>230,000	L		R	Dillon et al. (2007)
Maize	*Zea mays*	Diploid, 2.3 Gbp	>2 M	A	A	A	Schnable et al. (2009)
Rice	*Oryza sativa*	Diploid, 389 Mbp	>1.2 M	A	A	A	IRGSP (2005)
Wheat	*Triticum aestivum*	Hexaploid, 17 Gbp	>1 M	A	R	R	http://wheat.pw.usda.gov/GG2/index.shtml
Potato	*Solanum tuberosum*	Polyploid, 850 Mbp	>230,000	A	A	R	http://potatogeme.net/
Sugar beet	*Beta vulgaris*	Diploid, 758 Mbp	>26,000	A	A		Catusse et al. (2008)
Cassava	*Manihot esculenta*	Diploid, 760 Mbp	>1.5 M		A		http://www.phytozome.net/
Soybean	*Glycine max*	Diploid, 1.1 Gbp	>1.4 M	A	A	A	Schmutz et al. (2010)
Tomato	*Solanum lycopersicum*	Diploid, 950 Mbp	~300,000	A	A	A	http://solgemics.net/gemes/Solanum_lycopersicum/
Barley	*Hordeum vulgare*	Diploid, 5 Gbp	>501,616	A	A	A	Schulte et al. (2009)
Cotton	*Gossypium hirsutum*	Tetraploid, 2.5 Gbp	>260,000	A	A		Van Deynze et al. (2009)
Sweet potatoes	*Ipomoea batatas*	Hexaploid	>22,000	A			Srisuwan et al. (2006)
Watermelon	*Citrullus lanatus*	Diploid, 424 Mbp	~8000	A	R	R	Joobeur et al. (2006)
Banana	*Musa acuminata AAA Group*	Diploid, 600 Mbp	>11,000	A	R		Hippolyte et al. (2010)
Onion	*Allium cepa*	Diploid, 16 Gbp	>20,000				Jakše et al. (2008)
Cabbage	*Brassica rapa subsp. Pekinensis*	Diploid, 529 Mbp	~150,000	A			Kim et al. (2009)
Sorghum	*Sorghum bicolor*	Diploid, 730 Mbp	>200,000	A	R		Paterson et al. (2009)
Apple	*Malus x domestica*	Diploid, 750 Mbp	>320,000	A	R	A	Han and Korban (2008)
Grape	*Vitis vinifera*	Diploid, 487 Mbp	>360,000	A	A	A	Jaillon et al. (2007)
Rapeseed	*Brassica napus*	Tetraploid, 1.1 Gbp	>640,000	A	A		http://brassica.bbsrc.ac.uk/welcome.htm

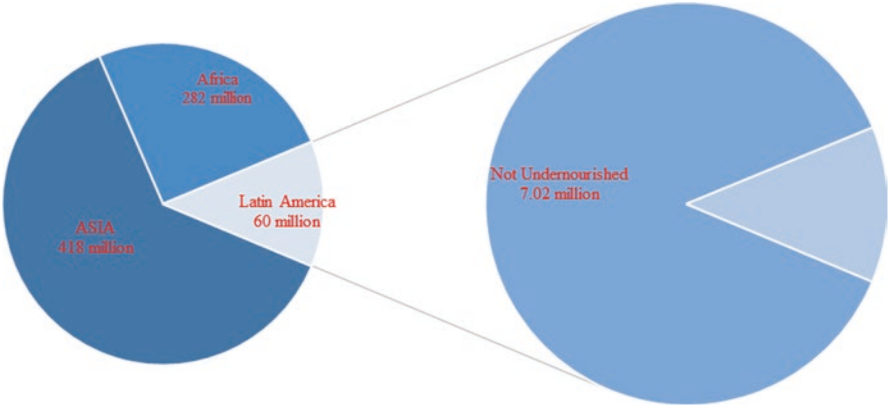

Fig. 18.4 Affected peoples by hunger in 2020 mostly in Asia 418 millions. (Adopted from FAO et al. 2021)

decade from 2030 due to climate change if compared with the estimated yield without climate change (Ray et al., 2019). Two billion extra people will be needed to feed by 2050 when the population of the globe is estimated to reach 9 billion (Ray et al., 2019), and to feed such a population an increase of 70% in the world food production will be required (FAO, 2011). Climate change increased the frequency of floods and droughts leading to decrease agricultural production and increase malnutrition and food insecurity (Schmidhuber & Tubiello, 2007). With each 1 °C rise in temperature, a loss of US$28/hectare/year by African farmers is estimated (Begna, 2022). Competition for land will increase due to more demand for food and, thus, will lead food security and access to safe and adequate food one of the hot topics in public (Rehber, 2012).

Conclusion

OMICS, a collection of high-throughput genome analytical techniques, is extremely beneficial for studying the structural and functional features of plant cellular mechanisms on a global scale, and these technologies are opening up new possibilities in plant genetics, trait characterization, and manipulation. Integrating diverse omics technologies to investigate the regulation of the link between plant metabolism and physiology might also be a good strategy. The potential of genomics, proteomics, transcriptomics, and metabolomics as techniques for crop genetic studies has been recognized, and they are now regularly used to better understand genotype-phenotype interactions, thereby assisting in the improvement of crop plant quality and productivity for the global population's food and nutritional security. To overcome the obstacles, various aspects of omics, such as genomics, metabolomics, proteomics, and transcriptomics, must collaborate with various basic disciplines of genetics, plant physiology, plant breeding, and biochemistry to develop strategies to address the problem- and process-oriented goals, resulting in long-term crop improvement.

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Chapter 19
Nutritional Enhancement in Horticultural Crops by CRISPR/Cas9: Status and Future Prospects

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Introduction

Worldwide, every country is affected by various forms of malnutrition. It occurs when the body does not get enough nutrients from the food. According to WHO report, malnutrition is linked to around 3.5 million deaths of people of which 45% alone account for children. Therefore, nutrient-rich balanced diet is very important for good health and development of people. Several ingredients of food are very important in promoting well-being and health of an individual like vitamins, minerals, antioxidants, aminoacids, fat, and dietary fiber. All these are present in different food groups like pulses, cereals, oilseeds, legumes, fruits, and vegetables. Undernutrition due to vitamin deficiency may cause severe underlying conditions. For instance, vitamin A is one of the essential nutrients for normal growth and vision, maintenance of epithelial cells, and reproduction, and its deficiency leads to blindness, increased risk of dying from infections, anemia, and also impairment of fetus growth in pregnant women. Over 250,000 to 500,000 children lose their eyesight due to lack of enough vitamin A in their daily diet (Boyd et al., 2022). Another important vitamin, vitamin C, is responsible for healthy immune system, and its deficiency weakens the immune system, thereby slowing the wound healing process. Also, 30% of people worldwide are anemic due to the consumption of iron-deficient foods (Kumar et al., 2022).

Fruits and vegetables play crucial role in healthful living. Green leafy vegetables such as spinach, bathua, mint, fenugreek leaves, amaranth, and roots and tubers such as carrots and potatoes are rich sources of vitamins, minerals, proteins, antioxidants, carbohydrates, calcium, and iron (Wan et al., 2021). Currently, fruits and vegetables meet only half of the requirements of different vitamins and minerals. Fortification of such fruits and vegetables by formulation of different value-added products is an important source of nutritional security. Apart from that, different factors such as affordability, life style, dietary habits, accessibility, and bioavailability contribute to nutritional security. With the rapid rise in population, there is a dire need to raise the production of fruits and vegetables, and also concerted research should be directed toward the fortification programs. To reach that stage where sufficient nutrients are available as recommended dietary allowance (RDA), there is a need to improve quality of fruits and vegetables via biofortification and increasing shelf life. Fortification of fruits and vegetables can be achieved by selective breeding program or via some cutting-edge biotechnological approaches (Garg et al., 2018). However, breeding programs are very time-consuming, laborious, and complicated (Jangra et al., 2017, 2018, 2019a, b, 2021a, b; Rani et al., 2021). With an urge to resolve the complexity and surpass the drawbacks of breeding programs, researchers developed the GM technology (Yadav et al., 2018). However, the non-acceptability of the GM technology prompted the researchers to develop various genome editing tools like zinc finger nuclease (ZFNs), meganucleases, transcriptionally activator-like effector nuclease (TALENs), and clustered regularly interspaced small palindromic sequence associated nucleases (CRISPR/Cas, Carroll, 2011; Mahfouz et al., 2011; Li et al., 2012; Gaj et al., 2013; Jangra et al., 2022).

Acceleration in sequencing technologies had provided genomic information on a number of plant species and has created ample opportunities to target desired traits using genome editing for further augmenting the crop improvement program (Priti et al., 2018).

One of the latest versatile and dynamic genome editing tools is CRISPR-Cas9, which has the potential to carry out desirable, precise, efficient, and permanent changes in the target crops. Due to its efficacy, simplicity, and accuracy, CRISPR/Cas9 has become very popular among researchers (Karkute et al., 2017; Corte et al., 2019). CRISPR/Cas9 has improved hybrid-breeding processes, making it easier to eliminate undesirable characteristics or add desired features to elite varieties, allowing crop traits to be accurately modified even within a single generation. As a result, CRISPR/Cas9 has the potential to improve global food security and agriculture sustainability (Rani et al., 2016; Chen et al., 2019). Using CRISPR technology nutritional improvement programs can be sped by introducing precise and predictable mutations (insertion and deletions) directly in horticultural crops. There are several examples of improvement of various key traits like yield, growth improvement, quality, and biotic and abiotic stress tolerance in a wide range of horticultural crops (Fiaz et al., 2019; Zafar et al., 2019; Ahmad et al., 2020; Wang et al., 2019). CRISPR/Cas9 can also be exploited for complete knockout of genes or silencing for the production of antinutrient compounds like lectins, oxalate, and tannins, which reduces the bioavailability of nutrients or can change the taste of fruits (Sinha & Khare, 2017; Sango et al., 2016) such as berries, grapes, and pomegranate (Smeriglio et al., 2017; Lamy et al., 2016). Anthocyanin (Meng et al., 2015), lycopene (Li et al., 2018c), malate (Ye et al., 2017), and gamma-aminobutyric acid (Nonaka et al., 2017) are bioactive compounds. Editing genes regulating the metabolic pathways of these bioactive compounds via CRISPR/Cas9 can increase nutrient levels in horticultural crops.

In this chapter, we provide insight into the CRISPR/Cas9 technology strategy for targeted mutagenesis in plants and recent research done in horticultural crops to enhance shelf life, quality improvement, and biofortification. Further, the possible application of this technology in crop improvement for nutritional security and potential challenges and future prospects are discussed.

CRISPR: A Brief Summary

CRISPR is clustered regularly interspaced short palindromic repeats, and Cas9 is an enzyme encoded by a gene adjacent to CRISPR region. CRISPR has been found in almost half of all sequenced bacterial genomes and all the genomes of archaea. Bacteria and archaea having CRISPR in their genomes depict the evolution of their immune system to resist themselves against viruses known as phages (Jiang & Doudna, 2017). The genetic feature of CRISPR comprises of two alternative stretches of sequences. First one is repeated sequence (23–47 bps) of nucleotides that is already present in bacterial genomes prior to viral infection and is highly

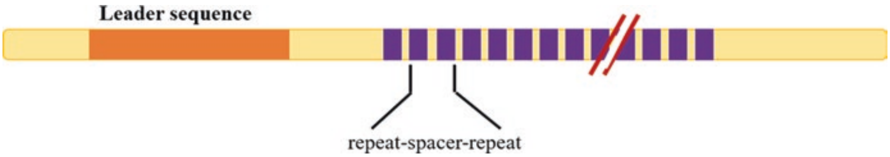

Fig. 19.1 The organization of CRISPR locus. The repeated and spacer sequences are arranged alternatively in the genome. Proximal leader sequence is often A-T rich and ~500 bp in length

conservative for a given cluster (Fig. 19.1) (Makarova et al., 2011). Another one is spacer sequence (21–72 bps), which is highly divergent but similar in length (Makarova et al., 2011). First clue of their origin came from a bioinformatics analysis where it was found that spacer sequence was identical to the regions of known phage and plasmids, and this quickly leads to the idea that these sequences are involved in defense mechanism against foreign nucleic acid entering the bacterial cell. Cas9 is an endonuclease found in close association with CRISPR and contains two active domains, HNH and RuvC, for targeting individual strands of the DNA double helix (Jia and Wang, 2014). CRISPR works by acting as a pair of DNA scissors and Cas9 as the protein in CRISPR system that unzips DNA (Nekrasov et al., 2013).

CRISPR/Cas9 Mechanism of Action

In 2012, two different individual laboratories at the same time came across the fact that CRISPR/Cas can be constructed in vitro and had biological function. This in vitro constructed CRISPR/Cas can also cut the individual DNA sequence (Gasiunas et al., 2012; Jinek et al., 2012). This finding laid the foundation for the fact that CRISPR/Cas can be used as a genome-editing tool. Their studies in combination with some others found that to make CRSIPR/Cas editing tool functional, three components are required, namely, Cas enzyme, crRNA, and tracRNA. In CRISPR/Cas system, CRISPR loci transcribe into pre-crRNA and further processed into each individual short crRNAs (part of guide RNA) as described by Makarova et al. (2011). Each crRNA comprises single spacer between two half repeats, and these repeats are also called 5′ and 3′ handles, representing the conserved part of every crRNA. Cas9 loci get translated into Cas9, an endonuclease enzyme (Deltcheva et al., 2011). Upon infection of any virus CRISPR/Cas scan, these viral DNA and if any viral DNA fragment is already present in CRISPR is detected in turn forming the complementary spacer-viral DNA pairing (Cong et al., 2013). In this way, Cas9 enzyme cuts the viral DNA and confers the resistance to bacteria. The Cas9 recognizes only those viral sequences complementary to the guide crRNA, where target sequence is directly 5′ to a protospacer adjacent motif (PAM). Commonly used Cas9 recognizes PAM sequence, which is present in two to six nucleotides downstream of target DNA sequence where Cas9 will make a cut. PAM is 5′-NGG-3′

Table 19.1 Summary of Cas and other nuclease variants used in CRISPR experiments and their PAM sequences

SpCas9	*Streptococcus pyogenes*	NGG
SaCas9	*Staphylococcus aureus*	NGRRT or NGRRN
NmeCas9	*Neisseria meningitidis*	NNNNGATT
CjCas9	*Campylobacter jejuni*	NNNNRYAC
StCas9	*Streptococcus thermophilus*	NNAGAAW
LbCpf1 (Cas12a)	*Lachnospiraceae* bacterium	TTTV
AsCpf1 (Cas12a)	*Acidaminococcus* sp.	TTTV
AacCas12b	*Alicyclobacillus acidiphilus*	TTN
BhCas12b v4	*Bacillus hisashii*	ATTN, TTTN and GTTN
Cas14	*Uncultivated archaea*	T-rich PAM sequences, eg. TTTA for dsDNA cleavage, no PAM sequence requirement for ssDNA
Cas3	In silico analysis of various prokaryotic genomes	No PAM sequence requirement

Source: https://www.synthego.com/guide/how-to-use-crispr/pam-sequence

where N can be any nucleotide. The necessity of presence of PAM sequence may create obstacles for researchers. Thankfully, lot of different Cas endonucleases are present in different bacterial species, which can recognize different PAM, which further makes it a hassle-free approach for genome editing (Table 19.1).

Researchers have also resolved this complication of using naturally occurring enzymes by engineering them to recognize different PAM sequences. For example, SpCas9 is an engineered enzyme that recognizes PAM sequences other than NGG. As CRISPR/Cas relies mainly on recognizing, binding, and introducing sequence-specific nucleic acid cleavage, CRISPR/Cas9 and other CRISPR/Cas system can be easily engineered to introduce double-stranded break at desired target sequence at minimal cost (Mali et al., 2013). Since its first application in plants (Shan et al., 2013), it has been used for genome editing in a number of crop species including horticultural crops (Cermak et al., 2015; Ito et al., 2015; Pan et al., 2016; Hayut et al., 2017; Zhang et al., 2018).

Targeted Mutagenesis Using CRISPR/Cas9

Cas9-gRNA mutagenesis requires the engineered bacterial Cas9 protein to function in a particular plant species efficiently. Firstly, a target locus or gene is selected where mutation is to be introduced (20 bp sequence), and it should be followed by the PAM (NGG) sequence. A sgRNA construct is designed where the crRNA and tracrRNA are engineered into a single-guide RNA (sgRNA) using online bioinformatics tools. Suitable modifications such as codon optimization, addition of promoter (small RNA promotor such as U6p or U3p), and a nuclear localization signal (to enable its efficient import into the eukaryotic nuclear compartment) are required for stable expression of Cas9 cassette into the plants. Cas9 and gRNAs are further delivered into the plant tissues by different methods such as protoplast transformation, *Agrobacterium tumefaciens* or *A. rhizogenes*-mediated transfer, and particle bombardment. Regenerated transgenic plants are screened for gene editing events using restriction enzyme site loss, T7E1 assays, surveyor assays, and next-generation sequencing (NGS) methods (Fig. 19.2).

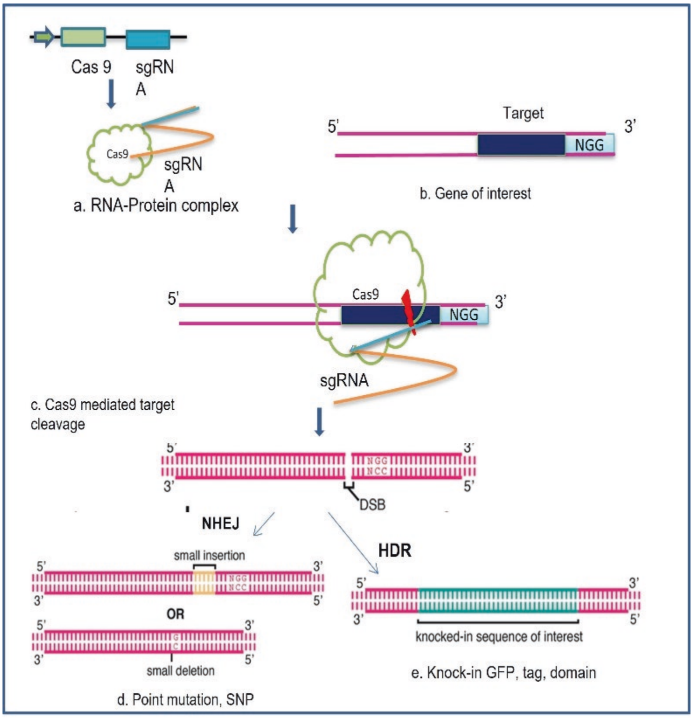

Fig. 19.2 Targeted mutagenesis using CRISPR/Cas9. (**a**) The user-generated Cas9 protein and sgRNA associate to form the RNA-protein complex, which consists of 20 nt like target DNA of gene of interest. (**b**) The genomic DNA target, which must lie adjacent to a protospacer adjacent motif (PAM), is specified by a 20 nt user-generated guide sequence in the sgRNA or crRNA. (**c**) Cas9-sgRNA complex binds to the target sequence, cleaving both strands of the DNA at the site 3 nt upstream of the PAM. Cleavage results in a DSB, which is repaired by NHEJ or HDR DNA repair mechanisms. (**d**) Due to NHEJ, formation of random short indels, point mutation thus frameshift mutations, and disruption of gene function take place, which leads to CRISPR-mediated gene breakdown. (**e**) HDR occurs when artificial repair template is provided from outside, leading to the introduction of an exogenous DNA sequence at a specified genomic location. It forms the basis for gene knock-in, tagging, and precise pre-specified insertions or deletions using CRISPR

CRISPR/Cas9 for Improving Vegetable Crops

CRISPR/Cas9 is a relatively new biotechnological technology that is altering plant breeding practices by manipulating specific DNA regions inside plant genomes (Chen et al., 2019). It is similar to how we use the "cut" and "paste" capabilities in a word processor. This approach might allow plant breeders to modify the genomes of better kinds to create new variety in a single generation, regardless of existing variability or the requirement to pick favorable allele combinations. However, to build the right sgRNA and forecast the editing outcome, such strategies need information about the nucleotide sequence and function of the targeted genome. CRISPR-Cas9 systems have been successfully implemented in improvement of vegetable crops during the last few years for generating the disease resistance line/variety (Chandrasekaran et al., 2016; Nekrasov et al., 2017; Andersson et al., 2017), enhancement of nutrient profile, and for increasing shelf life. For example, parthenocarpic tomato fruits have been developed by targeting SIAGL6 and SlIAA9 genes (Klap et al., 2017). The CRISPR/Ca9-based improvement of nutritional quality in various vegetable crops is described below. However, to date, only few reports are available where this technology has been utilized to add nutritional value to vegetable crops (Table 19.2).

Potato

Potato (*Solanum tuberosum*) is a tetraploid and mainly heterozygous crop, and its tetrasomic inheritance makes standard cross-breeding difficult for potato study and breeding (Muthoni et al., 2015). One or a few features can be introduced to a commercial variety using specific genome editing techniques, avoiding cross-breeding. In comparison to other possible food crops, it may produce more carbs, proteins, minerals, and vitamins per unit amount of land, and it is an important staple food in many poor nations. These factors have prompted researchers to focus their efforts on improving the nutritional value of potatoes while preserving production. It has been demonstrated that raising Amaranth Albumin-1 seed protein content, vitamin C content, carotene level, triacylglycerol, tuber methionine content, and amylose content, among other things, enhanced the nutritional value of potato tubers. To increase potato tuber quality, researchers are working to remove anti-nutritional chemicals such as steroidal glycoalkaloids, acrylamide, and food toxins (Hameed et al., 2018).

In recent years, genome editing techniques such as TALEN and CRISPR/Cas9 have been used to study and develop commercially important traits in potatoes, traits that would otherwise be difficult to incorporate using traditional breeding techniques (Sawai et al., 2014; Clasen et al., 2016; Andersson et al., 2017). The starch quality of potatoes is a critical factor in their use as food or other industrial uses. Andersson et al. (2017) used CRISPR/Cas9 editing technology to create

Table 19.2 Different CRISPR-Cas systems used for improvement of nutritional value in horticultural crops

Sl. No.	Crop	Description	Reference
1.	Potato	SgRNAs to target *ALS* gene (*acetolactate synthase I*) in potato by CRISPR/Cas 9	Butler et al. (2015)
2.		Targeted exon 2 of *StIAA2* gene in double haploid DM potato	Wang et al. (2015)
3.		Targeted *GBSS* gene for starch quality	Andersson et al. (2017)
4.		Targeted gene *WRI1, DGAT1,OLROSIN,* and 100-fold increase in triacylglycerol content were obtained in potato	Liu et al. (2017a)
5.		CRISPR-Cas 9 mediated genome editing in protoplasts of potato	Andersson et al. (2018)
6.		In vitro activities of sgRNAs targeting different regions of phytoene desaturase in potato by the application of CRISPR/Cas9 system	Khromov et al. (2018)
7.		Targeted *St*ALS1, *St*ALS2 for starch biosynthesis	Kusano et al. (2018)
8.		Targeted gene *St*16DOX for removal of steroidal alkaloids using CRISPR-Cas9	Nakayasu et al. (2018)
9.		Designing and construction of single-guide RNA (sg RNA) vector	Nadakuduti et al. (2019)
10.		Targeted gene SBE1, SBE2 for starch improvement	Tuncel et al. (2019)
11.		Targeted gene *PPO* for anti-browning in potato using CRISPR/Cas9	Gonzalez et al. (2020)
12.		Compared *Agrobacterium*-mediated transformation and protoplast transfection with CRISPR/Cas 9 in potato	Gonzalez et al. (2021)

13.	Tomato	Targeted ANT-1 gene for Anthocyanin content	Cermak et al. (2015)
14.		Targeted rin gene for fruit ripening	Ito et al. (2015)
15.		Targeted SIPDS gene for carotenoid synthesis	Pan et al. (2016)
16.		Targeted SIPIF4 gene for carotenoids	Hayut et al. (2017)
17.		Fusion of CRISPR/Cas9 and activation-induced cytidine deaminase by using sgRNAs in rice and tomato	Shimatani et al. (2017)
18.		Breeding of parthenocarpic tomato plants using CRISPR/Cas 9 system	Ueta et al. (2017)
19.		Targeted ORRM4 gene in cultivar Micro-Tom for fruit ripening	Yang et al. (2017b)
20.		Editing of *Psy1* and *CrtR-b2* genes of carotenoid synthesis using CRISPR/Cas9 in tomato	Ambrosio et al. (2018)
21.		Engineered mutations in Inc RNA 1459 using CRISPR/Cas 9 resulting in delay in ripening of tomato	Li et al. (2018a)
22.		Increase in gamma-aminobutyric acid content by targeting genes SlGABA-TP1, SlGABA-TP3, SlCAT9, SlSSADH	Li et al. (2018b)
23.		Genes *SGR-1, LCY-E, LCY-B1,* and *LCY-B2* were targeted for lycopene biosynthesis using CRISPR/Cas9	Li et al. (2018c)
24.		Targeted six loci SELF-PRUNING (SP for general plant growth habit), OVATE (O for fruit shape), MULTIFLORA (MULT for fruit number), FASCIATED and FRUIT WEIGHT 2.2 (FAS and FW2.2 for fruit size), LYCOPENE BETACYCLASE (CycB for functional quality), which resulted in increased fruit size (threefold) and yield (tenfold) and lycopene content fivefold	Zsögön et al. (2018)
25.	Lettuce	Targeted gene *LsGGP2* for improving ascorbate content using CRISPR/Cas9	Zhang et al. (2018)
26.		Upregulation of KN1 homolog by CRISPR/Cas9 promotes leafy head development in lettuce	Yu et al. (2020)
27.		CRISPR/Cas 9-mediated mutagenesis of homologous genes in Chinese kale	Sun et al. (2018)
28.		Targeted editing of the carotenoid isomerase gene *BoaCRTISO* through CRISPR/Cas system	Sun et al. (2020)

(continued)

Table 19.2 (continued)

Sl. No.	Crop	Description	Reference
29.	Carrot	Targeted *F3H* gene for anthocyanin biosynthesis	Klimek-Chodacka et al. (2018)
30.		Targeted mutagenesis in orange and purple carrot plants through CRISPR/Cas 9	Xu et al. (2019)
31.		Inhibition of carotenoid biosynthesis by CRISPR/Cas 9 triggers cell wall remodeling in carrot	Oleszkiewicz et al. (2021)
32.	Brassica	CRISPR/Cas9-mediated genome editing using sgRNA in *B. napus*	Yang et al. (2017a)
33.		Genome editing of the *fatty acid desaturase 2* gene by CRISPR/Cas9 in *B. napus*	Okuzaki et al. (2018)
34.		Genome editing of *B.oleracea* and *B. rapa* protoplasts using CRISPR/Cas 9 ribonucleoprotein complexes	Murovec et al. (2018)
35.		Targeted gene *BnSFAR4* and *BnSFAR5* for increased seed oil content in *B. napus*	Karunarathna et al. (2020)
36.		Targeted *FAD2* gene leads to increased oleic acid content in *B. napus*	Huang et al. (2020)
37.		Targeted gene *BnITPK* in *B. napus* for increase in protein level in oil	Sashidhar et al. (2020)
38.	Eggplant	Targeted *PPO* gene leads to reduction in fruit flesh browning after cutting	Maioli et al. (2020)

starch-free potato tubers by altering the granule-bound starch synthase (GBSS) gene. They used the technique for developing multiple viral resistance in plants by employing gRNAs that target numerous viruses in conjunction with a single Cas9 gene. The "waxy genotype," which produces exclusively amylopectin-containing starch, has been successfully created in hexaploid potato by mutating the GBSS (granule-bound starch synthase) gene. Nakayasu et al. (2018) used CRISPR/Cas9 to test the efficacy of several potential sgRNAs in knocking out the *St16DOX* gene in tetraploid potatoes (cv. Mayqueen). 16-Hydroxylase is a gene that is involved in the manufacture of steroidal glycoalkaloids (SGAs). SGAs cause bitter taste and are harmful to a variety of species. They created two distinct lines devoid of SGA in root hairs using nine sgRNAs that target the *St16DOX* gene. Gonzalez et al. (2021) evaluated *Agrobacterium*-mediated transformation and protoplast transfection for potato genome editing using CRISPR/Cas9 components. Two sgRNAs were created to drive Cas9 to the *StPPO2* gene, which encodes a tuber PPO, polyphenol oxidase (to make it acrylamide free). In protoplasts, a binary vector (CR-PPO) was used for either *Agrobacterium*-mediated transformation or temporary expression, while ribonucleoprotein complexes (RNP-PPO) were also tested. From *Agrobacterium*-mediated transformation, RNP-PPO temporary expression in protoplasts, and CR-PPO transient expression in protoplasts, respectively, 9.6%, 18.4%, and 31.9% of altered lines were obtained.

Tomato

Tomato (*Solanum lycopersicum*) is the most fascinating vegetable crop for biofortification. It is an important food crop as well as a model plant species for investigating gene functions, particularly with fruit biology. It is an attractive option for gene editing because of its routine use and easily available resources (mutant populations, genome sequences, and transformation methods). Because of the availability of efficient transformation methodology (Van Eck et al., 2006), the genome's diploidy, a high-quality genome sequence (Tomato Genome Consortium, 2012), and its economic importance, tomato is an ideal candidate for testing CRISPR/Cas9 gene editing in a dicot crop (fresh and processed). The first tomato genome editing method, CRISPR/Cas-9, was published in 2014 for the "*AGRONAUTE-7*" (*SIAGO-7*) gene, which is responsible for leaf formation (Brooks et al., 2014). Efficiency of CRISPR/Cas assembly in generating targeted mutations in the first generation transgenic tomato plants was demonstrated by Parkhi et al. (2018) where they edited phytoenedesaturase (PDS) gene in tomato plants. PDS gene product is involved in carotenoid biosynthesis pathway (Parkhi et al., 2005). CRISPR/Cas edited lines showed albino phenotypes and accumulation of transcripts of *Cas9* gene in tomato transformants. This methodology demonstrates the usefulness of CRISPR/Cas in future for creation of commercial hybrids of tomatoes for desired traits. Various CRISPR-Cas systems utilized in the tomato have been discussed in Table 19.2.

The arabinosyl-transferase gene activity has been investigated using CRISPR/Cas9 technology as novel components of the CLAVATA signaling pathway necessary for tomato meristem size regulation (Xu et al., 2015). The shelf life of a fruit is an essential attribute, and studies have revealed that cell wall remodeling has a larger role in ripening and shelf life, which determines the life span of tomato fruit and, as a result, minimizes crop postharvest loss (Changwal et al., 2021). In a similar vein, significant work has been done utilizing CRISPR/Cas9 to decode the involvement of cell wall-modifying enzymes such pectate lyase (PL), polygalacturonase 2a (PG2a), and beta-galactanase (TGB4) in fruit ripening (Wang et al., 2019). CRISPR/Cas edited PL lines resulted in firmer fruits, and on the other hand, PG2a and TBG4 influenced fruit color and weight also. The study indicated that PL, PG2a, and TBG4 act on different cell wall domains, and importance of increased pectin accumulation is reflected in different lines. Cermak et al. (2015) used genome editing for developing anthocyanin-rich tomatoes by targeted modification of the *anthocyanin 1* (*ANT1*) gene for carotenoid bio-fortification by mutating carotenoid biosynthesis genes such as *phytoene desaturase* (*SlPDS)* and *phytochrome interacting factor* (*SlPIF4*) (Hayut et al., 2017). Research team at Cold Spring Harbor Laboratory used CRISPR-Cas9 to edit the promoter region of the tomato CLAVATA-WUSCHEL (CLV-WUS) stem cell gene *CLV3* using eight sgRNAs to obtain more in number and larger fruits than wild-type fruits (Ma et al., 2015). Li et al. (2018b) targeted five key genes in the g-aminobutyric acid (GABA) shunt in tomatoes by designing a multiplex pYLCRISPR/Cas9 system. They obtained 53 genome-edited plants, which included mutants ranging from single to quadruple. The GABA (bioactive compound) contents were significantly increased in edited mutants.

Lettuce

Lettuce (*Lactuca sativa* L.) is a fresh green crop that is often used in salads and sandwiches. Apart from being a valuable agricultural crop, lettuce has a number of commercial applications, including nicotine-free cigarette manufacture, edible oil extraction from seeds, and a sedative generated from dried latex found in stems and other tissues (Hassan et al., 2021). Lettuce naturally produces vitamins A and C and thiamine, however, produced in limited amounts. Some successful reports are present for genetic transformation (Michelmore et al., 1987; Curtis et al., 1994; Mohebodini et al., 2014) as well as genome editing approaches (Bertier et al., 2018). Few reports are there of CRISPR/Cas editing in lettuce. Park et al. (2019) reported DNA-free genome editing via ribonucleoprotein in lettuce. They claimed to increase success rate of whole plant regeneration by reducing damage to the transfected naked protoplast cell.

According to a recent report, Alexander Vainstein and Yarin Livneh at Hebrew University in Israel tricked lettuce into producing nutrients in higher quantities by modifying some regulatory regions via CRISPR/Cas (https://www.foodnavigator.com/Article/2022/01/04/Nutritionally-charged-lettuce-developed-with-CRISPR-

Cas-gene-editing-tech). There is no other report available regarding enhancing nutrient profile in lettuce by using CRISPR/Cas technology.

Kale

Chinese kale (*Brassica oleracea* var. Chinensis Lei) is a popular vegetable crop that is highly regarded for its nutritional benefits and flavor. It contains significant quantities of glucoraphanin, which has been shown to have cancer-preventive qualities (Higdon et al., 2007; Jeffery, 2014). Phytoene desaturase (PDS) catalyzes the dehydrogenation of colorless phytoene to z-carotene, which is one of the essential rate-limiting enzymes in the carotenoid biosynthesis process (Huang et al. 2008). Sun et al. (2018) cloned the promoters of two Chinese kale *PDS* gene family members (*BaPDS1* and *BaPDS2*). The findings show that both gene promoters comprised a variety of cis-acting elements, both common and unique. *BaPDS1* was stimulated by red and blue, blue, and strong light and repressed by blue light and dark, whereas *BaPDS2* was induced by weak light, GA3, and MeJA (methyl jasmonate), and suppressed by blue light and dark. ABA (abscisic acid) and SA elicited responses in both *BaPDS1* and *BaPDS2* (salicylic acid).

Sun et al. (2020) employed a CRISPR/Cas9 gene-editing technique in Chinese kale to show functional variations among members of the PDS family, which are essential genes in the carotenoid biosynthesis pathway. The edible organs of most kinds of Chinese kale are green, with the exception of a few cultivars with red bolting stems. The goal of this study was to modify the hue and pigment concentrations of Chinese kale by performing targeted editing of BoaCRTISO using the CRISPR/Cas9 system.

Carrot

Carrot (*Daucus carota* L.) is one of the most widely farmed root vegetables in the Apiaceae family. It has high content of carotenoids, anthocyanins, dietary fiber, vitamins, and other minerals making it the most frequently used storage root. Carrot extracts, which are high in antioxidants, have a key role in the prevention of a variety of ailments. Carotenoids in carrots have been extensively investigated for their production, metabolism, and therapeutic effects. Genome sequence of carrot is available (Xu et al., 2014; Iorizzo et al., 2016), which will facilitate the identification of gene of interest and may further enhance the carrot breeding approaches. However, genetic factors involved in growth development and phytochemical accumulation have not been well studied. So research community needs to understand the underlying genome imparting respective functions and their regulation mechanisms for carrot improvement. Recently, CRISPR/Cas system has been proved as much efficient technology for genome editing and can be utilized for rapid agricultural trait improvement.

The CRISPR/Cas9 technology was used for the first time in carrot study in 2018. Klimek-Chodacka et al. (2018) knocked down the *F3H* gene in carrots, demonstrating its role in anthocyanin production. However, due to the absence of stable gene-edited plants in carrots, Xu et al. (2019) build a genome editing system based on CRISPR/Cas9 in carrot. They employed the CRISPR/Cas9 system to edit the *DcPDS* gene in orange carrots and the *DcMYB113*-like gene in purple carrots, with 35.35% and 36.4% editing efficiencies, respectively (Table 19.1). These findings indicate that the CRISPR/Cas9 system will be a valuable and helpful tool for future studies into carrot gene activity.

Brassica

Brassica genus has a vast variety of species and subspecies that are consumed as shoots, leaves, roots, turnip roots, or as seeds. They are commonly utilized as food and fodder, and their contribution to human nutrition and health benefits are much valued (Kumar & Andy, 2012). Due to domestication and continued breeding, the species has diversified into a vast variety of agriculturally significant morphotypes. *Brassica oleracea,* which comprises morphotypes of cabbage, kale, Chinese kale, savoy cabbage, Brussels sprouts, kohlrabi, broccoli, and cauliflower, is the most common vegetable species among Brassicas. Rapeseed offers raw materials for industrial operations such as biodiesel generation, as well as edible oils for human meals and high-quality animal feed proteins. The key breeding aims in rapeseed production have traditionally been to achieve large oil yields and higher oil and meal quality (Zhai et al., 2020). Triacylglycerols (TAGs) are the primary source of energy in oil plant seeds during germination. Seed oil content (SOC) and fatty acid composition (FA) are important factors that influence yield and quality (Karunarathna et al., 2020). Modern biotechnology techniques for breeding and study of Brassica species are still limited, despite their significant economic relevance. Some of the examples of CRISPR/Cas system in Brassica are mentioned in Table 19.2. Due to well-balanced amino acid and protein levels, commercialization of *B. napus* (oilseed rape) meal as a protein diet is gaining traction. Although phytic acid (PA) is a primary source of phosphorus in plants, it is anti-nutritive in monogastric animals, including humans, due to its negative effects on vital mineral absorption (Sashidhar et al., 2020).

Zhai et al. (2020) used CRISPR/Cas9 for the first time to create yellow-seeded mutants in rapeseed. The targeted mutations in the *BnTT8* gene were passed down through generations, and phenotyping was done on a variety of homozygous mutants with loss-of-function alleles of the target genes. The *BnTT8* double mutants generated seeds with higher seed oil and protein content, as well as changed fatty acid (FA) composition, without any severe abnormalities in yield-related parameters, making them a viable resource for rapeseed breeding. The oilseed *Camelina sativa* has been employed in wide range of applications like biolubricants and biofuel and used as major source of omega-3 fatty acid for aquaculture feed industry. Seed meal coproduct is the main source of protein for animal feed. Low-quality nutrient profile in seed will hinder its overall value and profitability. Lyzenga et al. (2019) exercised the CRISPR/Cas9 genome editing tool to target three homologous genes encoding the seed storage protein CRUCIFERIN C (CRUC), which in result created an identical premature stop codon resulted in a CsCRUS knockout line. They observed that it did not affect the total seed protein content but the ratio of other cruciferin isoforms and other seed storage protein altered. CsCRUS knockout seeds also did not had changed total oil content, but fatty acid profile altered with increased abundance of all saturated fatty acids.

Eggplant

Eggplant is a plant species of Solanaceae family, grown worldwide for its edible fruit. CRISPR/Cas technology was first time employed in brinjal by Maioli et al. (2020) for reducing the fruit flesh browning after cutting. Eggplants mainly characterized by significant amount of phenolic compounds mainly chlorogenic acid (5-o-caffeoylquinic acid). Chlorogenic acid has remarkable therapeutic properties (Naveed et al., 2018). Polyphenol oxidases (PPO) are group of enzymes that negatively affect the quality of eggplant upon cutting due to enzymatic browning (Mishra et al., 2012). Thankfully eggplant genome sequence is available (Barchi et al., 2019), which enables the homology-based thorough characterization of ten PPO (*SmePPO10-PPO10*) genes. *PPO* gene expression was studied after fruit cutting and based on which *SmelPPO4*, *SmelPPO5*, and *SmelPPO6* were targeted for generation of knockout mutants using CRISPR/Cas9 technology. Induced mutation was stably inherited in T1 and T2 and characterized by reduced level of PPO activity and less browning of fruit flesh after cutting.

CRISPR-Cas9 in Fruit Crop Improvement

Fruits are very important part of healthy and balanced diet as they are vital sources of essential nutrients, fiber, and trace elements and are also consumed as staple foods in many countries of the world. Climate change and the expanding human population have necessitated the production of fruit crops with enhanced nutritional values and acclimatization to the environment, for which traditional breeding is unlikely to keep up the pace. Nutritional improvement of fruit crops such as banana, mango, apple, banana, kiwi, and strawberry via plant breeding is a challenging task due to their lengthy life cycle, ploidy level, and nature of parthenocarpic fruit development (Dalla Costa et al., 2017). Hence, these limitations have led to the advancement in the CRISPR mediated genetic modification, which has paved the way for improving the nutritional aspects of fruit crops and consequently revolutionizing fruit crop improvement programs. Many reports have since been published in

Table 19.3 List of target genes and traits modified via CRISPR-Cas9 technology in fruit crops

Sl. No.	Crop species	Target	Transformation method	Trait modification	References
1.	Kiwifruit	*CEN*	Agrobacterium-mediated transformation	Fruit development	Varkonyi-Gasic et al. (2019)
2.	Banana	*PDS1, PDS2*	Agrobacterium-mediated transformation	Chlorophyll, carotenoid	Kaur et al. (2018)
3.	Banana	*MaGA20ox2*	Agrobacterium-mediated suspension cells transformation	Semi-dwarfing size	Shao et al. (2019)
4.	Apple	*IdnDH*	Agrobacterium-mediated transformation	Biosynthesis of tartaric acid	Osakabe et al. (2018)
5.	Apple	*PDS*	Agrobacterium-mediated leaf discs transformation	Albino phenotypes	Nishitani et al. (2016)
6.	Grape	*VvPDS*	Agrobacterium-mediated transformation	Albino phenotype	Nakajima et al. (2017)
7.	Groundcherry	*ClV1*	Agrobacterium-mediated transformation	Fruit size	Lemmon et al. (2018)
8.	Strawberry	*Auxin Response Factor 8* (*FvARF8*) and *Auxin biosynthesis gene* (*FveTAA1*)	Agrobacterium-mediated transformation	Auxin biosynthesis	Zhou et al. (2018)
9.	Strawberry	*PDS*	Agrobacterium-mediated leaf and petiole transformation	Albino phenotypes	Wilson et al. (2019)

fruit-producing species such as citrus, watermelon, apple, banana, grape, and strawberry for improving fruit quality and shelf life. Table 19.3 summarizes published reports of CRISPR-Cas9-based genome editing in fruit crops. Most of CRISPR-Cas9-mediated genome editing studies targeted the *phytoene desaturase* (*PDS*) gene, which encodes an enzyme in carotenoid biosynthesis pathway. The disruption of this gene thus results in an albino phenotype owing to inhibition of chlorophyll and carotenoid production (Qin et al., 2007). The albino phenotype can be easily identified in plants, thus validating the efficiency of CRISPR-Cas9 genome modification system. CRISPR-Cas9-mediated PDS mutation leading to albino phenotype has been reported in apple (Nishitani et al., 2016), pear (Charrier et al., 2019), kiwifruit (Wang et al., 2018), and grapes (Nakajima et al., 2017). The following sections will give a detailed description of the achievements of CRISPR-Cas9 in fruit crop improvement.

CRISPR-Cas9 in Grape and Grapevine

People have cultivated grapes (*Vitis vinifera* L.) for thousands of years for their use in winemaking and also for preparing various dishes. Grapes offer lots of health benefits, primarily due to their high nutrient and antioxidant contents. Grapes are of tremendous economic value; therefore, whole-genome sequence of grape was reported and released in 2007 (Jaillon et al., 2007). Despite the huge progress in genetic engineering based on *Agrobacterium*-mediated transformation, some cultivars are still recalcitrant to transformation (Torregrosa et al., 2002). So, there is a need to develop a stable as well as efficient transformation method for the application of CRISPR/Cas9 in grapes. A study was conducted by Ren et al. (2016), targeting *L-idonate dehydrogenase gene* (*IdnDH*) in the biosynthesis of tartaric acid to verify the efficacy of CRISPR/Cas9 in grapes. A CRISPR/Cas9 construct containing two sgRNAs via *Agrobacterium* was transformed into the "Chardonnay" embryonic suspension cells, yielding 100% mutational efficiency. This study proved that CRISPR can be utilized for investigating the function of candidate genes and conducting genetic improvement in grapes. A second report targeting phytoene desaturase in grapes (*VvPDS*) resulted in albino leaves at efficiencies ranging from 0% to 86% (Nakajima et al., 2017).

Banana

Bananas are one of the most consumed and popular fruits worldwide. They contain essential nutrients that provide beneficial impact on health. A medium banana provides almost 9% of a person's daily potassium needs, essential vitamins, and soluble fibers. Traditional breeding in banana is difficult due to its triploid genome and sterility (Dash & Rai, 2016). Banana genome has been edited at MA-ACO1 loci, which encode a key component of the ethylene biosynthetic pathway; the mutant plants were characterized by extended shelf-time. The edited plants have smaller size and smaller fruit length and weight. The edited bananas were still bright yellow upon the 60th day, whereas the control ripened in 21 days. Complete ripening time restoration of edited fruits was done by treatment with growth regulator ethephon. MA-ACO1-edited lines also exhibited an increased content of vitamin C (Hu et al. 2021). A single-guide RNA was designed to target the conserved domain of two phytoene desaturase (*RAS-PDS)* genes (*RAS-PDS1* and *RAS-PDS2*) in the embryonic cell suspension cultures of banana cv. Rasthali (Kaur et al. 2018). Albino and variegated phenotypes among the regenerated plantlets were observed at a mutation rate of 59%.

CRISPR/Cas9 for Non-transgenic Horticultural Crops

To meet the global demands of the stakeholders, significant improvements have been made in yield, quality, and biotic and abiotic stress tolerance. Most of the successful outcomes have come through conventional breeding. The use of conventional breeding in horticultural crops is limited due to the presence of high heterozygosity, crossing incompatibilities, long juvenile phase, and genome complexity (Anderson, 2006; Bisognin, 2011). Over the past two decades, genetically modified (GM) technology was extensively used to overcome these limitations, although it has its own limitations (Yadav et al., 2018). In GM crops, target gene along with a selectable marker is introduced into the elite cultivars. The foreign DNA randomly gets integrated into the genome of the target crop and may affect other genes (Bawa & Anilakumar, 2013). CRISPR/Cas9 allows researchers to develop non-transgenic crops. Therefore, the problem of transgene associated with GM technology can be resolved with the help of CRISPR/Cas9-mediated genome editing. The location of the CRISPR/Cas9 cassette and the target site are distinctly located, which opens the possibility of removal of CRISPR/Cas9 cassette in the subsequent generations through selfing and crossing. This strategy was employed to genome-edited non-transgenic tomatoes. Tomato (cultivar moneymaker) plants were edited to induce resistance to powdery mildew by introducing two sgRNAs. The transformants were selfed to eliminate the sgRNA cassette (Nekrasov et al., 2017).

The removal of genome editing cassette through selfing and crossing cannot be utilized in case of fruit trees. Fruit trees are characterized by long juvenile phase, which restricts the use of this strategy in case of fruit trees. Along with this, fruit trees possess high level of heterozygosity and have a complex genome and are generally propagated through vegetative mode. To overcome this, transient expression of CRISPR/Cas9 system in nucleus can be utilized. This strategy was applied to apples to develop transgene-free edited apple lines (Charrier et al., 2019). However, as per study, this system was very less efficient and had an efficacy of 0.4% only. Therefore, further studies are needed to improve the efficacy of this system, so that it can be utilized in routine genome editing process.

Transgene-free genome-edited plants can also be obtained by delivering preassembled Cas9 protein and gRNA ribonucleoproteins in plant cells. Once delivered to the plant cells, these RNPs can edit the target site and would be eliminated by degradation through endogenous proteases. Hence, the plants are transgene-free (Woo et al., 2015; Malnoy et al., 2016). Transgene gene-free lettuce was obtained by transforming the protoplast with pre-assembled complexes of Cas9 protein (Woo et al., 2015). Similar strategy was used to obtain genome-edited DNA-free grapevine and apple (Malnoy et al., 2016). Protoplasts of pepper and capsicum were transformed using PEG-mediated transformation to DNA-free edited plants (Kim et al., 2020). Further, a stepwise protocol with high accuracy and efficacy was developed for design and delivery of CRISPR/Cas9 system in grapevine and apple. The mutated DNA-free plants were regenerated within 2–3 weeks by employing this protocol (Osakabe et al., 2018). However, the plasmid-mediated transformation takes over more than 3 months to regenerate plants. Researchers have also come up with several other strategies to develop non-transgenic genome-edited plants (Kanchiswamy et al., 2015; Liu et al., 2020; et al. 2021).

Development of DNA-free through CRISPR-Cas9-mediated genome editing is of prime importance due to rigorous and costly regulations faced by genetic modification. Furthermore, regulations on genome-edited crops are a matter of debate at present. DNA-free edited plants may not have to undergo the present regulatory check (Schaart et al., 2016; Jansson, 2018), thereby saving time and money to carry out these regulation processes. We hope that the plants developed through this DNA-free technology do not face similar regulatory burden as traditionally bred varieties and are adopted worldwide. This will be a step forward toward meeting the global demands and promoting competitiveness of the agro biotech sector by reducing chemical input. These plants are not considered transgenic and may gain public acceptance, which is foremost essential to commercialize improved crops.

The Regulatory Status of CRISPR/Cas9 Horticulture Crops

Recent advances in gene-editing technologies such as CRISPR/Cas9 have led to a new era of crop improvement in terms of productivity (Zhang et al., 2016; Xu et al., 2016), resistance to various diseases (Wang et al., 2016), better nutritional quality (Li et al., 2018b), and resistance to abiotic stress (Shi et al., 2016; Nieves-Cordones et al., 2017; Haque et al., 2018). Research in CRISPR/Cas9 involves precise gene editing and is preferred over traditional breeding crop improvement methods. Both mutagenesis and CRISPR/Cas9 systems are considered similar in terms of inducing mutation, but the CRISPR/Cas9 system is quite specific including wide targeting flexibility, ease of use, and less expensive. This gene-editing technology potentially represents the biggest breakthrough in several areas including agriculture, medicine, healthcare, veterinary, and industrial production processes. Small insertions and deletions that are equivalent to spontaneous genetic variation have been created using this technology (Woo et al., 2015), which are then repaired using either the NHEJ or a donor template-based HDR pathway. The null segregants are identified through selection against transgenic components like Cas9 and selection markers. Whole-genome sequencing can also be used to guarantee that no CRISPR/Cas9 elements are present (Kim & Kim, 2016) and to examine the potential for off-target impacts. Because the NHEJ pathway is the most common repair route in plants, most CRISPR/Cas9-based gene-editing research has focused on it (Hilscher et al., 2016). The NHEJ mechanism aids in the creation of transgene-free plants that are not subject to regulatory scrutiny (Ishii & Araki, 2017), as they do not contain foreign elements found in GMOs, and the mutations caused are comparable to those seen in natural mutagenesis. As a result, once null segregants are obtained, plants created using NHEJ-based editing are currently unregulated.

In comparison to traditional breeding, which takes more than ten years to generate a variety (Wieczorek & Wright, 2012), NHEJ offers quick crop breeding, that is, less than five years (Waltz, 2016; Sander & Jeschke, 2016). Exogenously given, homologous DNA sequences (template) are similarly used in HDR to accurately repair DSBs in DNA (Kim & Kim, 2014). To some extent, HDR gene addition may make it transgenic. The produced crops may not be called transgenic if the repair template is cisgenic, meaning it uses genes from the same species, related interbreeding, or wild species. Nonetheless, depending on the country, the regulatory framework for NHEJ and HDR-mediated gene editing is seen and characterized in different ways (Ishii & Araki, 2016). The CRISPR/Cas9 technology is full of potential, but the ultimate utilization depends on how these gene-edited crops are regulated. The most significant advantage of CRISPR-edited crops over GM crops is that they do not always contain foreign DNA and so are not deemed GMOs (Mubarik et al., 2021). GMOs are created by tinkering with a living organism's DNA, and many GM crops contain genetic material from other organisms, such as *Bacillus thuringiensis* genes in Bt cotton and Bt maize. These alterations have raised health and environmental concerns, resulting in a reluctance to grow or consume such crops in many regions of the world (Munawar & Ahmad, 2021).

Different countries adopt different approaches in terms of a regulatory framework. In the United States, various agencies that look after the regulation of genetically modified organisms (GMOs) are the US Department of Agriculture (USDA), the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA) (Globus & Qimron, 2017). Because transgenic elements have been removed from plants, regulatory agencies in the United States do not consider CRISPR-edited plants to be GMOs. Plants that have been CRISPR-edited can be broadly distributed in less time and at a lower cost than traditional plant breeding (Liu et al., 2017b). In 2016, the USDA approved a gene-edited waxy corn null segregant line (https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/15-352-01_air_response_signed.pdf) and an *Agaricus bisporus* mushroom with an anti-browning trait (https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/15-321-01_air_response_signed.pdf.) Lines that have been certified by regulatory agencies can be directly tested in the field, which benefits researchers and biotechnology businesses (Songstad et al., 2017) and aids in the development of novel cultivars in a short amount of time and at a lower cost. Genome editing has been approved in the United States as a possible extension of existing plant breeding technologies for crop enhancement (https://www.usda.gov/media/press-releases/2018/03/28/secretary-perdue-issues-usda-statement-plant-breeding-innovation). In the United States, there is a database of regulatory inquiries and approvals for gene-edited crops (https://www.aphis.usda.gov/aphis/%20ourfocus/biotechnology/am-i-regulated/Regulated_Article_Letters_of_Inquiry). The US regulatory agencies promote genome editing as a powerful technology as compared to traditional breeding and have been used in the development of new varieties with desirable traits including tomato, citrus, soybean, sugarcane, rice, orchid, camelina, petunia, and flax. Similarly, in May 2020, the USDA-APHIS issued the latest edition of biotechnology regulations defined as SECURE (Sustainable, Ecological, Consistent, Uniform, Responsible, Efficient) rule (Barrangou, 2020). This framework provides three exemptions for genetic modifications in any plant species: (i) changes resulting due to DSB in DNA in the absence of an external repair template, (ii) targeted single base pair substitution, and (iii) introduction of a known gene that exists in the plant's gene pool. (https://www.aphis.usda.gov/aphis/ourfocus/%20biotechnology/biotech-rule). The legislative framework in each country or group of countries determines how gene-edited crops are regulated. Only if the gene-edited product (plant, feed, or food) is unique, that is, distinct from what is already on the market, is it subjected to pre-market approval in Canada (https://inspection.canada.ca/plant-varieties/plants-with-novel-traits/eng/1300137887237/130013793963 5). CRISPR/Cas9 is governed by strict laws in Europe (Wight, 2018). The Court of Justice of the European Union, Europe's highest court, recently declared that gene-edited crops should be subject to the same restrictions as genetically modified (GM) species (Callaway, 2018). Australia, on the other hand, has chosen a medium path, permitting gene editing without the introduction of foreign genetic material (Mallapaty, 2019).

In Asian countries like China and Japan, where multiple field trials of gene-edited crops have been reported (Metje-Sprink et al., 2020), there is also a suitable climate for gene-edited crops. Genetic engineering is regulated in India, and it is defined as "modification of an organism or a cell by deletion and removal of parts of the heritable material." In 1986, the country's Environmental Protection Act (EPA) was implemented by the Ministry of Environment, Forests, and Climate Change (MoEFCC). The Genetic Engineering Appraisal Committee (GEAC), which was established under the EPA and MoEFCC, is India's highest regulatory authority for GM crops. Before they may be sold for public consumption, all GM crops must be approved by the GEAC (http://www.geacindia.gov.in/resource-documents/13_2). The MoEFCC also adheres to the CPB and has developed institutes to assess biosafety laws for GM crops, one of which is the Biosafety Clearing House, which was founded in 2017 (Shukla et al., 2018; Kolady & Herring, 2014). Hazardous microorganisms and genetically altered organisms are referred to as "hazardous substances"" by the EPA. The Recombinant DNA Advisory Committee (RDAC), Review Committee on Genetic Manipulation (RCGM), GEAC, and different institutional biosafety committees (IBSC) (Shukla et al., 2018) are among the groups formed under India's EPA. The Indian Parliament has established three laws covering the creation, approval, and commercialization of genetically modified crops. The EPA 1986, which is administered by the Ministry of Environment, Forest and Climate Change (MoEFCC), the Seeds Act 1966 & Seeds Order (a historical seeds order replaced by a plant quarantine order under the Ministry of Agriculture and Food Safety) (Kaur, 2020) and the Food Safety and Standards Act 2006, which is administered by the Ministry of Health and Family Welfare (Choudhary et al., 2014), are among them. The Biotechnology Regulatory Authority of India (BRAI) was also established in India to examine current GMOs and raise farmer awareness of modern GM products. To now, the only GM crop permitted for commercial farming in India is Bt cotton, which has been certified for seed, fiber, and feed production. Bt eggplant was approved in 2009, but the MoEFCC halted it in 2010.

Improvement of GM crops for the public sector is typically performed by government agencies such as CRIDA, ICAR-IIOR (Hyderabad), ICAR-CICR (Nagpur), CPRI (Shimla), and UPCSCR (Shahjahanpur) for insect resistance, herbicide and drought tolerance, greater nutritional value, and higher yields. Banana, cauliflower, chickpea, cabbage, mustard, wheat, papaya, potato, cassava, tomato, rice, and watermelon are examples of public-sector GM crops. Private enterprises including Monsanto, JK Agri Genetics, Nath Seeds, and Bayer Bioscience are more focused on cabbage, corn, mustard, okra, peas, tomatoes, cotton, and cauliflowers. Field experiments have only been undertaken for cotton, corn, and rice due to disagreements over state government approvals (Shukla et al., 2018; Kaur, 2020).

GMO rules are in place in South Africa, but genome-editing-specific legislation is still needed (Pillay & Thaldar, 2018). GMOs are creatures with a “new combination of genetic material generated through current biotechnological processes,” according to Argentina (Whelan & Lema, 2015). However, whether a plant was developed utilizing transgenic procedures, a final product with no transgenes will be designated as non-GM. Depending on the situation, Argentina’s regulatory system can be divided into two categories: product-based and process-based. Because Argentina’s GM crop regulatory structure is based on a case-by-case analysis, each new plant change can be regulated separately if necessary (Whelan & Lema, 2019).

CRISPR-Cas9-Mediated Genome Editing Limitations and Future Prospects

Even though the CRISPR/Cas9 system is an excellent tool for genome editing, but many important aspects related to CRISPR function remain obscure. Its tendency to create off-targets even with one single mismatch is a matter of concern. Mutations caused due to off-targets and cleavage efficiency require investigation in more detail. The necessity of NGG (PAM) adjacent to the target site is another limitation of this approach. With gene editing, it will be difficult for regulators and farmers to identify a CRISPR engineered crop, that is, whether it has been mutated conventionally or genetically engineered. It poses further questions to regulatory agencies, as to how to regulate gene-edited plants. Consequently, the status of crops generated through gene editing is debatable, whether to be included in GMOs or not. Crops modified using CRISPR-Cas9 are edited in their genome and technically no gene has been added from other species. This is particularly beneficial for those crops where lesser variability is present among plant varieties. Indeed, the developments in CRISPR/Cas9 have emerged as an alternative to classical breeding and transgenic methods for improving nutritional value in horticultural crops. This scientific knowledge should be implemented in future to accomplish the goal of nutritional security worldwide. However, to achieve the full potential of this technology, many important questions and challenges must be addressed.

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Chapter 20
Physiological Interventions of Antioxidants in Crop Plants Under Multiple Abiotic Stresses

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Introduction

The current environmental changes across the globe are serious threats to agriculture and all living organisms (Devendra, 2012). As a result of these environmental changes, global temperature may rise up to 3–4°C (WMO, 2014), which may lead to serious problems such as food shortage and starvation. Owing to these climate changes, plants are persistently facing different stresses such as salinity, heavy metals, drought, chilling, heat, increased sunlight, etc. Due to aforesaid stresses, yield of crops is being dwindled (Lamaoui, 2018; Dhamgaye & Gadre, 2015; Gao et al., 2014) that will certainly affect increasing human population (Poljsak et al., 2013).

From chemistry point of view, oxidative stress on plants due to the abovementioned external stimuli imbalances the ROS-antioxidant interrelations (Fig. 20.1). Excess ROS production in response to various stresses has also been known to speed up peroxidation of lipids, DNA impairment, and carbonylation of proteins (Munns, 2008). Plants produced enzymatic and nonenzymatic antioxidants as a

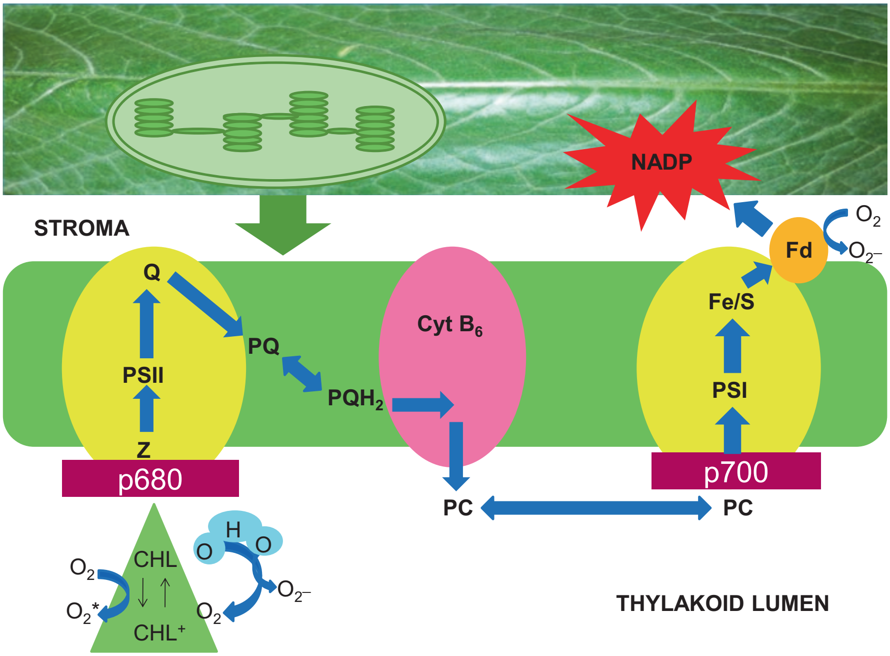

Fig. 20.1 Production of superoxide radical and singlet oxygen in chloroplast at PSII and PSI, respectively

defensive strategy to avoid cytotoxic effects (Shalata et al., 2001) but ultimately hindered plant growth obtained under saline conditions (Taarit et al., 2012a, b). Therefore, there is a need to grow plants under simulated saline conditions using exogenous enzymatic and nonenzymatic antioxidants. In this way, the toxic effects of salinity on the plant growth can be diminished, which is witnessed by various studies (Khan et al., 2013a, b, 2017; Husen et al., 2018).

Strategies of Plants to Cope with Stress

Plants adapted different strategies at cellular, physiological, biochemical, and molecular levels to cope with salinity stress (Gupta & Huang, 2014; Tamang & Fukao, 2015; Wang et al., 2018). For instance, plants respond to salinity by Na^+ selectivity and compartmentalization of Na^+ ions at cellular as well as tissue levels. Mechanisms of salt tolerance thus could be categorized into two major groups, that is, physiological and molecular.

Physiological mechanisms can further be explained by osmotic adjustment and/or water homeostasis, ion exclusion/inclusion and/or ion homeostasis, ROS scavenging, and hormonal biosynthesis (Batool et al., 2015). Among these mechanisms, water and ion homeostasis are mainly thought to counter antagonistic impacts of salinity on plant growth.

Moreover, many transcription factors such as heat shock factors (HSF) or ABA-responsive elements (ABF/ABRE) may induce salinity tolerance in plants (Vinocur & Altman, 2005). Stress-induced activation of molecular networks, signal transduction (starting from the roots toward cellular and whole plant levels), metabolites and specific gene expression are among decisive factors of plants to adapt against environmental stresses (Nguyen et al., 2018; Ismail et al., 2014; Ashraf, 2009; Vinocur & Altman, 2005). In plants, resistance to biotic stresses is usually controlled in a simple way, but salinity tolerance as an abiotic factor is controlled by the interaction of several genes (i.e., a few major genes along with several minor genes) (Ashraf & Harris, 2004; Batool et al., 2015). At the transcriptomic level, genes related to stress signaling, transcription regulation, ion transport mechanism, and biosynthesis of specific metabolites of complex signaling pathways are responsible for salt stress tolerance in plants (Cotsaftis et al., 2011; Kawasaki et al., 2001; Kumar et al., 2013; Walia et al., 2007).

Abiotic Stresses and Plants

Plants growth is significantly affected by various abiotic stresses, which resulted in low crop yields. Abiotic stresses included salinity, heavy metals, drought, chilling, temperature, water logging, and increased exposure to UV radiations (Dhamgaye & Gadre, 2015). Figure 20.2 explained the response of plants to various stresses.

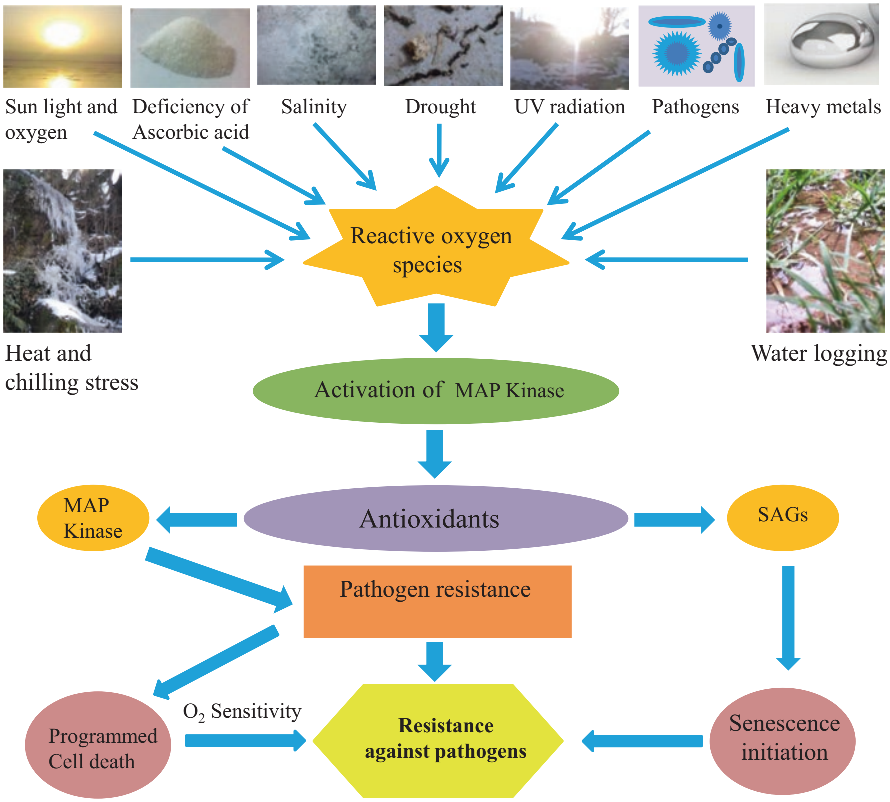

Fig. 20.2 Diagram showing response of plants to various stresses

Among multiple abiotic stresses, salinity is considered the most alarming, which constrained the agricultural production and adversely affected growth and yield of chief crops. Salt stress has affected 25% of the agricultural lands all over the globe due to application of saline irrigation water. Salinity reduced the water availability to crop plants (Taffouo et al., 2010; Ashraf, 2009). High concentrations of salts inhibited the growth of plants due to stumpy osmotic potential of lands, imbalanced nutrition, and selected ion effects (Parvaiz & Satyawati, 2008).

Drought, another abiotic factor, is threatening and has disturbed the economy of the world by reducing crop production (Cenacchi, 2014). Transgenic plants have been prepared to use genes that encoded proteins involved in drought tolerance. Accumulation of osmolytes is also one of the important mechanisms that helped the plants to tolerate drought stress in plants(Bechtold & Field, 2018).

Chilling stress also affects plant metabolism thus hindering plant growth and reproduction. The plants changed their pattern of gene expression to cope with chilling stress, thereby producing a suite of metabolites to protect plants against chilling (Sanghera et al., 2011).

UV radiations also cause a serious threat on the environment and oxidative damage in plants (Du et al., 2011). Due to stress environment, plants switched on their antioxidant system to reduce the toxic effects of stress (Carletti et al., 2013). Although UV-absorbing compounds mainly protected the DNA (Stapleton & Walbot, 1994), these compounds also played a key role in the plant antioxidative defense system and pathogens (Tutejaet al., 2001).

Moreover, heavy metals in the environment is a serious threat to agro-ecosystem and crop plants (Ashraf et al., 2015, 2017a, b; Mani & Kumar, 2014). Toxic levels of heavy metal in plants often result in the oxidative damage and disruption of structural and functionality of plant cells (Ashraf et al., 2018, 2020; Ashraf & Tang, 2017). The oxidative stress disturbs the equilibrium between prooxidant and antioxidant homeostasis (Flora et al., 2008). Waterlogging and salinity go parallel with each other and create severe problems for plant growth. One of the first responses to plant against waterlogging is reduction in stomatal conductance (Folzer et al., 2006). The deficiency of oxygen due to waterlogging generally leads to the substantial decline in photosynthetic efficiency (Kaur et al., 2018a, b; Ashraf et al., 2011; Ashraf & Arfan, 2005) and causes oxidative stress. Due to oxidative stress, reactive oxygen species produced and disturbed the metabolic process of plants (Ashraf et al., 2011; Ashraf, 2009). Excess of water also inhibit electron transport chain, respiration, and ATP formation due to hypoxia (low oxygen concentrations (Ashraf et al., 2011). The nutrient uptake and growth of the plants reduced due to the contrary effects of waterlogging (Ashraf et al., 2011).

Furthermore, the magnitude of temperature stress increased as the ambient temperature increases from a threshold level, which results in alteration in physio-biochemical mechanisms in plants (Kong et al., 2017). The extent of possible damage owing to increased temperature in plants depends on plant developmental stage experiencing the high temperature stress (Slafer & Rawson, 1995; Wollenweber et al., 2003).

Antioxidants Combat Plant Abiotic Stresses

Antioxidant defense system is the best strategy adapted by the plants to ameliorate the abiotic stresses. Plants produce a variety of enzymatic, nonenzymatic antioxidants, and hormones endogenously in response to aforesaid stresses (Albaladejo et al., 2017; Almeselmani et al., 2006; Kandil et al., 2017; Massoud et al., 2018). Enzymatic antioxidants included enzymes such as superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), and polyphenol oxidase (PPO), whereas α-tocopherol, carotenes, and ascorbic acid (vitamin C) are nonenzymatic antioxidants produced by the plants to alleviate the impacts of abiotic stresses on plants. It is noticed that the abovementioned endogenous production of antioxidant secondary metabolites is the best way but not enough in amount to cope with elevated salinity conditions (Srinieng et al., 2015). It has been reported that antioxidants being supplied exogenously to plants are also fruitful to mitigate plant stresses, particularly salinity (Agada, 2016). Al Kharusi et al. (2019) performed an experiment on date palm to induce salinity tolerance by application of antioxidants.

Ascorbic acid has been found to be involved in cell wall expansion, enhancement of cell division, leaf area, biosynthesis of photosynthetic pigments, and improvement in plant tolerance against multifarious stresses by scavenging ROS (Dey et al., 2016; Kasote et al., 2015). Moreover, salicylic also modulates the important physiological processes such as photosynthesis, osmoregulation, and nitrogen metabolism (Khan et al., 2013a, b). Salicylic acid also plays its role in the tolerance of plants against chilling, drought, salinity, UV radiations, pathogen, heavy metals, waterlogging, and heat stresses (Farheen et al., 2018; Khan et al., 2015; Palma et al., 2013). Exogenous application of trehalose alleviates the adverse effects of salinity stress in wheat by changing the physiological process (Alla et al., 2019; Mervat & Sadak, 2019; NematAlla et al., 2019). Ellagic acid, a natural polyphenolic antioxidant in various vegetables and fruits (Lima et al., 2014), is distributed in the vacuoles as water-soluble ellagitannin and played a vital role in plant defense against a number of stresses by capturing ROS effectively (Nagarani et al., 2014; Priyadarsini et al., 2002; Saul et al., 2011).

In addition, brassinolide captured ROS effectively and protects the plants from oxidative stress. Various literature reports confirmed its oxidative potential when applied exogenously against different stresses (Zhou et al., 2015; Behnamnia 2015; Javid et al., 2011; Li & Chory, 1999). Moreover, tocochromanols are effective and useful group of lipophilic phenolic antioxidants (Housam et al., 2014), which could protect key cell components by scavenging free radicals before prior to lipid peroxidation and/or DNA damage. The tocochromanols break the chain reactions involved in lipid peroxidation and shield the cellular membranes by repair and replacement of lipid in bilayer membranes (Falk and Munnè-Bosch, 2010; Mène-Saffrané & DellaPenna, 2010). On the other hand, exogenous GABA application improved stress tolerance by regulating the physio-biochemical processes and redox balance (Jin et al., 2019; Li et al., 2016). Similarly, carotenoids are important antioxidants used as photosynthetic apparatus in plants, algae, and cyanobacteria, which protected against photooxidative damage and contributed to light harvesting for photosynthesis (Ksas et al., 2015).

Mode of Exogenous Applications of Antioxidants

Folair Applications of Antioxidants

Foliar spray of an antioxidants is considered a shotgun approach to ameliorate the toxic effects of salinity. Foliar application is very economic mode of application to save the nutrients. Previously, Khan et al. (2006) and Athar et al. (2009) have performed experiments on wheat by applying different concentrations (0 and 100 mg

L^{-1}) of ascorbic acid and found substantial improvements in the growth and development of wheat plants. Malik and Ashraf (2012) also performed experiment on wheat by applying different concentration of ascorbic acid and hydrogen peroxide to mitigate the effects of drought. Ahmad et al. (2014) studied the effect of salicylic acid and hydrogen peroxide (each 0, 20, and 40 mgL^{-1}) on maize at low temperature stress. Noreen et al. (2009) reported that exogenous salicylic acid application improved salt stress tolerance in sunflower. Baber et al. (2014) also reported that salicylic acid application improved the performance of fenugreek under saline conditions. Noreen and Ashraf (2008) reported that exogenous salicylic acid application improved the physiology and growth of sunflower under saline conditions. Li et al. (2014a, b) stated that foliar spray of salicylic acid improved the photosynthesis and antioxidant system in *Torreya grandis.* Exogenous salicylic acid application improved the germination and early growth of wheat under salt stress (Sahli et al., 2019). Moreover, Desoky and Merwad (2015) performed an experiment on foliar applications of ascorbic acid and salicylic acid and found that co-application of both resulted in improved the growth and development of wheat under saline conditions (Hamideldin et al., 2017; Morsi et al., 2018; Rihan et al., 2017).

Applications of Antioxidants Through Rooting Media

In a number of studies, much attention has been given on stress tolerance by the application of antioxidants through rooting medium. It was observed that antioxidants increased photosynthetic rate via stomatal regulation, which was positively associated with stress tolerance. For example, Athar et al. (2009) found improved growth of wheat when ascorbic acid was applied through rooting medium at vegetative stage under salt stress. Malik and Ashraf (2012) also conducted experiment on wheat and applied ascorbic acid through rooting medium under drought stress and found substantial improvements in growth. In another study, Xu et al. (2015) evaluated the positive effects of ascorbic acid on *Festuca arundinacea* through rooting medium under water stress. Arfan et al. (2007) found improved growth of wheat when applied with the salicylic acid through rooting under saline conditions.

Applications of Antioxidants by Seed Soaking

Exogenous application of antioxidant compounds as a pre-sowing treatment has gained a considerable attention in ameliorating the adverse effect of salt stress. In this regard, El-Soud et al. (2013) observed that seed treatment of chickpea seeds with ellagic acid improved seed germination under PEG-induced stress. Seed soaking of soybean and other crops in distilled water or ASC solution for 4 h improved germination under associated physio-biochemical mechanisms under saline conditions (Çavuşoğlu and Bilir 2015; Malik & Ashraf, 2012; Dehghan et al., 2011). Kasim et al. (2016) soaked radish seeds in *Pterocladia capillacea* and *Codium taylorii* extracts and found improved growth under saline conditions. Khan et al. (2006) observed positive effects of pretreatment of wheat seeds with ascorbic acid under saline conditions. Overall, pre-treatment of seeds with antioxidants increased endogenous level of ascorbic acid that had a protective effect on photosynthetic pigments against salt-induced oxidative stress; thus, antioxidants are involved in the regulation of many physiological functions to improve the performance of plants under stress conditions.

Nonenzymatic Antioxidants

To fight against stresses, plants produced antioxidants (Fig. 20.3) that maintained the growth and provided strength under stress and non-stress conditions. Most commonly studied nonenzymatic antioxidants to mitigate the stresses on plant growth included ellagic acid, ascorbic acid, salicylic acid, α-tocopherol, anthocyanins, brassinolides, and carotenes.

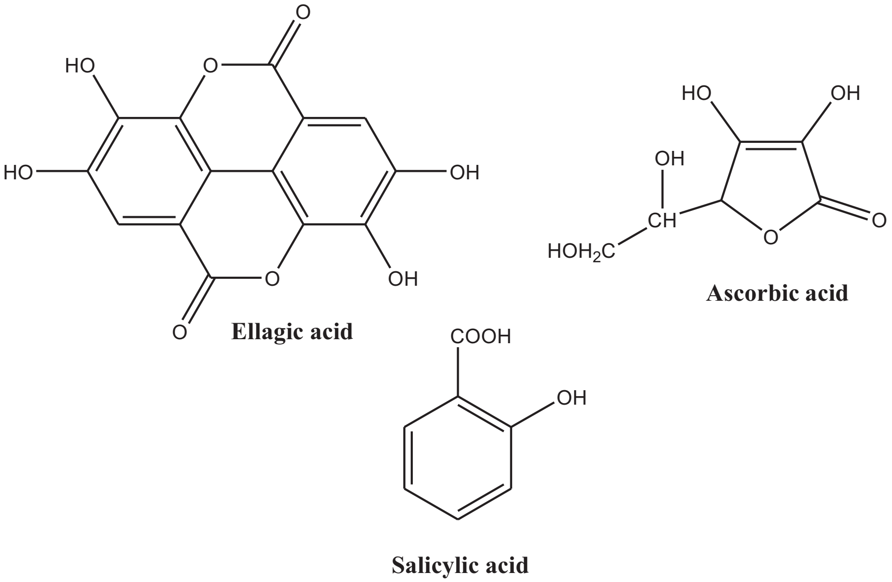

Fig. 20.3 Structures of some important carboxylic acids used as antioxidants against salinity stress

Ellagic Acid

Ellagic acid is a naturally occurring polyphenolic antioxidant that is present in several fruits including grapes, nuts, pomegranate, and a wide variety of berries as well as in vegetables (Malini et al., 2011). However, ellagic acid played several essential roles in plants under stress conditions such as DNA binding, scavenging of ROS, and inhibition of ROS production (Fig. 20.4) and protection of DNA from alkylating injury (Barch et al., 1996). Ellagic acid is also responsible for the restoration of normal functioning of various biomolecules. ROS depolarized cell membranes and hence disturbed the cell metabolism through seepage of essential ingredients from the cell (Hasanuzzaman, 2013). Ellagic acid has displayed antioxidant (Han et al., 2006; Sepúlveda et al., 2011), antibacterial (Han et al., 2006; Sepúlveda et al.,

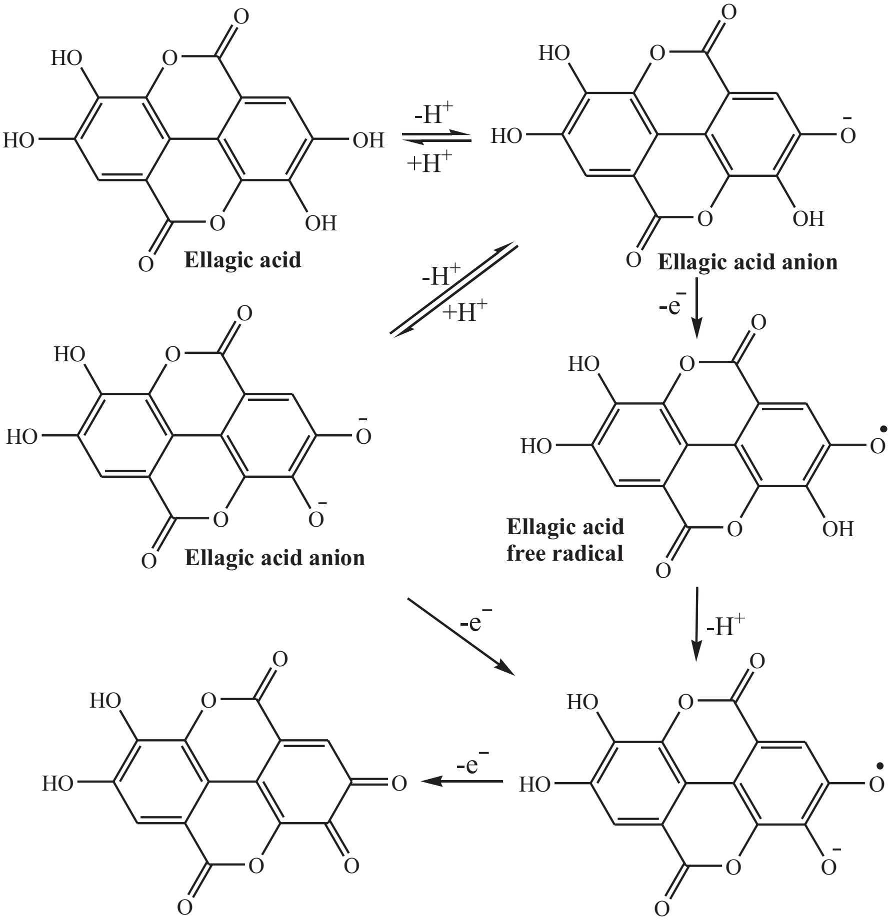

Fig. 20.4 Plausible free radical capturing mechanism of ellagic acid

2011), antiviral (Han et al., 2006; Sepúlveda et al., 2011), anti-inflammatory (Mehan et al., 2015), and anticancerous activities (Han et al., 2006; Mehan et al., 2015; Sepúlveda et al., 2011) in humans and inhibited UV-induced oxidative stress in plants with protection against lipid peroxidation (Bhandari, 2012).

More importantly, it effectively captured ROS at physiological pH to protect cells against toxic effects. Under such conditions, the ellagic acid anion is well known for its protective role, which continuously regenerated after capturing two free radicals, and thus proves more beneficial even at low concentrations (Galano et al., 2014). Moreover, the ellagic acid metabolites have also the ability to scavenge free radicals efficiently showing that its working performance is not reduced after being metabolized. This is an uncommon and constructive characteristic of ellagic acid, which made it particularly valuable against oxidative damage (Galano et al., 2014). Moreover, it has also been reported that ellagic acid provided better protection against oxidative stress and lipid peroxidation than vitamin E.

In another study, it has been investigated that the antioxidant activity of ellagic acid is mainly due to the presence of two pairs of neighboring hydroxyl groups in its structure, and it is very effective in inhibiting lipid peroxidation even at micromolar (low) concentrations. The scavenging activity of ellagic acid resembled those of other antioxidants such as vitamins E and C (Parthasarathi & Park, 2015; Galano, 2014; Indira et al., 2002).

A recent study showed that ellagic acid is bound to DNA by intercalating with the minor groove because of its planar structure. In this function, it activated various signaling pathways such as apoptosis, protected from oxidative DNA damage, and altered growth factor expression (Parthasarathi & Park, 2015). However, detailed investigations are still needed on bioavailability and absorption capacity of ellagic acid.

In a study, ellagic acid (50 ppm) was applied as a pre-seed treatment on chickpea seedlings, and it was found that ellagic acid accelerated the germination and growth with enhanced total antioxidant capacity and contents of compatible components (proline and glycine betaine) and antioxidant enzymes. Furthermore, ellagic acid decreased the lipid peroxidation levels, glutathione content, and seepage of solutes. Thus, the study discovered an improved salt tolerance of gram seedlings under osmotic stress by decreasing contents of H_2O_2 and increasing total antioxidant capacity after ellagic acid treatment (Aguilera-Carbo et al., 2008; El-Soud et al., 2013).

Ascorbic Acid

Ascorbic acid (Fig. 20.3) is a potential antioxidant to scavenge ROS produced under stresses (Kumar et al., 2014). Ascorbic acid possesses antioxidant and cellular reductant abilities, promotes plant growth and development, and regulates plant cellular mechanisms against environmental stresses (Hameed et al., 2015). Generally, ascorbic acid is present in all plant parts, subcellular compartments including the cell wall and vacuole (Fernie & Szilvia, 2015) except dry seeds (Davey et al., 2000), and its concentration varies in different parts of plants (Klause et al., 2016). Ascorbic acid is synthesized by almost all higher plants, while animals capable to oxidize L-gulono-1,4-lactone can synthesize ascorbic acid. It has been discovered that synthesis of ascorbic acid is regulated by the presence of jasmonate, which induced the transcription level and enhanced its production inside the cell (El Hariri et al., 2010; Smirnoff, 2005).

Plants release ascorbic acid in response to stresses. It not only captured free radicals but also activated complex biological defense mechanisms at cellular levels (Conklin & Barth, 2004) (Fig. 20.5). Exogenous ascorbic acid application reduced lipid peroxidation in seedlings of *S. fruticose* (Hameed et al., 2012) and *Brassica napus* (Dolatabadian et al., 2008) and *Phaseolus vulgaris* (Saeidi-Sar et al., 2013) and in perennial halophytes (Hameed et al., 2015) under salinity stress. Shalata and Neumann (2001) described the protective role of exogenous ascorbic acid that appeared to be associated to its antioxidant activity. Ascorbic acid via rooting medium, pre-sowing seed treatment, and foliar spray has been found reliable to reduce the effect of salinity in wheat (Azzedine et al., 2011; Raafat et al., 2011; Athar et al., 2008, 2009; Khan et al., 2006; Shalata & Neumann, 2001; Janda et al., 1999). It can also mitigate the toxic effects of oxidants, inhibit the uptake of sodium, and enhance the uptake of potassium (Conklin & Barth, 2004).

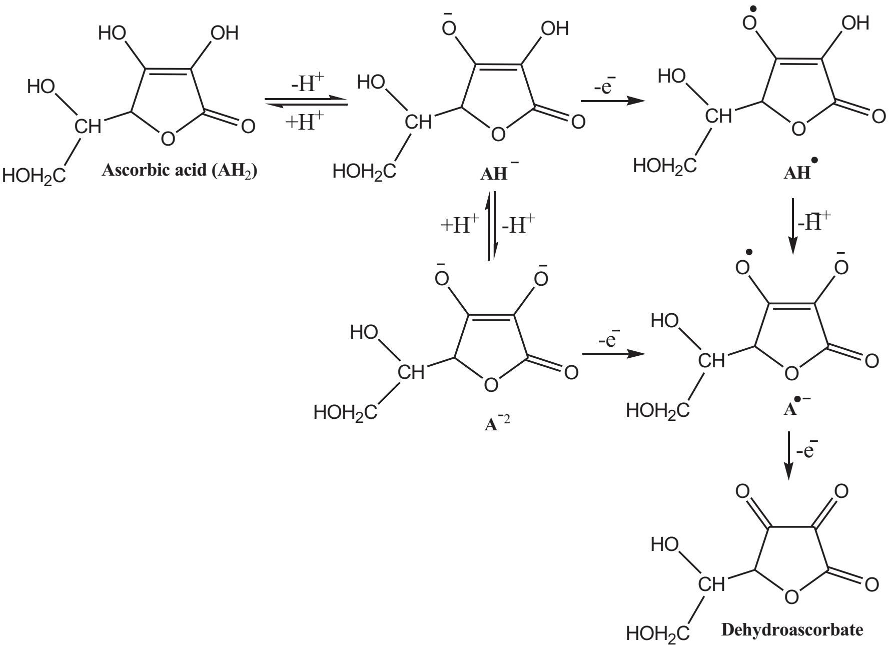

Fig. 20.5 Plausible free radical capturing mechanism of ascorbic acid

It has been indicated that ascorbic acid is centrally correlated with different physiological processes that involved plant growth and production (Hameed et al., 2012; Younis et al., 2010) and rapidly reached the target area owing to its greater solubility in water (Herschbach et al., 2010). Therefore, foliar application of ascorbic acid improved salt tolerance of crop plants in a number of ways (Athar et al., 2008; Dolatabadian et al., 2008; El Hariri et al., 2010; Farahat et al., 2013).

Chemically, ascorbic acid acts as a strong reducing agent and oxidized reversibly to dehydroascorbic acid. The investigation on the interactions of various chemicals with ascorbic acid and metal ions has shown that ascorbic acid, its oxidation product (dehydroascorbic acid), and intermediate, monodehydroascorbic acid free radical might function as cycling redox couples in electron transport and membrane electrochemical potentiation. It quenches oxidizing free radicals and other highly reactive oxygen-derived species such as the hydrogen peroxide, hydroxyl radical, and singlet oxygen by inactivating them in water-soluble compartments such as the plasma, cytosol, and extracellular fluid (Nimse & Pal, 2015).

Exogenous ascorbic acid application induces salt tolerance in wheat and improved Na^+ ions accumulation, leaf chlorophyll contents, and photosynthetic machinery (Akram et al., 2017; Khan et al., 2006). Folair application of ascorbic acid enhanced the plant biomass accumulation, photosynthetic pigments, and absorption of potassium and calcium ions (Khan et al., 2013a, b).

Application of ascorbic acid on roots not only enhanced the root growth, antioxidant activities, and photosynthetic rate but also improved the antioxidant activities (Athar et al., 2009). Ascorbic acid helped in the accumulation of potassium and calcium ions in the leaves; however, application of ascorbic acid on roots did not improve the growth of salt-stressed wheat plants (Athar et al., 2008). The exogenous application of ascorbic acid on leaves or via irrigation accelerated the antioxidant activities with enhanced contents of proline in wheat (Batool et al., 2012). The pre-treatment of barley with ascorbic acid improved seed germination traits, early growth, biomass accumulation, and anatomical features of barley under saline conditions (Çavuşoğlu & Bilir, 2015).

Foliar spray of ascorbic acid on *Cyamopsis tetragonoloba* grown under sea salt irrigation improved plant growth, photosynthetic pigments, protein contents, and potassium contents (Gul et al., 2015). Similarly, ascorbic acid improved seed germination, growth, yield, and ionic composition of eggplant under salt stress (Jan et al., 2016).

Salicylic Acid

Salicylic acid is one of the important antioxidants owing to its involvement in endogenous signal mediating local and systemic plant defense response against stresses. Salicylic acid is a growth regulator that promoted the growth of plants under stress and non-stress conditions (Rivas-San & Plasencia, 2011) (Fig. 20.3). Salicylic acid acts as a potential nonenzymatic antioxidant, which plays a key role in regulations of various physiological processes in crop plants (Jayakannan et al., 2015; Arfanet al., 2007). It has also been found that plants release salicylic acid in response to multiple abiotic stresses such as heavy metal toxicity, water stress or drought, chilling stress, temperature, and osmotic stress (Jayakannan et al., 2015). Some earlier reports showed that exogenous application of salicylic acid could minimize the damaging effect of drought on wheat (Waseem et al., 2006) and heavy metals in rice (Khan et al., 2015).

Salicylic acid is a phenolic compound involved in many physiological and biochemical processes such as nitrogen metabolism, photosynthesis, proline metabolism and production of antioxidant system, glycine betaine, and plant water relations under stress conditions and thereby provided protection in plants against abiotic stresses (Viehweger, 2014; Miura & Tada, 2014; Khan et al., 2013a, b). In another study, salicylic acid was reported to induce salinity tolerance and increased biomass of *Torreya grandis* owing to improved chlorophyll content and antioxidant activity that eventually alleviated the oxidative stress (Li et al., 2014a, b).

The deficiency of salicylic acid in plants could make the effects of salt stress more worse and lead to substantial decline in plant growth (Mirdehghan & Ghotbi, 2014). Salicylic acid-induced pre-adaptation status in plants remained helpful in the acclimation to subsequent salt stress via reducing lipid peroxidation in terms of reduced malondialdehyde (MDA) content (Li et al., 2014a, b; Deng et al., 2012). In wheat, exogenous salicylic acid negated the salt stress-induced growth inhibition (Arfan et al., 2007).

Salicylic acid has variable effects on plants regarding plant adaptation to salt stress; however, the magnitude of protective effects depends on plant species, application dose, application method, and time of application (Metwally et al., 2003). Salicylic acid has obtained special attention owing to its protective effects on plants under NaCl salinity. Several studies have shown that the effects of cytotoxicity induced by salt stress can be overcome by exogenous application of salicylic acid (Dong et al., 2015). Salicylic acid can also act as an endogenic phytohormone, which may regulate various physiological and biochemical processes in plants (Abedini & Hassani, 2015). Foliar application of salicylic acid promoted growth, enzymatic, and photosynthetic activities in salt-stressed sunflower plants (Noreen et al., 2009). Foliarly applied salicylic acid on maize grown in saline soil showed positive effect at the vegetative stage of maize plants. Exogenous salicylic acid application prominently improved sugar, protein, and proline contents and antioxidant enzyme activities. On the other hand, chlorophyll, carotenoids, osmotic potential, and membrane stability index were reduced (Fahad & Bano, 2012).

In addition, exogenous application and salicylic acid concentrations significantly improved plant growth and development (Akhtar et al., 2013). The foliar spray of salicylic acid also protected citrus seedlings subjected to salt stress. Growth, chlorophyll (Chl) contents, relative water contents (RWC), maximal quantum yield of PS-II photochemistry, and gas-exchange attributes were negatively affected by salinity. In addition, cell membrane damage and proline contents were enhanced by salinity. It appeared that the best ameliorative remedies of salicylic acid were obtained when citrus seedlings were sprayed by 0.50 and 1.0 mM salicylic acid solutions (Khoshbakht & Asgharei, 2015). Cucumber seedlings were treated with foliar salicylic acid applications at low concentrations, and it was noted that salt stress negatively affected the growth, chlorophyll content, and mineral uptake of cucumber plants. However, foliar applications of salicylic improved plant biomass accumulation. Moreover, salicylic acid application improved water contents of salt-stressed cucumber plants and reduced electrolyte leakage (Yildirim et al., 2008).

Tocopherols

Tocopherols (Fig. 20.6) are lipophilic antioxidants, which are synthesized in plants and some photosynthetic microorganisms. Four isoforms (*α, β, γ, δ*) of tocopherols and tocotrienols, which vary in the positions and number of methyl groups in the chromanol ring, are found in nature (Eitenmiller et al., 2007). Plants mainly

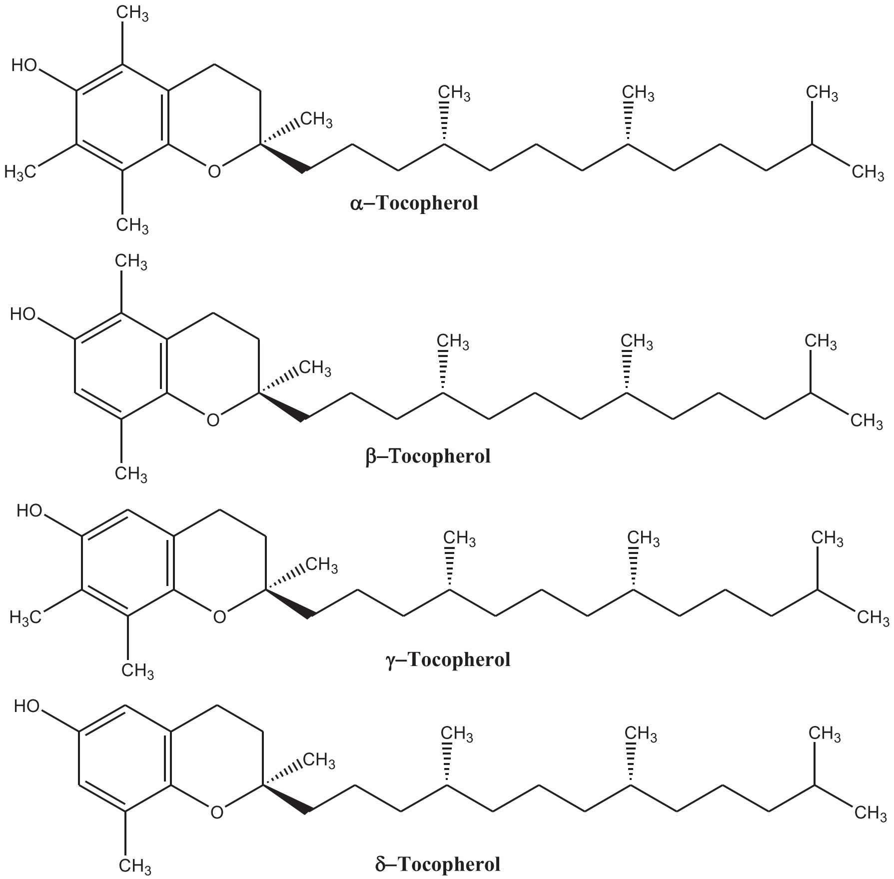

Fig. 20.6 Structures of important tocopherols

accumulated tocochromanols to reduce the lipid oxidation (Falk & Munné-Bosch, 2010). Some evidences suggested that the effectiveness of antioxidant may vary between natural and synthetic source of tocochromanols (Ahsan et al., 2015). To date, little is known about the specific roles of α- and γ-tocopherols in different plant tissues. Tocopherol biosynthesis happens at inner envelope membrane of chloroplasts of photosynthetic organisms (Fritsche et al., 2014), which provides protection to photosynthetic machinery from oxidative damage and lipid peroxidation owing to enhanced ROS production under stress conditions. The important aspect of the biosynthetic pathway of tocopherols in plants has already been identified, whereas the enzyme tocopherol cyclase has been identified as a key enzyme of tocopherol biosynthesis (Ali et al., 2015).

Up to the 1990s, the function of α-tocopherol in plants is believed to be associated only with antioxidant activity and maintenance of membrane integrity. Later on, it was found that α-tocopherol has the ability to transmit cellular signals in plants as well as in animal cells. Experiments performed on mutant plants, which are unable to synthesize tocopherols, have proved this assumption. Tocochromanols are the most effective group of lipophilic phenolic antioxidants, which protect key cell components by neutralizing free radicals before they can cause damage to cellular structures and functions (Espinosa-Diez et al., 2015).

Among tocopherols, α-tocopherols (vitamin E), which contain three methyl groups, have an excellent antioxidant activity (Kamal-Eldin & Appelqvist, 1996). Protective mechanism of vitamin E is the quenching of ROS and removal of the polyunsaturated fatty acid radical species (Fig. 20.7), which are generated during lipid peroxidation (Shin et al., 2016; Raederstorff et al., 2015; Munne-Bosch, 2013; Bramley et al., 2000).

Vitamin E reduced the effect of seawater stress on growth, yield, and physiological and antioxidant responses of faba bean plant. Similarly, foliar application with α-tocopherol on faba bean plants alleviated injuries and caused diluted seawater irrigation. The positive effects are related to the enhancement of protective parameters such as antioxidant enzymes, proline, carotenoids, and inorganic ions ($K^+$and Ca^{2+}). Tocopherols also improved faba bean plant growth, yield, and quality of seeds (Orabi & Abdelhamid, 2016). Foliar application of tocopherols increased relative growth rate, plant nitrogen contents, and net assimilation rate and showed positive changes in all other parameters and productivity of soybean plants when grown under irrigation with moderately saline water (Rady et al., 2015). The antioxidants appraised to alleviate salinity-induced stresses in plants, which has been mentioned in Table 20.1.

Exogenous application of α-tocopherols substantially improved salt stress tolerance in onion plants by inhibiting endogenous H_2O_2 and lipid peroxidation and enhancing enzymatic (i.e., SOD, CAT, APX, and GR) and nonenzymatic (i.e., ascorbic acid and glutathione) antioxidant activities. Moreover, α-tocopherol application improved photosynthetic efficiency and plant water status. Therefore, foliar application of α-tocopherols could be used to induce salt tolerance in plants (Semida et al., 2014).

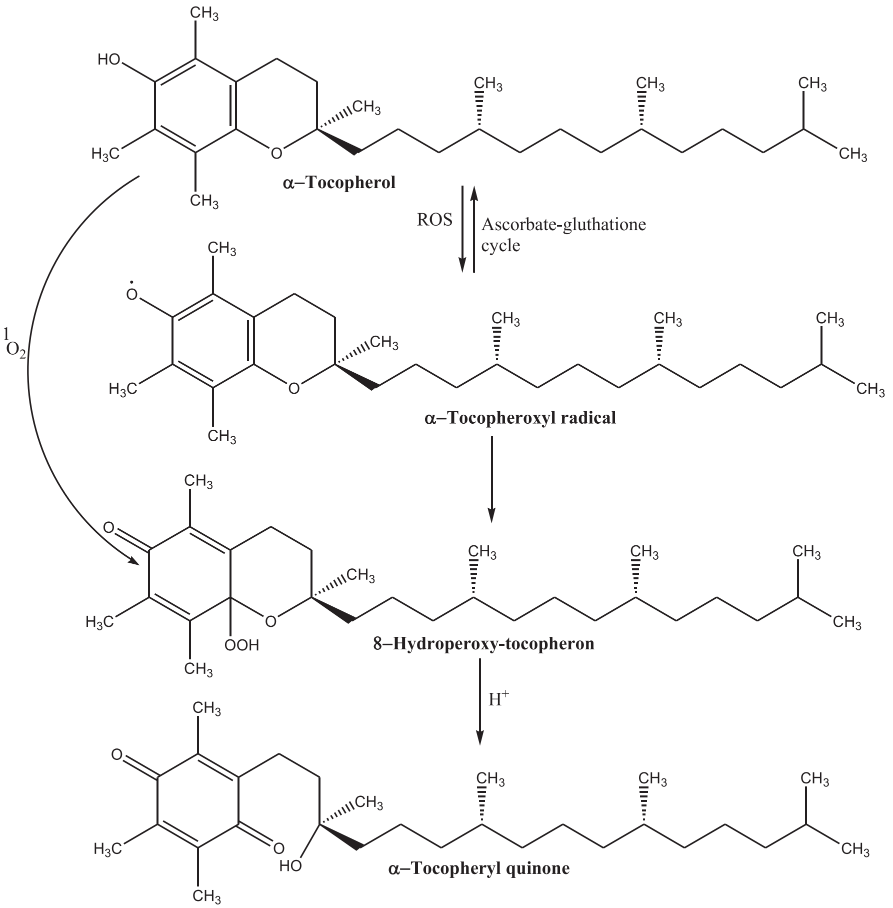

Fig. 20.7 Conversion of α-tocopherol into α-tocopherylquinone by its reaction with ROS

Anthocyanins

Anthocyanins are water-soluble, polar, and pigmented flavonoids (Bendary et al., 2013; Prior, 2006; Harborne, 1998; Holton & Cornish, 1995), which also contributed to the antioxidant properties (Longo & Vasapollo, 2006) in plants grown under saline conditions. Major sources of anthocyanins are cherries, strawberries, blueberries, raspberries, purple grapes, and black currants (Mazza, 2007) and found in the vacuoles of the epidermal and mesophyll cells (Chalker-Scott, 1999). Anthocyanins accumulated in expanding juvenile tissues and autumnal senescing leaves of deciduous species under stress (Amal et al., 2015; Close & Beadle, 2003). Anthocyanin supplementation through foods and beverages plays an important role in the prevention of diverse cardiovascular diseases, cancer, and a plethora of other diseases due to their strong antioxidant, detoxification, anti-proliferation, anti-angiogenic, and anti-inflammatory activities (Ames et al., 1993; Nikkhah et al., 2008).

Table 20.1 Antioxidants used in various crops to alleviate salinity-induced effects

Sr. No.	Antioxidant	Crop	Mode	References
1	Ellagic acid	*Cicer arietinum* (chickpea)	Seed soaking	El-Soud et al. (2013)
2	Ascorbic acid	*Triticum aestivum* (wheat)	Foliar	Khan et al. (2006)
			Rooting	Athar et al. (2008)
			Seed soaking	Athar et al. (2009)
		Helianthus annuus (sunflower)	Foliar	Khan et al. (2013a, b)
		Saccharum spontaneum (wild sugarcane)	Foliar/rooting	Batool et al. (2015)
		Solanum melongena (eggplant)	Foliar	Elwan et al. (2007) and Jan et al. (2016)
		Hordeum vulgare (barley)	Seed soaking	Çavuşoğlu and Bilir (2015)
		Cyamopsis tetragonoloba (guar)	Foliar	Gul et al. (2015)
		Sesamum indicum (sesame)	Seed soaking	Tabatabaei and Naghibalghora (2013)
3	Salicylic acid	*Helianthus annuus* (sunflower)	Foliar	Noreen et al. (2009)
		Zea mays (maize)	Foliar	Fahad and Bano (2012)
		Vigna radiata (mung bean)	Foliar	Akhtar et al. (2013)
		Citrus sinensis (citrus)	Foliar	Khoshbakht and Asgharei (2015)
		Cynara scolymus (artichoke)	Foliar	Bagherifard et al. (2015)
		Cucumis sativus (cucumber)	Foliar	Yildirim et al. (2008)
4	Tocopherols	*Vicia faba* (horsebean)	Foliar	Orabi and Abdelhamid (2016)
		Glycin max (soybean)	Foliar	Rady et al. (2015)
		Mung bean seedling (*Vigna radiata*)	Soaking	Farheen et al. (2018)
		Allium cepa (onion)	Foliar	Semida et al. (2014)
		Linum usitatissimum (Linseed)	Foliar	Sadak and Dawood (2014)
5	Anthocyanin	Role of anthocyanin in plant defense	Endogenous	Lev-Yadun and Gould (2009)
		Higher plants	Endogenous	Eryılmaz (2006)
		Hibiscus esculentus (okra)	Endogenous	Dkhil and Denden (2012)
		Triticum aestivum (wheat)	Endogenous	Tereshchenko et al. (2012)
		The photoprotective role of anthocyanin pigments in leaf tissues	Endogenous	Hughes (2007)
6	Flavonoids	Flavonoids production in plants under stress condition Giovanni	Endogenous	Agati et al. (2013)

(continued)

Table 20.1 (continued)

Sr. No.	Antioxidant	Crop	Mode	References
7	Brassinosteroids	*Triticum aestivum* (wheat)	Foliar spray	Shahbaz and Ashraf (2007) and Eleiwa et al. (2011)
			Seed soaking	El-Feky et al. (2014)
		Lycopersicon esculentum (tomato)	Foliar	Behnamnia et al. (2015)
		Leymus chinensis Trin.	Foliar	Niu et al. (2016)
		Oryza sativa (rice)	Seed soaking	Sharma et al. (2015)
		Citrullus lanatus (water melon)	Spray	Cheng et al. (2015)
		Lycopersicon esculentum (tomato)	Spray	Slathia et al. (2012)
		Leymus chinensis (Chinese lyme grass)	Spray	Jin et al. (2015)
		Zea mays (maize)	Foliar	Anjum et al. (2011)
		Solanum lycopersicum (garden tomato)	Foliar	Hayat et al. (2012)
		Gossypium hirsutum (cotton)	Seed soaking	Fathima et al. (2011)
		Raphanus sativus (radish)	Seed soaking	Mahesh et al. (2013)
8	28-HBL	*Cicer arietinum* (chickpea)	Seed soaking	Ali et al. (2007)
		Zea mays (maize)	Seed soaking	Arora et al. (2008)
		Triticum aestivum (wheat) *Brassica juncea* *Triticum aestivum* (wheat)	Foliar Foliar	Eleiwa et al. (2011) Hayat et al. (2012)
			Foliar	Yusuf et al. (2011)
			Seed soaking	El-Feky (2014)
		Brassica juncea (Chinese mustard)	Foliar	Alyemeni et al. (2013)
9	24-EBL	*Pisum sativum* (garden pea)	Rooting	Fedina (2013)
		Pisum sativum L. (pea)	Seed soaking	Shahid et al. (2011)
		Cucumis sativus (cucumber)	Foliar spray	Fariduddin et al. (2013)
		Solanum melongena (eggplant)	Rooting	Wu et al. (2017)
			Rooting	Ding et al. (2012)
		Cajanus cajan (pigeon pea)	Rooting	Dalio et al. (2013)
		Capsicum annuum (pepper)	Foliar	Abbas et al. (2013)
		Lactuca sativa	Foliar and Seed soaking	Ekinci et al. (2012)
		Brassica juncea	Seed soaking	Kaur et al. (2018a, b)
		Triticum aestivum (wheat) *Triticum aestivum* (wheat)	Foliar	Qayyum et al. (2007) and Shahbaz et al. (2008)
			Foliar	Talaat and Shawky (2013)
		Acacia gerrardii	Foliar	Abd Allaha et al. (2018)
		Cucumis sativus (cucumber)	Foliar	Anwar et al. (2018)
		Phaseolus vulgaris	Foliar	Rady (2011)
		Hordeum vulgare (barley)	Seed soaking	Kartal et al. (2009)
		Cajanus cajan	Rooting	Dalio et al. (2011)
		Fragaria x ananassa	Foliar	Karlidag et al. (2011)
		Grass seedlings	Rooting	Wu et al. (2017)

The promising antioxidant properties of anthocyanins in humans caused also an interest to study their role on plant growth under saline conditions. However, the ecophysiological roles of anthocyanins are manifold as compatible solutes in osmotic regulation, antioxidants, and photoprotectants by masking photosynthetic pigments and capturing ROS (Carletti et al., 2013; Hatier & Gould, 2008; Nakabayashi et al., 2014; Steyn et al., 2002). Anthocyanins are well recognized as an important component of *Quinoa* grains owing to their high nutritional value and health benefits (Alvarez-Suarez et al., 2014). The induced synthesis and accumulation of anthocyanins under stress at grain filling could be an important functional trait for grain nutritional quality of *Quinoa*. Anthocyanin captures free radicals generated from the cyanidin oxidation (Castañeda-Ovando et al. (2009) as well as defends plants against environmental stresses such as ultraviolet radiation, drought, temperature variations, and attraction of pollinators (Chalker-Scott, 1999; Close & Beadle, 2003; Leão et al., 2014; Stone et al., 2001).

Anthocyanins improve drought resistance in plants due to its ability to stabilize the water potential and thus hypothesized to be involved in osmotic regulation (Chalker-Scott, 2002; Oosten et al., 2013). Ploenlap and Pattanagul (2015) suggested that the increase in anthocyanin levels under water stress is mainly due to the photoprotection of chlorophylls by anthocyanins. The anthocyanin level was increased in the juvenile leaves under drought stress, however the accumulation of anthocyanins inhibited under severe stress conditions. Similarly, flavonoids with radical scavenging activity mitigated oxidative and drought stress in *Arabidopsis thaliana* (Nakabayashi et al., 2014). Moreover, it has been demonstrated that anthocyanins are potent antioxidants, displaying up to four times the ROS scavenging potential of trolox (Wang et al., 1997), an industry standard in gauging antioxidant potential. Moreover, in vivo monitoring of an oxidative burst (following mechanical wounding) showed that H_2O_2 decreased more rapidly in red (anthocyanic) *Pseudowintera colorata* leaves than green ones. While the vacuolar storage of anthocyanins was found against their action as direct scavengers of ROS produced in the chloroplast, possibly due to cytoplasmic anthocyanins, which act as antioxidants. For example, Zhang et al. (2012) showed that leaves of an acyanic *Arabidopsis thaliana* mutant subjected to a high irradiance displayed a reduced DPPH (2,2-diphenyl-1-picryl-hydrazylhydrate) scavenging potential and increased oxidative damage (estimated by cell membrane permeability) as compared to wild-type anthocyanic leaves. It was further observed that anthocyanins in *Sambucus* spp. peduncles are responsible for ameliorating light stress during senescence, and anthocyanins may additionally prolonged the senescence period. This dichotomy in anthocyanin research is unwarranted, and its significance is still poorly acknowledged. Identification and exploration of those functions that anthocyanins perform in either reproductive or vegetative organs are necessary to understand the adaptive significance of anthocyanin production in plants.

Brassinosteroids

Brassinosteroids belong to a group of steroid plant hormones with significant growth promoting potential (Bishop & Yokota, 2001; Clouse & Sasse, 1998; Chory et al., 1989). Brassinosteroids have multiple effects on seed germination, growth, leaf abscission, and senescence (Sasse, 1997) although its mechanism is still obscure (Mathur et al., 1998). Moreover, brassinosteroids exert anti-stress effects on plants such as those caused by cold, heat, drought, and salt (Anuradha & Rao, 2003; Dhaubhadel et al., 2002; El-Feky, 2014; Kagale et al., 2007; Ogweno et al., 2008; Sharma et al., 2018). The brassinosteroids' stress response is an intricate sequence of biochemical reactions such as induction of protein biosynthesis, activation or suppression of key enzymatic reactions, and the production of multiple chemical defense compounds (Bajguz & Hayat, 2009; Jin et al., 2015).

Exogenous applications of brassinosteroids under salinity have long been known to improve growth and yield in many economically useful plant species (Cheng et al., 2015). In cereals, brassinosteroids promoted growth and yield attributes, whereas in leguminous crops, the number of pods per plant and total seed yield remained higher after the exogenous application of brassinosteroids (Rao et al., 2002). Growth and seed yield of rapeseed plants were also promoted by brassinosteroid application (Hayat et al., 2012; Sharma et al., 2018), and the same was reported for seed yield in cotton (Ramraj et al., 1997). Brassinosteroids removed the salinity-induced inhibition of seed germination and seedling growth in rice (*Oryza sativa*) and improved the chlorophyll biosynthesis and enhanced nitrate reductase activity under salt stress(Anuradha & Rao, 2003; Bajguz & Hayat, 2009).

Furthermore, brassinosteroids had no prominent effect on the leaf cell ultrastructure under normal conditions; however, damages imposed by salt stress on nuclei and chloroplasts were significantly reduced by brassinosteroid treatment in barley (Krishna, 2003). When salt solution was supplemented with brassinosteroids, the inhibitory effect of salt on rice seed germination was considerably reduced that is possibly associated with enhanced levels of nucleic acids and soluble proteins (Anuradha & Rao, 2009). The exogenous application of 28-homobrassinolide on *Pusa Basmati*$^{-1}$, a commercially important rice variety, resulted in reduced growth and protein and chlorophyll contents and increased proline and MDA contents of at early growth stages (Sharma et al., 2015).

The plants resulting from the seeds soaked in 28-homobrassinolide exhibited higher activities of nitrate reductase (23%) and carbonic anhydrase (31%), improved dry mass (34%) and nodule number (30%), content of leghemoglobin (28%), and nitrogenase activity (30%), while contents of nodule nitrogen and carbohydrate were decreased by 5% and 6%, respectively, with ultimate increase in yield (26%) in chickpea (Ali et al., 2007). The structures of some important brassinolides are given in Fig. 20.8.

In addition, the activities of antioxidative enzymes and protein contents were promoted in 28-HBL-treated maize plants. Moreover, application of 28-HBL reduced lipid peroxidation in salt-treated maize plants (Arora et al., 2008). Similarly,

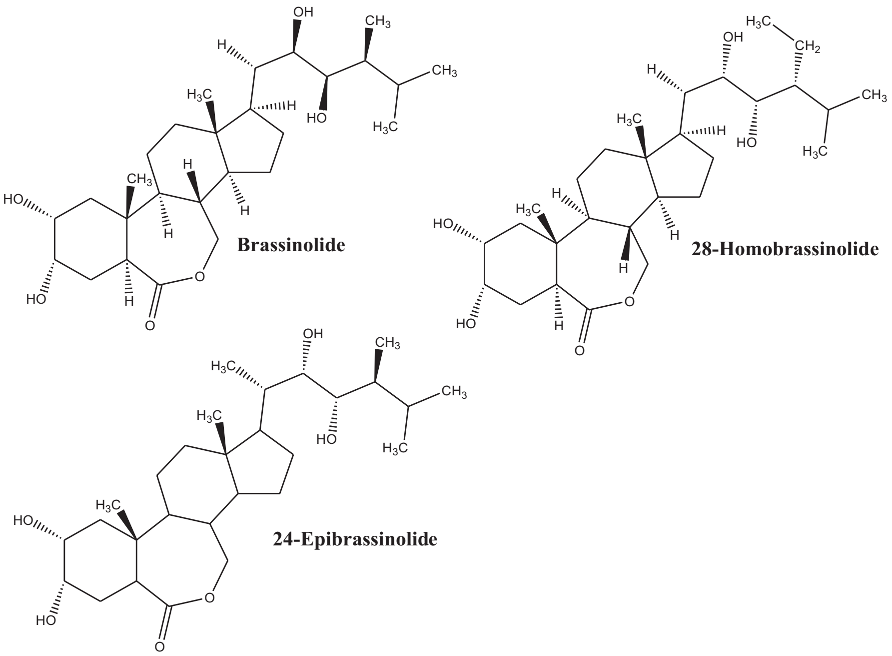

Fig. 20.8 The structures of some selected brassinolides

foliar spray of 28-HBL increased growth and yield attributes and photosynthetic pigments in wheat (Eleiwa et al., 2011). Activities of nitrate reductase and carbonic anhydrase, photosynthetic rate, and seed yield were decreased along with content of chlorophyll under salt stress; however, application of 28-HBL solutions stimulated morpho-physiological attributes in *Brassica juncea* (Alyemeni et al., 2013). Application of 24-epibrassinolide (24-EBL) attenuated the hostile effects of salinity on *Eriobotrya japonica* plants; however, the effect of 24-EBL was significant at 0.5 mgL^{-1} under saline conditions (Sadeghi & Shekafandeh, 2014; Xue, 2012). Similarly, the grass seedlings were treated with 24-EBL and induce salinity tolerance (Wu et al., 2017). In similar studies, foliar spray of 24-EBL improved growth parameters of wheat and *Acacia gerrardii* plants significantly under saline and non-saline conditions; however, there was no prominent increase in the mineral contents of wheat plants (Abd Allaha et al., 2018; Ali et al., 2006; Shahbaz et al., 2008; Shahbaz & Ashraf, 2007). Exogenous application of brassinolide (1.0 mgL^{-1}) enhanced growth, carbohydrate, and total soluble proteins in roots and shoots of wheat and improved the activities of hydrolytic enzymes, amylase, and protease as well under salt stress (El-Fekyl, 2014; Durigan et al., 2011). No doubt, brassinosteroids alleviated the inhibitory effects of salinity on germination, seedling growth, and crop yields; however, further studies are needed to uncover the tolerance mechanism imparted by brassinosteroids under stress conditions.

Carotenoids

Carotenoids are among the most important nutrients in food and found in all plants as natural pigments. They are derived from acyclic C40 isoprenoid lycopene that can be classified as a tetraterpene (Heider et al., 2014). Carotenoids are lipophilic microconstituents that have beneficial effects on human health and provide protection against cancer, cardiovascular diseases, and muscular regeneration (Rao & Rao, 2007; Sommer & Vyas, 2012). Till date, there are approximately 700 known carotenoids that can be categorized as *α*-carotene, *β*-carotene, and lycopene and xanthophylls (zeaxanthin, lutein, and *β*-cryptoxanthin), which denote the oxygenated carotenoids fraction. The *α*-carotene, *β*-carotene, and *β*-cryptoxanthin are promoters of vitamin A and are represented in Fig. 20.9.

In plants, carotenoids function a crucial role in protecting chlorophyll owing to their antioxidant properties, and the endogenous carotenoid contents are affected by several factors such as environmental, genetic, or man-made strategies (Fanciullino et al., 2006). The carotenoid-rich extract is usually used in food supplements, food additives, medicines, and cosmetics (Mezzomo & Ferreira, 2016).

The extent of expression of carotenogenic genes varied with stress conditions. For instance, carotenoid molecules present in the tissues are capable of neutralizing

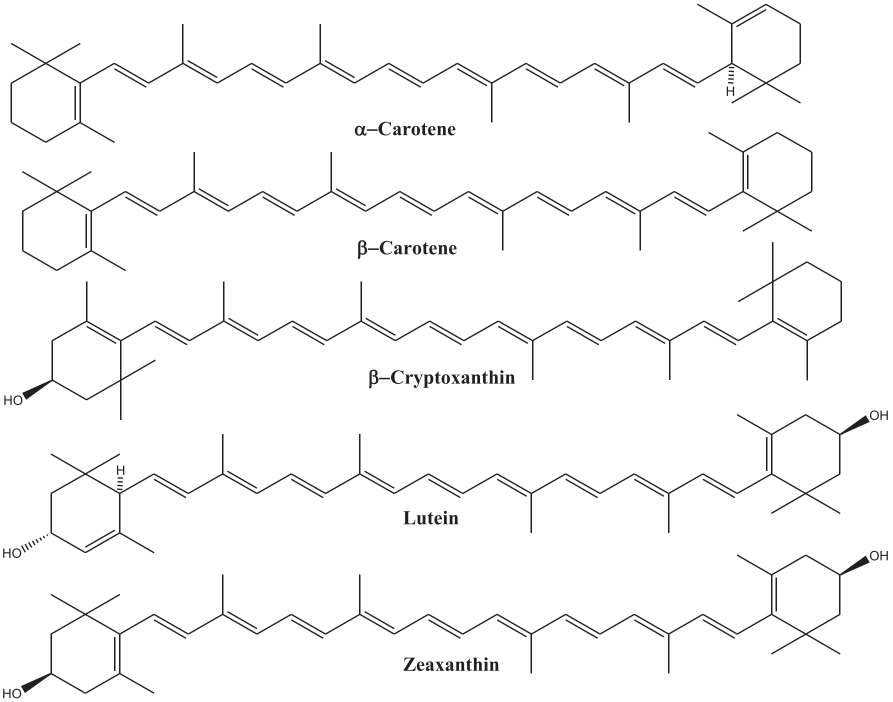

Fig. 20.9 The structures of some selected carotenoids

ROS; however, the mechanism of action of these molecules is based on the modifications of the cell metabolic functions, aimed at interacting with the polyunsaturated acyl groups of lipids to stabilize membranes and playing a protective role against ROS and synergic function with other antioxidants (Raposo et al., 2015). Table 20.2. shows uses and sources of some selected antioxidants.

Table 20.2 Sources and uses of some important antioxidants

Serial No.	Compound	Class	Sources	Uses	References
1	Ellagic acid	Polyphenolic	Vegetables, nuts, grapes, pomegranate, berries	Antioxidant, antiviral, antibacterial, anti-inflammatory, anticancerous	Galano et al. (2014), Bhandari (2012) and Malini et al. (2011)
2	Ascorbic acid	Vitamin	All plant parts except dry seeds, citrus fruit	Antioxidant, growth regulator, anti-saline	Kumar et al. (2014), Hameed et al. (2015) and Klause et al. (2016)
3	Salicylic acid	Phenolic carboxylic acid	Berries, dates, grapes, guavas, apricots, green pepper, olives, tomatoes	Antioxidant, growth regulator, anti-saline	Viehweger (2014), Li et al. (2014a, b) and Abedini and Daie Hassani (2015)
4	Tocopherols	Phenolics	Vegetables and vegetable oils, nuts, grains	Antioxidants	Ahsan et al. (2015), Fritsche et al. (2014) and Raederstorff et al. (2015)
5	Anthocyanins	Flavonoids	Blueberries, cherries, raspberries, strawberries, black currants, purple grapes, red wine	Antioxidant, detoxification activity, anti-proliferation activity, anti-angiogenic activity, anti-inflammatory activity	Chukwu et al. (2012), Leão et al. (2014) and Nakabayashi et al. (2014)
6	Brassinosteroids	Steroid	Plants	Antioxidant, anti-stress, enhanced growth and yield	El-Feky (2014), Cheng et al. (2015) and Jin et al. (2015)
7	Carotenoids	Tetraterpenes	Tomatoes, carrots, and apricots, all fruit or vegetable	Anticancer, cardioprotective, antioxidant	Fanciullino et al. (2006)

Enzymatic Antioxidants

The ROS are the by-products of aerobic metabolism, and their production is generally enhanced under stress conditions (Ashraf & Harris, 2013; Gónmez-Bellot et al., 2013; Mugnai et al., 2009) through enhanced oxidizing metabolic activities occurring in chloroplasts, mitochondria, and microbodies and disruption of electron transport system (Pinheiro & Chaves, 2011). In this context, enzymatic antioxidants, that is, CAT, POX, and SOD, served as efficient ROS scavenging systems to evade the oxidative damage (Mittler et al., 2011; Saisanthosh et al., 2018) in plants under stress conditions.

Catalases (CAT)

The CAT is a tetrameric protein of 244 kDa comprising four identical subunits of 59.7 kDa, and each subunit contains 527 amino acid residues, one haem group, namely, iron (III) protoporphyrin IX, and a tightly bound molecule of NADPH (Sofo et al., 2015). Stress conditions predispose the photosynthetic system of leaves to photoinhibition resulting in a light-dependent inactivation of the primary photochemistry associated with photosystem II (Ashraf & Harris, 2013). At low concentrations, H_2O_2 acts as a signal molecule involved in the regulation of growth and development, specific biological/physiological processes, cell cycle, photosynthetic functions, and plant responses to biotic and abiotic stresses (Kovalchuk, 2010; Seki et al., 2007; Vadez et al., 2012). Oxidative stress and eventual cell death in plants can be caused by excess H_2O_2 accumulation. Since stress factors provoked production of H_2O_2 in plants, severe damage to biomolecules can be possible due to enhanced and non-metabolized cellular H_2O_2 (Sofo et al., 2015;Foyer & Shigeoka, 2011; Apel & Hirt, 2004). Considering the key role of CAT in photorespiration, many authors focused on the role of CAT-catalyzed pathway under both drought and salt stress. Indeed, the maintenance of CAT activity in leaves of drought-stressed plants likely allowed the removal of photorespiratory H_2O_2 produced (De Pinto et al., 2013). Under stress conditions, the photorespiration works as energy sink preventing the over-reduction of the photosynthetic electron transport chain and photo-inhibition (De Pinto et al., 2013). On this basis, photorespiration and CAT pathway cannot be considered wasteful processes but appreciated as a key subsidiary component of photosynthesis and important parts of stress responses in green tissues for preventing ROS accumulation (Bauwe et al., 2012; Voss et al., 2013).

Enzymes, that is, APX, GPX, and CAT, are able to scavenge H_2O_2 with different mechanisms. Regulation of the CAT gene expression played an important role in the levels of CAT activity. The catalase gene expression is regulated by various mechanisms involving peroxisome proliferator-activated receptors (Ford et al., 2011; Sofo et al., 2015).

Peroxidases

Peroxidases (POXs) having molecular weight ranging from 30 to 150 kDa are widely distributed in nature. The POXs are involved in the detoxification of toxic pollutants, and its detoxification ability is dependent upon the reduction of peroxides such as H_2O_2 (Saxena et al., 2011). These enzymes are produced by a variety of sources including plants, animals, and microbes, whereas POXs have the potential for bioremediation of wastewater contaminated with phenols, cresols, and chlorinated phenols used for biopulping and biobleaching in paper industry (Malar et al., 2014). Moreover, the POXs are also used as biosensors. The term POX represents a group of specific enzymes such as NADH-POX, glutathione-POX, and iodine-POX as well as a variety of nonspecific enzymes that are simply known as POXs. These oxidases and POXs have been reported as excellent antioxidants to degrade dyes (Caverzan et al., 2012). Specifically, the POX activity involved donating electrons that are bound to other substrates such as ferricyanide and ascorbate to break them into harmless components. Moreover, the POX donates two electrons to reduce peroxides by forming selenols and eliminates peroxides as potential substrate for the Fenton reaction (Liochev & Fridovich, 2003, 2010).

In addition, the use of POX for the degradation of pollutants has thrown more light on sustainable bioremediation strategies for polluting compounds and environmental protection using different enzymes. Environmental protection is influenced by interwoven factors such as environmental legislation, ethics, and education. Each of these factors played an important role in influencing national-level environmental decisions and personal-level environmental values and behaviors. For environmental protection to become a reality, it is important for societies and the nations to develop each of these areas that together will inform and drive environmental decisions.

Superoxide Dismutase (SOD)

Plant-antioxidant defense machinery comprising antioxidant enzymes and nonenzymatic antioxidant components metabolized ROS and their reaction products to avert oxidative stress conditions (Gill & Tuteja, 2010; Hasanuzzaman et al., 2012). The SOD is a metalloenzyme and one of the most effective components of the antioxidant defense system in plant cells against ROS toxicity. The SODs catalyzed the dismutation of $O_2^{\bullet-}$ to H_2O_2 and O_2 in all subcellular compartments such as chloroplasts, peroxisomes, mitochondria, cytoplasm, nuclei, and the apoplast (Alscher et al., 2002; Gill & Tuteja, 2010). Moreover, the SODs are available at an intracellular concentration of 10^{-5} M and occur in all oxygen-metabolizing cells and all subcellular compartments (Alscher et al., 2002; Fink & Scandalios, 2002). The SODs constituted the first-line defense against abiotic stress-induced enhanced ROS production and its reaction products. Nevertheless, all the SOD isoforms are nuclear coded and, where necessary, transported to their subcellular targets by means of NH_2-terminal targeting sequences (Pan et al., 2006).

Four different isoforms of SODs have been distinguished depending on the metal at the active center, which is manganese, iron, copper, and zinc (Miller & Sorkin, 1997). Previous studies denoted that most of the SODs are intracellular enzymes; these are Cu/Zn SOD (which is also extracellular), Mn-SOD, and Fe-SOD. Cu/Zn-SODs are generally found in the cytosol of eukaryotic cells and chloroplasts. The Mn-SODs are found in mitochondria and reported in chloroplasts and peroxisomes in some plants. The dimeric Fe-SODs, which are not found in animals, have been reported in chloroplasts of some plants (Gomez et al., 2003; Droillard & Paulin, 1990; Camp et al., 1994; Fridovich, 1995; Salin & Bridges, 1980).

In summary, to detoxify ROS, enzymatic and nonenzymatic antioxidant systems become upregulated, whereas H_2O_2 is scavenged by CAT and POX. The SOD plays a determinant role in the protection against the toxic effects of oxidative stress by scavenging superoxide radicals and providing their conversion into O_2 and H_2O_2 (Verma et al., 2003; Bowler et al., 1992). Overall, the enzymatic antioxidants are first-line defense of plants against oxidative stress owing to multiple biotic and abiotic factors.

Conclusion

Throughout the world, environmental stresses are proved to be a fatal threat for agricultural productivity. Plants being sessile in nature have to face multiple abiotic stresses. Crops in arid and semiarid regions have to face uncertain periods of drought and extreme weather conditions. Thus, improving crop yields under such climatic conditions yield is vital to satisfy the increasing food demand. Phytohormones and plant growth regulators could play important role in this regard owing to their stress alleviatory role. Exogenous application of some phytohormones and plant growth regulators could substantially improve the enzymatic and nonenzymatic antioxidants to scavenge ROS and brought promising results regarding growth and productivity of crops under stress conditions. Moreover, the antioxidants play a diverse role in inducing abiotic stress tolerance in plants. Till now, a number of exogenous antioxidants have been reported, but still there is a need to discover more economical antioxidants so that they can be used for beneficial purposes.

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Chapter 21
Proteomics and Its Scope to Study Salt Stress Tolerance in Quinoa

Adnan Noor Shah, Mohsin Tanveer, Asad Abbas, Anis Ali Shah, Yasir Niaz, and Muhammad Mohsin Waqas

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Introduction

Salt stress is one of the most detrimental abiotic stresses that critically damage crops and causes major reduction in yield (Munns et al., 2006; Tanveer et al., 2017). Salt stress is characterized with deleterious effects on plant growth that are significantly associated with the production of different reactive oxygen species (which causes oxidative damage) (López-Gómez et al., 2017; Souza et al., 2012). Recent studies showed that ROS are not "bad guys" but also act as signaling compounds in plants to trigger numerous plant defense mechanisms, and those mechanisms significantly rely on the involvement of proteins. Proteins are important biological effectors in living systems, which influence a wide range of physiological and biological processes such as transportation, signaling, and/or metabolism (Scholzen & Gerdes, 2000). Proteins are involved in numerous physiological processes, which enhance tolerance against salt stress. Therefore, it is imperative to understand the role of proteins in salt stress tolerance in relation to ROS signaling (Shah et al., 2021). One technique is very helpful in answering the question "how proteins are involved in stress tolerance" called as proteomics.

Proteomics is a technique to study the function and the structural description of proteins and their higher-order complexes (Tyers & Mann, 2003). Proteomics complements other functional genomics approaches, including microarray-based expression profiles (Shoemaker & Linsley, 2002), systematic phenotypic profiles at the cell and organism level (Giaever et al., 2002), systematic genetics (Tong et al., 2001), and small-molecule-based arrays (Kuruvilla et al., 2002). This technique is well known from last two decades; during first decade, this technique was dominated by two-dimensional gel electrophoresis followed by protein staining techniques. This traditional way has been productive in providing relevant information about different biological systems, nonetheless problems found associated with sensitivity, sample conditions, and reproducibility of this method. Recent development in proteomics field results in the development of different powerful and complementary technologies, which are quite effective in quantitative comparison of protein production and expression among different biological systems (Fig. 21.1).

Proteomics Approach

Proteomics approach utilizes a combination of different techniques such as 2D gel electrophoresis, mass spectrometry, image analysis, sequencing, and bioinformatics for the quantification and characterization of proteins. During the early development era, proteomics approach was accompanied with 2D gel electrophoresis followed by staining; however, this protocol was found to be ineffective while doing comparative proteomics studies (Capriotti et al., 2015; Thelen & Peck, 2007). Some other problems, which have been encountered with such traditional proteomic technique, were problematic reproducibility of 2DE gel due to diverse protein properties, matching of 2DE spots among different spots on gel (Hajduch et al., 2007).

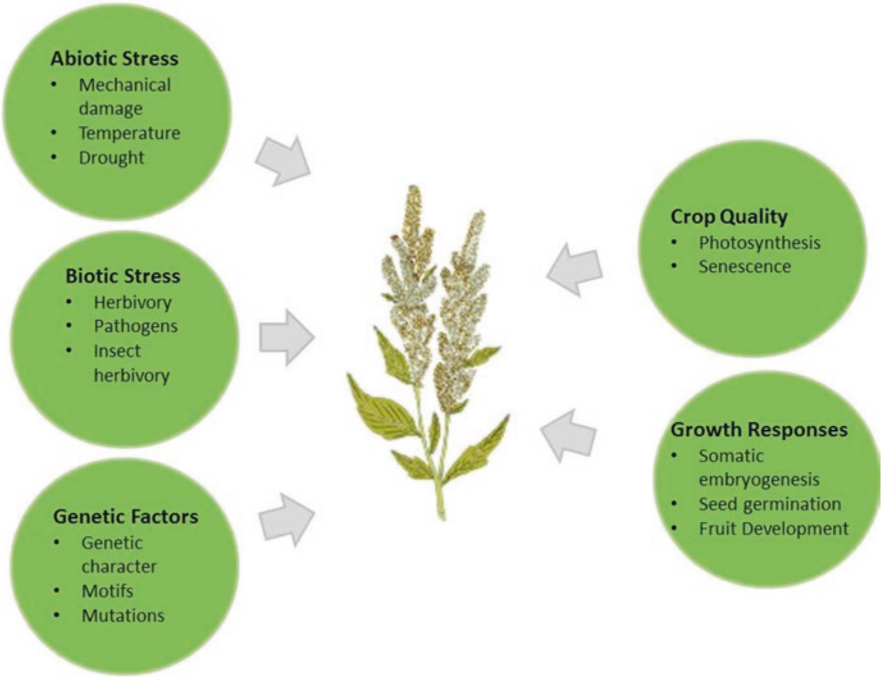

Fig. 21.1 Overview of proteomics application for quinoa crop improvement

Recent advancement in the field of proteomics results in the development of highly sophisticated protein analysis tools, software, and bioinformatics databases. These techniques are quite robust and reliable to address questions pertaining to protein involvement and salt stress tolerance. The key steps involved in the isolation, digestion, and separation by 2D gel followed by protein identification using mass spectrometry (steps illustrated in Fig. 21.2). Below section is explaining briefly each step of proteomics.

Protein Digestion and Solubilization

For effective 2DE gel electrophoresis, protein should be digested and solubilized properly; most common method to solubilize protein is protein lysis. Centrifugation is required after lysis to separate solubilized protein fragments from nonsolubilized protein fragment. These fragments can further be used to further prepare cellular compartments, for example, nuclear, chloroplastic, or mitochondrial fractions (Kleffmann et al., 2004; Rasmussen et al., 1994). It is also very important to solubilize hydrophobic and membrane-bounded protein separately, otherwise which may interact with electrophoresis. Different reagents/detergents can be used to solubilize

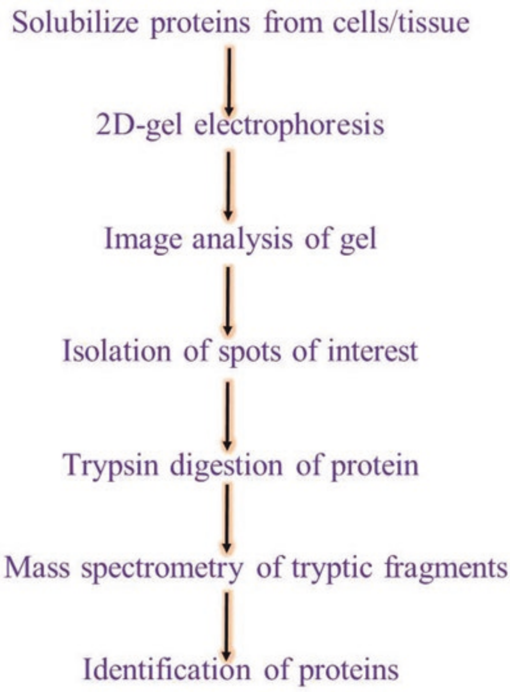

Fig. 21.2 An outline of steps involved in proteomics

protein, but the selection of reagents depends on the technique used. For instance, sodium dodecyl sulphate (SDS) is compatible with one-dimensional polyacrylamide gel electrophoresis (PAGE) but is not compatible with isoelectric focusing (IEF). Moreover, with time zwitterionic (e.g., 3- (3 - chloramidopropyl) dimethylammonio -1- propanesulphonate) or non-ionic (e.g., Nonidet P-40), buffers have been used as compared with ionic buffers (SDS).

All these three kinds of reagents can be used for one sample at one time to increase solubility, but enhanced solubility can cause problems relating to gel electrophoresis due to overloading gel with numerous overlapping proteins (Chamber et al. 2000). To solve this problem, sequential extraction of protein is very effective, which involved extraction of protein using tris base at first and then followed by extraction of insoluble protein fractions using urea and other reagents. This technique has been tested in *E. coli* and found 11 membrane proteins, and 5 out of 11 have not been identified previously (Herbet et al. 1999).

Protein Separation

Following protein solubilization, protein separation is very important step. Different methods can be used for separation, but most effective is two-dimensional polyacrylamide gel electrophoresis. This technique separates fragments using IEF and then polyacrylamide gel. This technique is very effective where fragments have to separate at specific pH (Humphrey-Smith et al., 1997; Sahu et al., 2013). This technique also helps in solving problems of pH gradient instability, pH restricted gradient, and difficulty in standardizing buffers. For visualization of proteins after 2D gel electrophoresis, different staining dyes can be used for the detection of different protein fragments. The recent development of new fluorescent dyes such as the SYPRO series of protein dyes may improve the sensitivity of protein detection without compromising mass spectrometric analysis (Steinberg et al., 1996).

Other than 2D gel electrophoresis, there is another method to separate protein fragments, which is known as 2D high performance liquid chromatography (HPLC). This system has been reported as more effective in separating fragments from a mixture of proteins (Opiteck et al., 1998; Ross et al., 2004). HPLC generate 2D chromatograms that are analogous to 2D electrophoresis gels, with discrete spots separated on an X ± Y axis. This method potentially offers higher throughput and faster sample resolution than 2D gel electrophoresis and could be very amenable to automation. Latest advancement coupled HPLC with IEF system, which resulted in better protein separation and visualization.

Image Analysis

Protein expression patterns from protein separation step are quite complex but can be analyzed by computer-based image analysis technique. Image analysis technique develops a 2D database and compares with already available databases to compare the protein size, dimension, amount, and structure. To facilitate such comparison, it is necessary to use specialized image analysissoftware. Some systems such as ELISE and HERMeS use a cluster-matching technique to compare gels, whereas GELLABE relies on the operator providing about 20 “landmark” matches, which “anchor” the two gel images under comparison. Software systems also vary in the way their databases are constructed and organized, from those using simple models, like ELISE, to those using more complex ones, like HERMes (Dunn, 1992).

Good gel software can be used to highlight protein expression patters at different level provided electrophoresis conditions are the same. Protein of interest can be excised from gel and then can be exposed to identification technique; nonetheless, recent advances in identification techniques further reduce any error, which might come during protein excitation. Now automatic proteomic systems are available, which allowed the coupling of spot-excising blot with image identifier. This type of automated machines is quite beneficial for high-throughput proteomics.

Protein Identification and Characterization

Numerous techniques are available to identify and characterize proteins. One of the most widely used methods of identifying proteins is through peptide mass fingerprinting (Henzel et al., 1993; Mann et al., 1993). After protein digestion, the masses of these peptides are then determined by mass spectrometry. This form of protein identification will only be successful if the protein being analyzed is represented in the databases. For proteins, which have incomplete sequence information, it is necessary to obtain sequence information for the protein by Edman degradation (Vandekerckhove et al., 1985) or by nanoelectrospray mass spectrometry (Wilm & Mann, 1996). This sequence information can then be used along with the mass spectrometry information to interrogate expressed sequence tag (EST) databases.

Though number of mass spectrometers are available with differences how they identify protein, MS analyses have a similar general workflow. After protein digestion, complex peptide mixture is separated by chromatography either directly coupled to the mass spectrometer (online separation) or prior to MS analysis (offline). The peptides are ionized to acquire the initial MS scan, a spectrum of the mass-to-charge ratio of peptide ions in that sample. Selected peptides from the MS scan are then individually fragmented for the MS/MS (or MS2) scan to collect amino acid sequence information about the peptides (Table 21.1).

Chenopodium quinoa and Scope of Proteomics Analysis

Quinoa (*Chenopodium quinoa* Willd.) is a pseudo-cereal of Andean origin and mainly cultivated in Bolivia, Chile, Peru, Ecuador, and Colombia on small scales, and the production has been growing in recent years. Quinoa just like buckwheat is also considered as "pseudo cereal" and quinoa produce starch-rich seed and high protein contents (Belton & Taylor, 2002). Quinoa, despite being poorly studied in the proteomics field, emerges as a good candidate for mainly three reasons: (1) It has exceptional nutritional values containing 15–17%proteins, (2) contains ten essential amino acids, and (3) is highly tolerant to numerous abiotic stress (Capriotti et al., 2015). Despite of these interesting characteristics of quinoa, very little information is available pertaining to the involvement of proteins in salt stress tolerance mechanism in quinoa so far; therefore, it would be very fruitful to employ proteomics technique and study salt stress tolerance mechanism in quinoa.

Table 21.1 Proteins responsive to salt stress in *Chenopodium quinoa*

Proteins	Stress	Specie	References
CqHSP70s	Salt stress	*Chenopodium quinoa*	Liu et al. (2018)
CqHSP20	Salt stress	*Chenopodium quinoa*	Hinojosa et al. (2018)
CqHSP83	Salt stress	*Chenopodium quinoa*	Hinojosa et al. (2018)
CqHsFs	Salt stress	*Chenopodium quinoa*	Tashi et al. (2018)

Because quinoa being non-model plant, proteomics approach can be performed by using 2DE electrophoresis, MS/MS analysis, and protein identification through a cross species homology search and de novo sequencing. This high resolving power technique is very important for protein identification and quantification. Furthermore, this technique can also distinguish between homologous and paralogous protein isoforms. This technique however is unfeasible if 2D gel would be replaced with one-dimensional SDS-PAGE due to lower resolution. Quantification needs thus to be performed at the MS level, which is challenged by reproducibility and the need to detect prototypic peptides since protein isoforms are not separated at the protein level. During protein digestion, multiple proteins digested simultaneously resulted in complex pool of multiple peptide pools that are troublesome in non-model plants such as *Chenopodium quinoa* or *Chenopodium album* for protein identification. Thus, when combination of nonrelated proteins is submitted to database, chances to find false-positive identification are more; therefore, it is crucial to analyze parent ion further by using MS/MS (Vertommen et al., 2011). During MS analysis, non-related peptides with high signal-to-noise ratio are selected for further fragmentation. In case if the peptides are less information or derived from complex mixtures, identification will fail; however, when peptide mixture is derived from a single protein, identification would be very positive (Vertommen et al., 2011). The 2DE technique would thus be an excellent choice for membrane proteomics in non-model plants.

So far, little research has been published. Capriotti et al. (2015) performed proteomics of quinoa seed and compared three different precipitation; MeOH/$CHCl_3$/double-distilled H_2O, acetone either alone or with trichloroacetic acid, and the isolated proteins were then in-solution digested, and the resulting peptides were analyzed by nano-liquid chromatography coupled to tandem mass spectrometry. Quinoa is a non-model plant, and there is no sequence available in database to match/compare the identified sequence with them. Therefore, this technique was found very useful identifying peptide (Capriotti et al., 2015). Studies from model plant showed that proteomic contributed significantly to understanding stress tolerance mechanism in model plants (Ndimba et al., 2005; Kim et al., 2006; Jiang et al., 2007). Another study showed high expression of stress-responsive protein in quinoa seed when exposed to salt stress (Aloisi et al., 2016). They used spot analysis by mass spectrometry followed by in silico analyses to identify proteins and found most proteins were related to antioxidants and flavonoids. In conclusion, lot of research is required to understand salt stress tolerance mechanism using proteomics approach.

Advantage of Proteomics over Other Techniques

Advantages of proteomics over other techniques are manifolds as follows:

- High-throughput transcriptomic analysis revealed the expression of numerous mRNA related to salt stress tolerance in plants; however, due to posttranscriptional and posttranslational modification, mRNA could not correlate with protein level. In that condition, proteomics is beneficial to encounter the protein type involved in stress signaling (Zhang et al., 2012).

- Proteomics is very useful in understanding complex mechanism of salt stress tolerance by studying plants response at very early stage (protein stage) (Zhang et al., 2012).
- Proteomics can yield new insights/pathways into the biological system, regardless of whether some information has been missed (Thelen & Peck, 2007).
- The use of mass spectrometers with high resolution and high mass accuracy allows improving the number of identified peptides and can increase the possibility of correct protein identification (Capriotti et al., 2015).

Limitations/Disadvantages

Through there are advantages, but there also some limitation in proteomics. Some of limitations are as follows:

- The inability of 2D gel electrophoresis to detect low abundance proteins.
- 2D gel electrophoresis cannot adequately separate hydrophobic proteins. Wilkins et al. (1998) reported 427 bacterial and yeast proteins and found a clear cutoff point beyond which 2D gel was unable to detect proteins.
- Abundant proteins such as beta-actin can cause problems by limiting the expression of other more minor proteins (Celis et al., 1995).
- Blackstock and Weir (1999) suggested that there are up to a quarter of the protein spots visualized in a 2D gel that are modified proteins, so it is not uncommon to isolate what is effectively the same protein from multiple spots on a gel.

Conclusion

Proteomics is very beneficial approach and has good scope in studying salt stress tolerance in plants. Quinoa being a pseudo cereal is also considered as halophytes. Proteomics can be useful in identifying proteins, which are actively involved in stress signaling, ROS production, and development of salt glands in leaves for the storage of toxic salts.

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Chapter 22
Sustainable Cotton Production in Punjab: Failure and Its Mitigating Strategies

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Introduction

Pakistan is one of the largest cotton producers and ranked fifth in cotton production and third in consumption of cotton yarn in the world (ICAC, 2019). Cotton is commonly called as "white gold" in cotton-producing regions (Razzaq et al., 2021), and it shared 33 million hectare areas worldwide in 2019 (Tarazi et al., 2019). Consumption of cotton is tremendously increasing due to a massive growth in global population (FAO, 2017). In Pakistan, out of 5 million, almost 1.3 million farmers are related to cotton cultivation, accounting for 15% of all cultivated land in the country. Cotton contributions in GDP have been dropped from 9% in 2004 to 0.8% in 2018 (Rehman et al., 2020). Total foreign exchange, which was 61.5% in 2006, also dropped to 51% in 2018.

Before the onset of cotton leaf curl virus in 1992, Pakistan has showed an incredible growth in cotton productivity, which increased from 9.6 million bales 1990 to 12.8 million bales in 1991–1992 (Pakistan Bureau of Statistics, 1997). This remarkable growth was ultimately translated in the form of a huge and widespread textile industry with almost 400 textile mills, 7 million spindles, more than 52,000 power loom including mill and non-mill sector, 700 knitwear units, 4000 garment units, more than 1000 ginning factories, and 5000 oil expellers (APTMA, 2019–2020).

Cotton production in 1947 was 0.735 million bales, yield 149 kg per hectare, and it was grown on 0.897 million hectare. This crop has gradually become the most important and major cash crop in Punjab Province. This crop was grown on 1.546 million ha with 62% increase in area in 2019–2020, production increased 575% with 4.964 million bales, and yield increased 266% with 546kg/ha (CRS, 2019–2020) (Fig. 22.1).

This steady growth in cotton production could not sustain. After 1991, the cotton production has been fluctuating in the range of 6.8 million bales (2018–2019) to a record production of 12 million bales (2011–2012). Since 2015, cotton production in Punjab has been stagnant hovering about 7 million bales against the target of 10 million bales (CRS, 2020).

Factors Affecting Cotton Productivity; We studied different biotic, abiotic, and socioeconomic elements that affected the cotton crop in Punjab and critically reviewed the situation. Technology gap, climate change, high temperature, availability of quality seed, limited number of approved cotton varieties, pest attack, disease losses, weed infestation, low plant population, pesticide quality and its efficacy, lack of awareness about production technology, cotton competing crops, and socioeconomic constraints are the major factors responsible for the decline of cotton crop. At the end, we recommended remedies to these problems and established comprehensive short- and long-term strategies for the cotton crop's resuscitation.

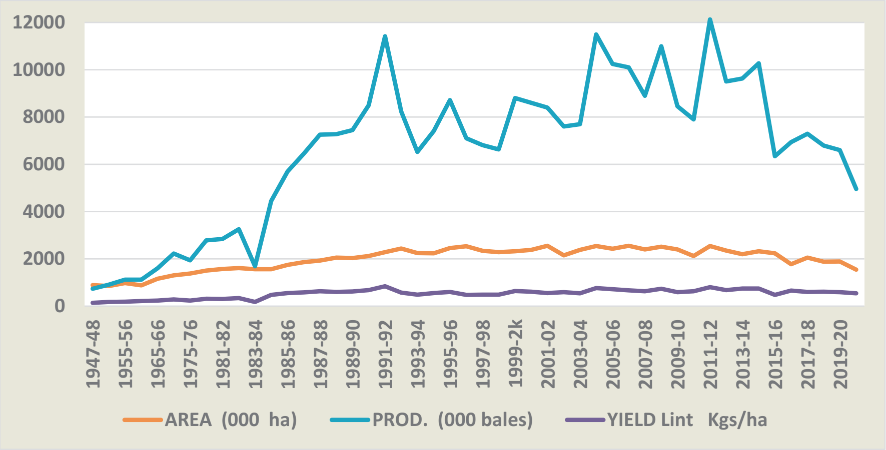


Fig. 22.1 Area, production, and yield of Punjab 1947–1948 to 2020–2021*. (CRS, 2020)

Table 22.1 Comparison of Pakistan with top cotton-producing countries (2019–2020)

Country	Area (million ha)	Production (million bales)	Yield (kg/ha)	Bt. technology		Price (cents/pound)	Certified seed (%)	Temperature (°C)
				Technology	Year			
India	12.2	32.01	447	Bollgard-II	2006	78.19	65	34/21 Jalandhar
China	3.37	35.53	1794	Fused[g] Cry1Ab, Cry1Ac Stacked CpTi	1999	108.54	>80	29/18 Xinjiang
USA	4.13	23.52	968	Bollgard-III	2017	78.8	>90	30/24 Texas
Australia	0.50	5.85	1990	Bollgard-III	2016	82.91	>90	34/19 Queensland
Pakistan	2.33	9.82	716	Bollgard-I	2010	65.12 (RS.4374)	60	37/25 Multan
World	32.60	151.47	790	-	-	70.50 (Rs. 4650)		

Obsolete Technology

Technological advancements have a key role for sustainable cotton production in the modern era of precision agriculture. About 95% of cotton in Punjab is genetically engineered Bt. cotton (Bollgard-I), which is being phased out in many developed nations due to its ineffectiveness against bollworms. Bollgard-I was superseded by Bollgard II and Bollgard III by top cotton producers such as the United States, China, Australia, and India (Table 22.1). Meanwhile, Bollgard-I was allowed for general cultivation in Pakistan in 2010 (Sajjad, 2013). This technology has become obsolete in most of the cotton-growing countries and has lost its effectiveness against the bollworms. The usage of this obsolete technology is becoming one of the major reasons for failure of cotton in Punjab, Pakistan, against bollworms.

Climate Change

Climate change is having an adverse impact on the overall productivity of cotton crop in the Punjab. Climate change is affecting not only the agriculture sector but also the related industries and beyond, such as manufacturing and services (Bandara and Cai, 2014). The consequences of climate change include the agricultural sector's vulnerability to food security issues, severe storms, floods, and droughts, putting the country at risk of socioeconomic losses (Kirby et al., 2016; Spash, 2007).

Temperature and weather patterns have become very unpredictable, with drastic changes occurring on a regular basis (Raza et al., 2019; Vaughan et al., 2018). Temperature rises, unpredictable precipitation, and significant droughts will all wreak havoc on key agricultural yields as a result of climate change (Sovidan et al., 2019). Temperature rise and unpredictable precipitation are two major issues that have harmed agricultural productivity.

Temperature

The minimum and maximum temperature of Multan (Fig. 22.2) shows that the maximum temperature is slightly on decreasing trend but the night temperature (minimum temperature) is increasing during the growth period of cotton crop particularly at flowering. At critical stages, thermal fluctuations result in lower yield due to reduced boll setting and high flower shedding. Moreover, higher temperature coupled with higher humidity encourage whitefly population (Pathania et al., 2020), which remained the major cause of yield reduction during 2019 and 2020.

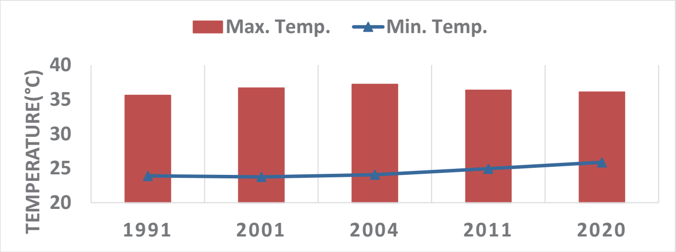


Fig. 22.2 Comparison of minimum and maximum seasonal temperature for 1991, 2001, 2004, 2011, and 2020. (Source: PMDC-ISB)

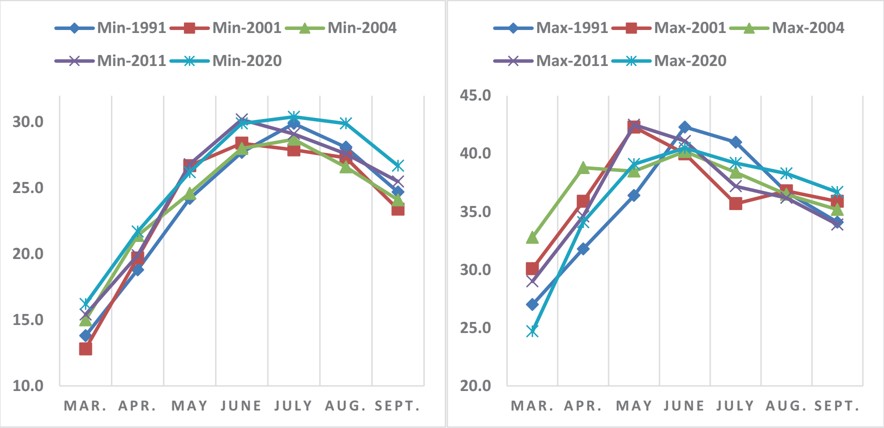


Fig. 22.3 Monthwise comparison of minimum and maximum seasonal temperature for 1991, 2001, 2004, 2011, and 2020

Monthwise comparison of minimum and maximum temperature also shows increasing night temperature from June to September in 2020 (Fig. 22.3).

Excessive Rains

High rains from July to September in 2020 compared with that of 2019 resulted in fruit shedding and consequently reduced crop productivity (Fig. 22.4). High moisture conditions also favored the epidemic growth of whitefly-mediated fungus sooty mold, which damaged cotton crop on a high scale in all over the province

Another effect of high rainfall during the first fortnight of May was delayed sowing in some core cotton areas. Cotton planting was delayed due to late harvesting of wheat because of rains. About 60% sowing was done in second fortnight of May (Fig. 22.5). Due to delay in planting crops, plant canopy remained below normal and also resulted increase in whitefly, which ultimately contributed in reduction in yield and overall production of Punjab Province. Development of heat, drought, and CLCuD-tolerant varieties is the need of hour to increase per acre yield and to boost cotton production

Availability of Quality Seed

Availability of quality seed to the farmers is another bottleneck in achieving higher outputs of cotton crop. According to the Federal Seed Certification and Registration Department, Punjab, Agri. Department, no more than 36% of certified cotton seed

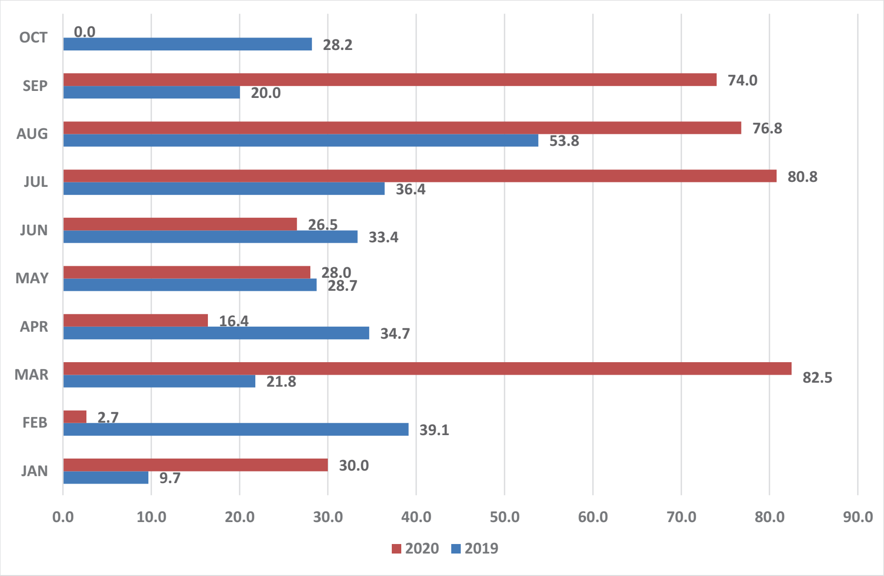


Fig. 22.4 Total rainfall (mm) in cotton-growing districts of Punjab from 2019 to 2020. (Source: PMD)

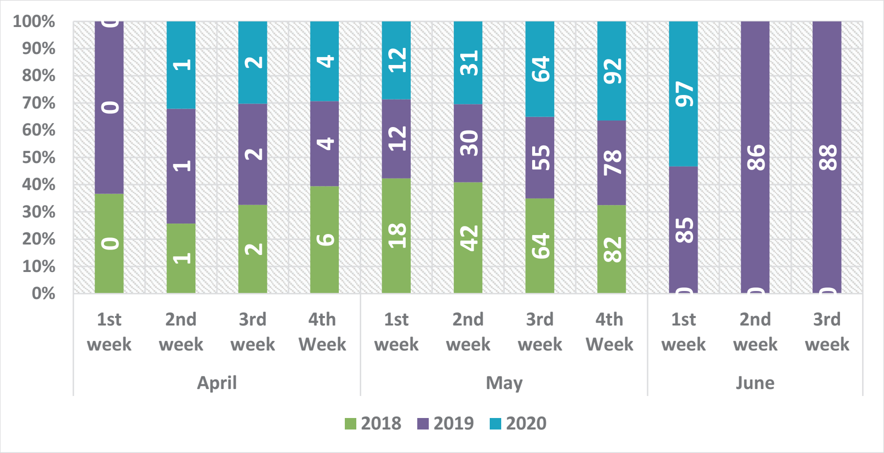


Fig. 22.5 Weekwise percent area sown in 2018, 2019, and 2020 in Punjab

was available to the farmers in 2019. Nonetheless, the informal seed sector (farmer-to-farmer sales) remains a major bottleneck in the availability of high-quality seeds to farmers. However, the cotton crop was severely harmed especially by whitefly, resulting in low plant yield and poor seed quality (Rizwan et al., 2021). Farmers had to suffer for good yield due to a serious shortage of high-quality seed.

Insect Pest Problem

Insect pest infestations are a major problem for successful cotton cultivation in Punjab, resulting in production losses of 1–2 million bales/year. Despite the extensive use of pesticides by farmers (over 80% insecticides used on cotton crop), such losses are not avoided. Cotton production was still plagued by whitefly and pink bollworm, two major insect pests. In the year 2020, whitefly remained on an upward trend (Fig. 22.6). This insect has become extremely difficult to eradicate because of its tolerance to a variety of pesticides. Pink bollworm is the same way. Infestations of pink bollworm (Fig. 22.6) were significantly greater in 2020, lowering per acre output and overall cotton productivity in Punjab.

Pesticide Quality, Efficacy, and Chemistry

Farmers are raising concerns about pesticide quality. Pesticides of poor quality result in increased number of sprays and high production cost (Popp et al., 2013). Cotton growers are reliant on old chemicals/sprays that provide partial control over pest control. The currently available pesticide chemistry is ineffective against pests, particularly whitefly, which has become a major problem in recent years. The number of sprays has gone up from four (1992) to ten and even in some cases to 15, which has increased overall cost of production and adversely effecting yield as well as overall productivity of the province. The efficacy of current insecticides for controlling whiteflies, on the other hand, varies from

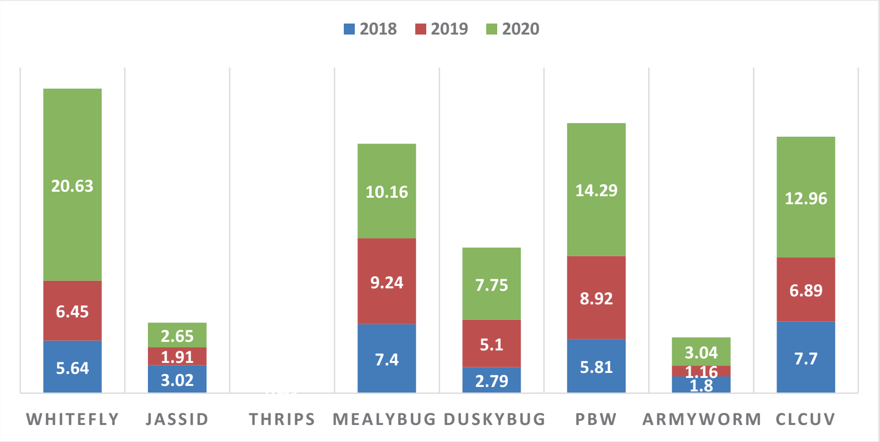


Fig. 22.6 Insect pests and disease position for the year 2018, 2019, and 2020. (Source: DG, PW&QC, 2020)

Table 22.2 Efficacy of different insecticides against cotton whitefly at Cotton Research Institute Multan during 2019

Sr.#	Name of insecticides (active ingredient, firm)	Dose/ acre (ml/gm)	Pre-treatment pop./leaf	% Mortality after		
				24 hours	48 hours	72 hours
1	Movento (Spirotetramate, Bayer)	150	13.8	21.4	27.1	37.3
2	Acetapmiprid (Generic)	200	16.5	17.4	19.2	22.1
3	Acetapmiprid (Arysta life sciences)	150	12.9	24.7	31.7	33.9
4	Ulala (Flonicamid), ICI	80	12.6	19.8	24.7	29.4
5	Buprofezin (Generic)	600	9.7	26.3	32.5	30.1
6	Buffer (Buprofezin, Jaffer Brothers)	600	13.4	29.5	34.1	37.2
7	Priority (Pyriproxifen, Kanzo Ag)	400	12.8	22.4	29.4	34.6
8	**Admiral (Pyriproxifen, FMC)**	**200**	**15.7**	**32.5**	**36.4**	**41.8**
9	Legend (Matrine, kanzo Ag)	500	13.1	21.4	28.3	36.6
10	Mavrik (Matrine, Suncrop)	500	14.8	11.7	23.4	29.4
11	Diafenthiuron (Generic)	250	12.9	35.5	39.3	42.3
12	**Polo (Diafenthiuron, Syngenta)**	**200**	**15.4**	**39.2**	**43.5**	**46.8**
13	Control	-	13.8	0.0	0.0	0.0

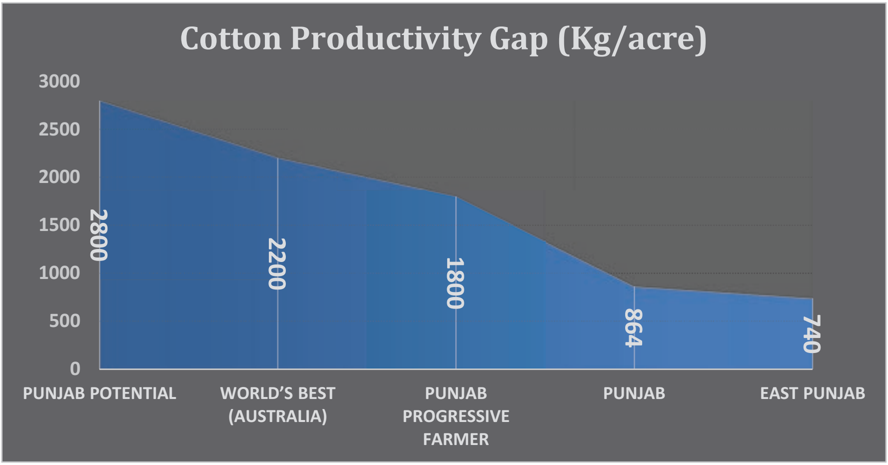
Table 22.3 Efficacy of insecticide against pink bollworm at Cotton Research Institute Multan during 2019

Sr. No.	Insecticide	Dose (ml/ acre)	Control (%)		
			After 3 days	After 5 days	After 7 days
1	Cedox (Triazophos+Deltamethrin)	600	33.58 a	44.05 a	48.20 a
2	Curator (Triazophos+Bifenthrin)	600	33.30 a	41.65 a	47.74 a
3	Coragen (Chlorantraniliprole)	50	24.06 b	26.77 c	30.50 b
4	Trizone (Triazophos)	800	30.66 a	34.59 b	40.33 a
5	Raddiant (Spintoram)	100	19.85 b	34.53 b	33.30 b
6	Belt (Flubendamide)	50	24.14 b	30.43 b	34.47 b
7	Proaxis (Gamacyhalothrin)	120	25.58 b	23.08 c	33.15 b
8	Talstar (Bifenthrin)	400	21.46 b	28.71 c	28.96 b
9	Bolton (Chlorpyriphos+Betacyfluthrin)	600	33.77 a	42.37 a	47.03 a
10	Capital Plus (Triazophos+Betacyfluthrin)	600	27.02 a	36.0 8b	40.71 a
11	Control	-	0.00c	0.00 d	0.00 c

29.4% to 46.8%. Polo (Diafenthiuron, Syngenta) was the most effective insecticide, with a mortality rate of 46.8% after 72 hours of spraying (Table 22.2). Similarly, the effectiveness of existing pesticides for controlling pink bollworm varies between 30.5% and 48.2% (Table 22.3). Cedox (Triazophos+Deltamethrin) was the most effective insecticide, with a 48.2% mortality rate after 72 hours of spraying (Table 22.3).

Lack of Awareness About Crop Production Technology

It is critical to have a good understanding of production techniques if you want to get the promising yield. The low literacy rate has hampered the acquisition of comprehensive understanding on cotton production techniques. Punjab's cotton output potential is 2800 kg/acre, which is higher than world's best average (2200 kg/acre). But on the other hand, small and progressive farmers get only 740 and 1800 kg/acre, respectively, just because of their lack of knowledge about innovative cotton production technologies. Growers with inadequate knowledge are unable to harvest a high output, resulting in a low yield and productivity compared to the production target (Liliane & Charles, 2020).



Cotton Leaf Curl Virus Disease Losses

Another hindrance is cotton leaf curl virus (CLCuV) disease, which resulted in a massive loss of 2–4 million bales per year. Despite continual concerted research efforts, the CLCuV problem has yet to be fully resolved. It is also stated that a massive project with the USDA was launched in which around 5000 accessions were imported and screened in Punjab, Pakistan, at several places, including Faisalabad, Vehari, Sahiwal, and Multan; however, none of them were found to be entirely resistant to the CLCuV disease. However, one of the introduced accessions, Mac-07, has been proven to be CLCuV-tolerant and is being used in cross-breeding studies to generate CLCuV-tolerant varieties. It's worth noting that this condition is very common in Pakistan, particularly in Punjab, Pakistan, and no research on it is being done anywhere else in the globe due to indigenous problem of South Asia. Figure 22.7 shows a comparison of CLCuV over time in different years.

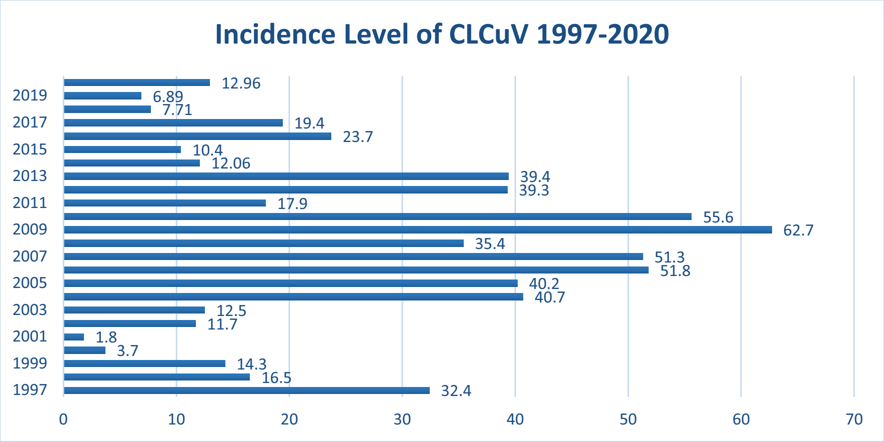


Fig. 22.7 Comparison of CLCuV % age since 1997–2020. (Source: PWQC-2020)

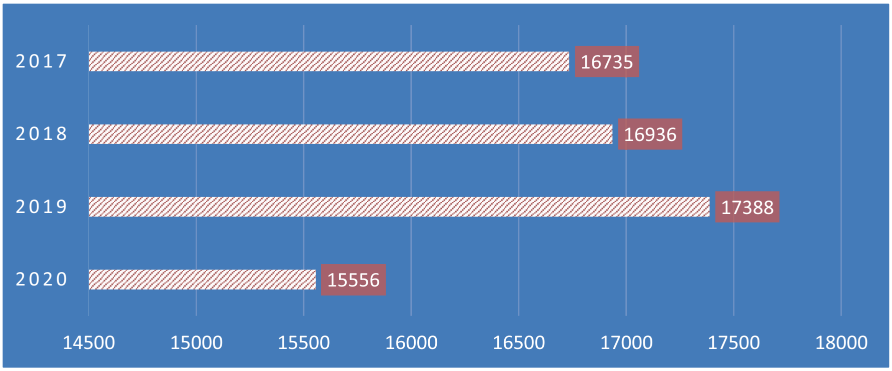
Weed Infestation

Weeds in the cotton field compete with the crop for nutrients and account for heavy losses ranging from 40% to 80%. These weeds can also serve as an alternate host plants for CLCuV, whitefly, and other insect pests.

Low Plant Population

Plant population is also one of the important components determining yield per acre. The recommended plant population by the Agriculture Department is 17,500, which is far behind when compared with that of about 400,000 plants per acre in the United States, Australia, and China. The average plant population in Punjab is even less than this and is about 16,500 plants per acre. Poor seed quality, soil salinity, water shortage and water quality, high temperature, and high rains during the sowing window cause mortality of the plants and further aggravate the problem by lowering plan population.

Plant population trend of cotton in Punjab 2017_2020 (CRS, LHR, 2020)



Cotton Competing Crops

Around the world, arable land is already dwindling. The diminishing area under cotton cultivation in Punjab is another reason for low cotton production. Cotton area decreased by 36.24% due to a continuous decline in farmer profitability. Other crops such as rice, maize, and sugarcane, on the other hand, are steadily rising. Rice, maize, and sugarcane, among other competing crops, compensated for the shortage of available land (Fig. 22.8).

Low Profitability

Cotton farmers in Pakistan are paid less than those in the rest of the world (Wei et al., 2020). Cotton prices in Pakistan are around 10 cents per pound (16 rupees per pound) cheaper than those in other countries. As demonstrated in Table 22.2, the

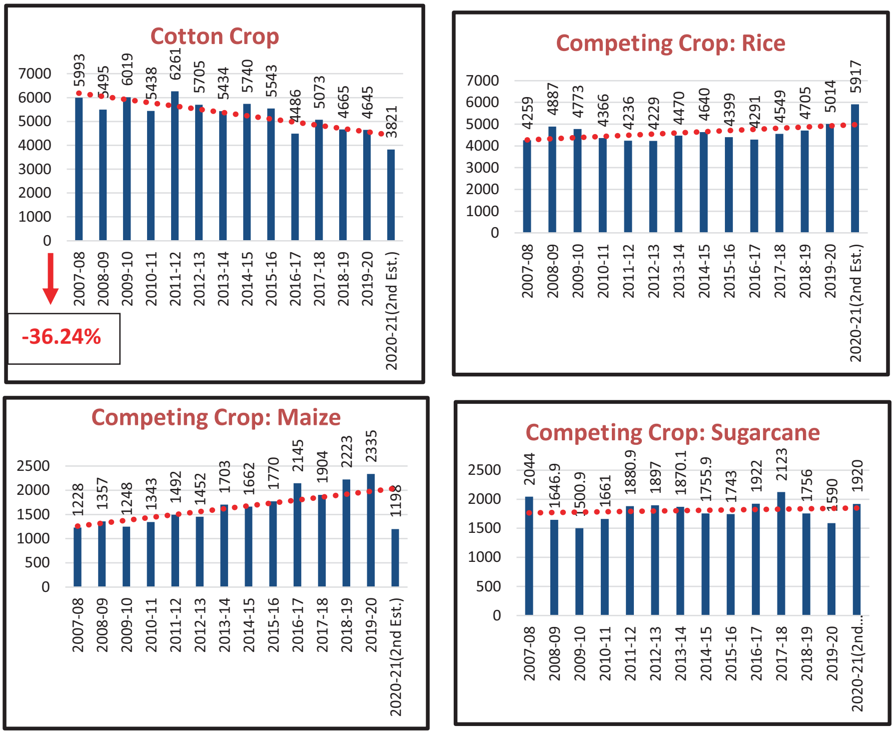


Fig. 22.8 Area for competing crops

Table 22.4 Comparison of cotton prices of Pakistan and the world since 2014

Year	Ave Cotlook A index (Rs./40 kg)	Ave KCA price (Rs./40 kgs)	Crop volume (Mil. Bales)	Crop value (Rs. Billion)		Farmer's loss (Rs. Billion)
				As per Cotlook price	As per KCA price	
2020	11,051	10,088	10.795	469	419	50
2019	10,229	9142	10.795	469	419	50
2018	9290	7911	10.847	428	365	63
2017	7149	6381	12.600	383	342	41
2016	6704	6072	11.039	337	305	32
2015	6104	5139	10.859	302	254	48
2014	6707	5943	13.488	412	365	47

Source: PCCC

price of lint in Pakistan remained low (Rs. 10,088 per 40 kg) compared to the foreign market (Rs. 11,051 per 40 kg). Despite the fact that cotton prices in 2020 were higher than in 2018 and 2019 (Table 22.4), they were still low when compared to the cost of production due to increases in input costs.

Socioeconomic Constraints

Due to urbanization, labor scarcity at the time of picking has emerged as a severe danger to quality cotton and seed production (FAO, 2017). Cotton's net profitability is lower than that of other competing crops such as sugarcane, rice, and maize due to rising production costs and stagnating yields. Farmers have moved from cotton to rice and maize crops, reducing cotton planting area and, as a result, lowering the province's overall productivity. All of this discouraged cotton growers from cultivating the crop and investing in better crop management.

According to the International Textile Manufacturing Federation (ITMF), Pakistan is the world's third most contaminated cotton producer, with its cotton fetching 2–3 cents per pound less in the international market, resulting in a loss of Rs. 7.0 billion yearly. As a result, it is critical to concentrate on clean cotton picking that is free of contamination.

Water Shortage

Water shortage has emerged as a major yield limiting factor particularly in those areas where groundwater is unfit for irrigation and farmers solely depend on canal irrigation water for cotton cultivation. Unavailability or limited availability of irrigation water at the time of sowing and different developmental stages result in late planting of cotton, which consequently is followed by less germination, increased pest pressure, stunted plant growth, fruit shedding, forced opening, and low productivity at the end of the cropping season.

Strategies for Sustainable Cotton Production

Approval and Adoption of Advanced Technology

In order to improve overall cotton production, genetically modified (GM) technology is critical. Pakistan needs GM new technology with herbicide-resistant genes that provide lepidoptera and pesticide resistance (Benbrook, 2012; Deguine et al., 2008). Introduction of Bollgard-III and glyphosate-resistant (Stacked Trait) technology should be introduced in Punjab, Pakistan. It will also lower the overall cost of production by obviating the use of pesticides.

Climate-Resilient Varieties

To combat the growing threat of climate change, agriculture needs an adaptation strategy that includes climate-resistant varieties with advanced technologies. Changing crop kinds, altering irrigation, and changing soil management practices are all options for mitigating or minimizing the detrimental consequences of environmental changes at the farm level (Manik et al., 2019).

Seed Quality Issues

Seed is basic unit for obtaining good yield of crop (Abebe & Alemu, 2017). Although 90% certified seed was available last year, yet it needs to be increased to 100%. Moreover, the quality of seed needs to be improved by adopting improved seed production system. Therefore, this sector needs priority for its improvement.

Punjab seed corporation has played a vital role in cotton production in Punjab in the past by providing quality seed to the growers. For the last few years, there are issues of land, staff, machinery, and capacity building in this organization. It is therefore recommended to resolve these issues on priority basis so as to enable this corporation to develop good-quality seed and its provision to cotton growers.

Launching Comprehensive IPM for Insect Pest Problem

It is recommended that a comprehensive IPM program for pink bollworm and whitefly control be implemented. The best tool for controlling pests and diseases is integrated pest management (IPM). It boosts the proliferation of natural enemies. Thus, all integrated measures should be taken to control pests, particularly whitefly and pink bollworm, and lower overall cost of production.

In order to control the emergence of pink bollworm during the off-season, farmers should take the following steps: grazing sheep and goats in the field (to destroy green bolls), stacking cotton sticks vertically and inverting them, removing infected seed from ginning facilities, early removal or rotavating of cotton sticks, early planting, which should be avoided, and using PB ropes.

Likewise, for control of whitefly: eradication of whitefly host plants; reduced sowing window: May 1 to May 30; sowing of approved varieties; treatment of the seed; no chemical spray for fist 60 days of planting; use of plant extracts (neem, castor oil, etc.) for 60–90 days; bio-control agents should be encouraged; application of nitrogen must be completed within 60 days of seeding; use of yellow sticky cards (1–1.5 feet) and replaced every 15 days; crop rotation, crop zoning, and the availability of certified seed.

Quality, Efficacy, and Chemistry of Pesticide

Research work should be accelerated to study the efficacy of available insecticides and increase in insecticide resistance. Pesticides must be manufactured locally in order to lower production costs. Since 1970, India has been working on this strategy, but Pakistan has been unable to establish itself as a domestic manufacturer. Hence, the import of new pesticide chemistry is direly needed to facilitate cotton growers for effective control of the insect pests of cotton.

Improvements to the Seed Industry Through Private Sector

Punjab Seed Corporation has limited resources and is unable to meet all of Punjab's cotton seed needs. As a result, in order to provide quality seed of approved varieties to Punjab's cotton growers, the private seed sector must be strengthened and regulated. Out of 1038 private seed companies, 316 companies failed to get their renewal of registration in Punjab. So, the remaining 722 private seed companies are now working in Punjab.

Farmers' Capacity Building

Farmers must be equipped with the ability to control critical pests such as whitefly and pink bollworm through better spraying techniques and knowledge of the best solutions for pest and disease control. Cotton growers and traders are being educated on cotton variety production technologies, spraying, and clean picking. Awareness about production technology is very important for obtaining maximum yield of cotton.

Zoning of Crop

Mixed cropping is widely regarded as the most significant barrier to effective pest management. To maximize agricultural output, zones should be identified for each crop. In cotton zones, maize, rice, and sugarcane should be restricted. In order to control diseases and pests, the sowing window should be set between 15 April and 30 May.

Laser Leveling

Laser land leveling is the solution to minimize the profitability losses for crop production. It is a simple operation to prepare the land before sowing and can reap massive returns such as increasing yields, saving water, and reducing greenhouse gas emissions. Laser land leveling considerably lowers irrigation time for rice by 47–69 h per hectare per season. It increases yields by an average of 8% for both crops. It is cost-effective. As demand increases, service providers rent out equipment, and farmers collectively share the costs. A study showed that it reduces greenhouse gas emissions from saving on energy, reducing cultivation time, and increasing input efficiency.

Farm Mechanization

Farm mechanization is the most significant part of precision agriculture. The majority of Punjabi farmers use ancient and obsolete farm machinery. We need to build new farm machinery, starting with sowing and ending with harvesting, to reduce production costs and increase cotton yield.

Moreover, the availability of picking labor is becoming a severe issue. Due to a lack of pickers, a large amount of phutti is lost standing in the field. It also has an impact on the quality of lint and seed. Promotion of mechanical picking as a solution to this problem is a step forward, and ideotypes/varieties suitable for mechanical picking should be produced as soon as possible. In addition, efficient mechanical pickers could be implemented employing a successful paradigm such as service providers.

Socioeconomic Constraint Barriers

There has been a consistent desire to secure farmers' profitability through intervention prices in order to ensure sustainable cotton output. The price governing bodies should declare an intervention price per mond to entice growers to plant cotton crops and invest in better crop management in order to increase production. It would also result in a larger cotton-growing area and, as a result, increased overall cotton yield. Moreover, investment of cotton research may be enhanced to align cotton system to the international level.

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Chapter 23
Biosafety and Biosecurity in Genetically Modified Crops

Asad Azeem, Sami Ul-Allah, Ahmad Sher, Muhammad Ijaz, Abdul Sattar, Waqas Ahmad, and Abdul Qayyum

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Introduction

Genetically modified (GM) crops have been swiftly adopted owing to prominent beneficial traits such as pest resistance during last decade (James, 2017). Such beneficial traits have played an essential role to enhance crop production and control environmental pollutions owing to the excessive use of pesticides (Wu et al., 2008; James, 2017). GM crops have special designed system to tackle a large diversity of pest such as in *Bt* crops a unique proteins (δ-endotoxin) is expressed against the herbivores of order Lepidoptera and Coleoptera which is toxic for pests and eliminate them entirely. Expression of δ-endotoxin may also alter plant-defensive system to some nontarget organisms. For instance, many *Bt* cotton and *Bt* maize line were found susceptible to aphid in comparison to non-*Bt* maize as well as cotton (Hagenbucher et al., 2013).

GM organisms are key players in the modern development and sustainability in the field of agricultural genetic engineering and agriculture. The GMOs are engineered, and gene of desired characteristics is introduced in relevant organism in vitro, but when such organism comes in outside environment, it may cause long-lasting negative effects (Prabhu, 2009), and in the last 10 years, the risks associated with GM crops have been increased at large scale (James, 2013). Biosafety deals with such types of novel risks and adverse effects of GM crops and suggests a lot of ways to tackle ricks in a unique method through the safe application of genetic engineering (Kumar, 2012). As day-by-day increase in the use of biotech tool, biosafety issues are ahead to ensure biosafety living organism as well as environment. Owing to increasing need of biosafety in biotechnology, a lot of countries worldwide have adopted an international multilateral agreement on biosafety "the Cartagena Protocol on Biosafety (CPB)."

Biosecurity is also complex and manifests various definitions in different literature. WHO defined biosecurity as the mechanism of establishment and maintenance of security of pathogen, toxins, and other relevant ways. It is the shield as well as accountability of biomaterials in in vitro condition to prevent their unauthorized access, misuse, loss, or deliberate release. While biosafety is the protection of human being from risky microorganism and biomolecules, biosecurity provides protection of these materials from human beings. Hence, biosecurity is something different from biosafety. For instance, biosafety directs tagging of the biomaterial during transport of hazardous biomaterial; however, in biosecurity angle, tagging of the biomaterial during transportation may lead to enhance jeopardy of misuse, theft, or loss.

A lot of issues regarding GM crops in terms of environmental risk for commercial application and public worker health safety are major issues (Coelho & García Díez, 2015). Owing to the anthrax attacks in the United States, the issues regarding biosafety and biosecurity have become more prominent after 2001. Unluckily, biosafety and biosecurity principles are still impractical in many parts of the world due to the lack of infrastructure. The judicial application of biotechnology plays vital role in the enhancement of better quality of GM crops. But the misuse of it may have partial adverse effects (Novossiolova & Sture, 2012). Nowadays, the use of biosecurity is a basic requirement to tackle bioterrorism and misleading production of super weeds as well as super pathogenic bacteria (Kumar, 2015).

Biosafety Objectives

Here are some objectives of biosafety protocols associated with genetically modified crops.

Safeguarding Life from Dangerous Biological Factors

From Pasteur and Koch's time, there is a solid evidence of biosafety practices in biotechnology, when first ailment was reported, and the need of safety measures was identified. Koch planned to switch these ailments by using glazed tabletop box plus oilcloth sleeves through which "bio-containment" thoughts were produced (Berlinger, 2003). The main and prime objective is to save living organism from hazardous agents.

Plant Health Protection

International Plant Protection Convention (IPPC) is working for the protection of the biological materials to tackle the risks associated with global trade such as plant pest outbreak, etc. The IPPC is a well-structured organization for the provision of protection of the phytomaterials as well as for the application of sanitary and phytosanitary measures of WTO. Specific "International Standards for Phytosanitary Measures" (ISPMs) deals to the analysis of pest risk or quarantine organisms or the special design of plant isolation stations (FAO/IPPC, 2019a).

Organism protection deals not only to control pathogens; in addition, it plays a vital role to control the vectors, which are actual responsible for whole process (Heil, 2014).

Environmental Protection

Discovery of genetic material led to a new discipline in bioscience and molecular biology, but after the discovery, a lot of experiments were performed to figure out its versatile function as well as structure, and along with these experiments, a lot of environmental risks also were associated when first experiments with DNA cloning and recombination were performed in United States (Jackson et al., 1972). This was the need of day to make some protective principles to safeguard the society as well as nature. The Second Asilomar Conference represented the following basic rules: There should be special consideration on containment, and its effectiveness must contest the risk possible.

Protection Against Theft, Loss, or Misuse

The WHO Bio-risk Management Laboratory Biosecurity Guidance (WHO, 2006) proposed the idea of biomaterial security. Valuable biomaterials are composed of toxins, pathogens and nonpathogenic organisms, vaccine, foods, and GMO. Now, the next step is provision of protection against theft, loss, and misuse of these valuable biomaterials. There are many societies as well as organization working to deal all types of risks and provide protection against theft, misuse, or diversion. The prime goal is to provide biomaterial safety as well as security where these materials are being used or stored (WHO, 2016).

Biosafety Levels for GM Crops

The National Coordination of the National Commission has developed the specific levels in biosafety for GM crops as well as for other GM organisms. These levels predict the level of overall risk to the use of GMOs in the environment. Following are the levels of biosafety, which categorize the entire GM organism in accordance with defined roles.

First Level

Development of a genetically modified crop from a spp. whose center of origin is not that area from where it was produced for which there will be no genetic diversity. For instance, there will be not a single wild relative present in that specific area. Least demand of this level is that warranty of monitoring system in that place or area must exist in relation to those environmental conditions in which GMO was released, and in case of certain environmental changes, specific biosafety measures as well as defined principles must be applied for protection (Kozajda et al., 2013).

Second Level

Development of a genetically modified crop from a spp. whose center of origin is not that area from where it was produced, but there exists its genetic diversity. For instance, Middle East is the place of origin of wheat, but its wild relative, which is *Aegilops cylindrical*, exists in Mexico. In the case of second level of biosafety, there is the presence of genetic resource information system in addition to the species data, which was involved in the GM organism construction as well as the necessary tools for the detection of genetic construct in genetically modified organism. By using this level, there is specific monitoring setup as well as detection of introgressed gene including specifically its effects as well as final consequences.

Third Level

Development of a genetically modified crop from a spp. whose center of origin is that area from where it was produced, but there exists its genetic diversity (Mourya et al., 2014). For instance, a genetically modified organism in third level has some additional biosafety as compared to the first two levels. Besides genetic recourses as well as detection system this level is also involved in the competent authorities which will update about the area either it is vulnerable or not through the procedure of Genetically Modified organism released in relevant environment (Pastorino et al., 2017). In this level, the competent authorities are designed in such a way to provide better protection as well as biosafety to the genetically modified organism keeping in view its positive as well as negative points. Center of origin as well as center diversity are the main places on which biosafety management system would be applied, which would play vital functions to warrant the management system to take steps as well as to adopt some precise measures to overcome associated risks in releasing GM crops and other GM organism (Wurtz et al., 2016). All the procedure from GMO formation to tackle its associated risks is in coordination with precise biotechnology and genetic engineering as well. Till now, a lot of GMOs have been released in Mexico, in 1989, there firstly released GMOs were done with little knowledge of their effects as well as results associated with bio risks to environment (Garcia-Alonso et al., 2014). Despite the fact, over 22 GM crops were experimented in field condition, and out of 22, only five were released in which three GM crops were most prominent in terms of specific genetic engineered trait; these are GM cotton, GM maize as well as GM soybean. Every GM crop has some unique bio-related risk, which can be tackled by using abovementioned biosafety levels, and in the case of genetically engineered soybean, its bio-related issues fall in use of technology package, for instance, use of glyphosate to tackle weeds in field. Recently, in apiculture items, its pollens were observed, which create a gap between utilization of honey and its production, and at this stage, the public is hesitant to purchase such honey containing pollens and its effects on vegetation of that ecosystem. In case of genetically engineered cotton, the recombinant proteins expressed in the genetic construct of GM cotton are detected in wild relatives mostly found in Mexico, which also falls in third level of biosafety. Genetically engineered maize is released in Mexico, which also falls in third level of biosafety.

Issues Associated with Biosafety and Biosecurity

Biosafety is the containment principles as well as practices, which lead to provide protection against accidental exposure to pathogens or any kind of toxins to the environment. Biosafety is not the need of a person, but it is the collective requirement of whole society to keep environment neat and free from biological risks. Releasing GMOs (GM plants or GM animals) has direct or indirect effects in environment in terms of transfer of gene or gene flow of a specie into its wild relatives, effect of traits on nontarget genus or species, and pest resistance as well (Lunshof & Birnbaum, 2017). The significance of GM crops is well elaborated in the sudden reduction in the utilization of agricultural chemical against pests for field crops. As many countries of the world are involved in the positive application of biotech tool for GM organism's formation especially in crop plant, the demand of biosafety increased and biosafety issues came in consideration with the increasing number of countries adopting molecular tools and techniques in their life science research and development activities, the biosafety issues are gaining importance to make sure safety especially for the people as well as for health environment (Kumar, 2012). After it, many countries globally adopted an agreement on biosafety "the Cartagena Protocol on Biosafety (CPB)" to ensure biosafety as per requirement.

On the other hand, the biosecurity is not simple; it has many meanings in different literature. The World Health Organization elaborated it in a unique way, which named biosecurity a well-planned and systematic system of security as well as management of toxins, harmful microorganisms, and other such biological resources. Biosecurity at laboratory level includes the security as well as accountability for biomaterials in order to check loss, misuse, or theft, which may play harmful effect on environment (Colussi, 2015). Biosafety plays a prime role in public protection from hazardous materials. So, the basic principle of biosecurity is different from biosafety, and both of these play a vital role in handling bio-related issue in all living organism. For instance, the hazardous materials must be tagged owing to biosafety during transportation, but biosecurity tagging of the hazardous materials may increase the risk of misuse or theft during transportation.

As the use and production of these GM organism increased, the application and adaptation of biosafety as well as biosecurity principles have been increased to overcome prevailing issues and biorisks related to ecosystem (Brizee et al., 2019). At present, many countries around the world are working for the insurance of protective measures and also for the proper regulation of made policies regarding risks related to genetically modified crop and genetically modified animals (Tierno & Plateau, 2012). Although regulatory bodies and policies exist to maintain check and balance between production and associated risks, there is still a need of more precise rules and regulatory bodies for proper functioning and biosecure environment.

Other than the abovementioned issues, there exist some technical issues regarding GM organism's production relevant to environment in terms of laboratory workers' safety and their uses at commercial scale.

A variety of ethical issues is also linked with biotech commercial products (GM organisms) (Novossiolova & Sture, 2012). Many ethical issues are derived from the transfer of gene from animals to plants, which may lead to social issues of genetically modified plants. A lot of question comes to mind regarding GM product, for example: Are GM foods safe to use or not? Are GM crops cause long-term negative effects on society and on other crops cultivated in field? Are GM crops more prone to risks in comparison to non-GM crops? What will happen in case of any damage to public? Are there some background system to tackle unexpected happening in the future owing to various genetically modified products and organism? Are GM products and organism release possible morally?

Solutions

Improvement of Biosafety and Biosecurity Capability Worldwide

Biosafety capability is much improved and enhanced the wide collaboration of different countries globally. The prominent incidents of disease outbreak and major loss of life in 2001 owing to anthrax (Meselson et al., 1994), in 2003 owing to SARS, in 2014 owing to Ebola (Cenciarelli et al., 2015), and in 2019 owing to Covid-19 pandemic (She et al., 2020) have made the importance to make well-structured and organized institutes to regulate and provide biosafety as well as biosecurity (Allix, 2013). The abovementioned objectives can only be achieved by developing better and standard institutions to provide safety and security globally.

Improvement and Establishment of Biosafety and Biosecurity Network Worldwide

Biological safety and security comprise a number of coordinated analyses and data collection to make secure about outbreak of plant or animal diseases in the future (Imperiale et al., 2018). The prime role of the development of safety and security network for biological materials is to make sure the reinforcement of biological safety and security podium and participation in coordination in a number of different areas and field including the protection and control of the outbreak of infectious disease which play role to response national and international outbreak (Zhou et al., 2019).

Improvement of Alert Mechanism to Deal Disease Outbreak

With the increase in population rate, urbanization and transportation by air market led to the evolution and disease transmission as well. Owing to the outbreak of novel-type diseases day by day, there must be a specified system, which must be responsible for the identification of diseases causing organism and the associated resources to it (Cipriano, 2002). The basic alert system must be proactive to tackle the emergence of disease-causing organism owing to GM organism.

Improvement of Identification Level and Control of Pathogens

Genetically modified organism must be checked and passed through a proper mechanism of channels to control unhappening and bioterrorism in society and ecosystem (Ahteensuu, 2017). Early identification of pathogen is very compulsory for proper handling and controlling of bio-risks and other associated bioterrorism (MacIntyre, 2015). There must be proper detection setup associated with subject specialist having skills to deal with a little bit of modification. By adaptation of precise biotech technologies, impact of bio-risks can be minimized easily.

Improvement of Biotechnology Applications and Administration System

By the improvement of the biotech application, a lot of bio-risks associated to GM crops and GM animals can be minimized (Chandra et al., 2010). As a matter of fact, the administration system dealing especially GM crops must be strong and should have backup strategies and technologies to control the spread of disease strain in various environments (Hulme, 2020). There must be collaboration of different country administration system and biotech researcher for better performance and advancement in technologies (Edwards, 2014). In case of gene transfer and genome editing, a specific administration system is working for the regulation (WHO, 2015) and implementation of set roles and provision of biological protection against bio-risks, for example, gene synthesis, etc. (Minshull & Wagner, 2009).

Improvement of Controlling Setup for Invasive Alien Species

In twenty-first century, one of the biggest challenges to the public, environment, and other biodiversity is invasion of alien species. Therefore, there must be active platform for proper and productive strategies on control of invasive alien species as well as detection system. Such strategies will create hurdle in the pathway of transmission of pathogen, which degrade the agricultural production and biological set mechanisms.

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