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Next-Generation Sequencing in the Development of Climate-Resilient and Stress-Responsive Crops – A Review

Amitava Roy[1], Suman Dutta[2], Sumanta Das[2,3] and Malini Roy Choudhury[3,*]

[1]*Division of Genetics and Tree Improvement, CFRE-Rain Forest Research Institute, Jorhat, Assam, India*
[2]*IRDM Faculty Centre, Ramakrishna Mission Vivekananda Educational and Research Institute, Kolkata, West Bengal, India*
[3]*School of Agriculture and Food Sustainability, The University of Queensland, St Lucia, Queensland 4072, Australia*

Abstract:
Current agricultural production is seriously threatened by climate change and global warming, which also bring new difficulties including the spread of pests and diseases and changes in the environment that can have a big influence on crop yields. Innovative strategies are required to guarantee food security and agricultural sustainability in light of these changing circumstances. To overcome these obstacles, molecular breeding techniques have become essential, particularly with the introduction of next-generation sequencing technology. Chain termination and enzymatic techniques, which were known for their low throughput and efficiency, were the methods used for DNA sequencing in the past. A paradigm change was brought about by the advent of the polymerase chain reaction (PCR) and PCR-based sequencing, which allowed for higher throughput and more effective sequencing procedures. On the other hand, next-generation sequencing, with its unmatched capabilities, represents the latest breakthrough. With the use of this technology, characteristics that govern how the body and metabolism react to different stresses in a changing environment may be examined in greater detail. These developments in molecular breeding technologies not only improve our knowledge of plant responses to global challenges to food production, but they also offer useful tools for creating crops that are climate resilient. Scientists and farmers alike may work towards developing crops that survive the effects of global warming and contribute to a more sustainable and secure food supply by decoding the genetic composition with unprecedented accuracy. In this review, we discuss the opportunities, mechanisms, and implications of next-generation sequencing in the development of climate-resilient crops.

Keywords: QTLs, Biotic and abiotic stresses, DNA, Molecular breeding, Next-generation sequencing, Climate change.

*Address correspondence to this author at the School of Agriculture and Food Sustainability, The University of Queensland, St Lucia, Queensland 4072, Australia; E-mail: m.roychoudhury@uq.net.au

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1. INTRODUCTION

Global food security is currently under massive threats of several challenges which involve the severe impact of climate change and the emergence of various negative impacts associated with it [1]. The ever so expanding global population along with such challenges of climate change led the global food security under massive threat. The emergence of new biotypes of various disease pests and the breakdown of available resistance against such agents is another area of challenge to be dealt with along with climate change [2]. Earlier, the breeding technologies majorly focused on increasing productivity levels without considering genetic diversity. As a result, there has been a significant reduction in the overall genetic variability making the crops genetically vulnerable to various insect

pests and diseases [3]. Climate change is one of the most important threats to the maintenance of sustainable production levels and at the same time increasing production levels to ensure global food security [1]. These changes not only will lead to reduced productivity levels but also will lead to lower quality of produce in terms of consumption and processing purposes [4]. Therefore, it is very important to focus on the development of such genotypes that will possess resilience against such adversities of nature.

Advancements in molecular marker technologies along with the development of the next-generation sequencing arena can lead to significantly better gains in terms of breeding for such climate-ready crops [5]. Identification of genomic regions controlling various important traits associated with higher productivity under adverse climatic and biotic conditions and subsequent transfer of such QTLs into superior genetic background utilizing marker-assisted selection has been proven to be an effective strategy for developing improved versions of different traditional cultivars to sustain under changing climate [6]. There is great potential for the technologies in terms of revolutionizing the current production scenario for different crops. The next-generation sequencing advances are cheaper, have high throughput, and produce results faster thus accelerating the overall breeding cycle of crop plants. These techniques also enable us to dissect the untapped genetic diversity and provide a finer understanding of complex genetic and physiological processes of plant behaviour under varying environmental circumstances. Identification of genomic regions associated with the control of such traits providing higher production potential under different varying stresses and incorporation into already adapted cultivars will lead to the development of materials capable of adjusting the growth and other physiological patterns with the environmental changes [5]. There is a huge scope for constructing such genetic materials, which will act as potential sources for getting plants climate change-ready with further exploitation of these newer advances in sequencing technology.

This comprehensive review delves into the evolutionary trajectory of various sequencing technologies and their profound impact on the field of crop plant breeding, particularly in the context of confronting diverse biotic and abiotic adversities. The exploration of sequencing technologies is pivotal in understanding how advancements in molecular tools have revolutionized the landscape of agricultural research. The narrative begins by tracing the historical development of sequencing methodologies. It elucidates the transition from earlier techniques such as chain termination and enzymatic methods, characterized by limitations in throughput and efficiency, to the transformative era marked by the advent of polymerase chain reaction (PCR) and PCR-based sequencing. This transition laid the groundwork for a more streamlined and high-throughput sequencing process. However, the true breakthrough emerges with the introduction of next-generation sequencing technologies. These cutting-edge tools not only represent a quantum leap in sequencing efficiency but also offer unprecedented resolution in deciphering the intricate genetic makeup of crop plants. The review underscores the pivotal role of next-generation sequencing in providing a finer dissection of traits governing the regulation of physiological and metabolic responses to diverse stressors imposed by biotic and abiotic factors in the environment. Moreover, the review explores the practical implications of these technological advancements in crop breeding strategies. It sheds light on how the insights gained through advanced sequencing techniques empower scientists and breeders to develop crops resilient to the challenges posed by evolving climates, emerging diseases, and pest pressures. By unraveling the genetic intricacies governing stress responses, researchers can now tailor breeding programs to enhance the adaptability and robustness of crop plants, ensuring sustainable agricultural practices in the face of an ever-changing environment. In essence, the review provides a comprehensive overview of the dynamic interplay between sequencing technologies and their instrumental role in shaping the future of crop breeding under varying biotic and abiotic conditions.

2. PROGRESS THROUGH THE GENERATIONS OF SEQUENCING

2.1. First-generation Sequencing

Deciphering the genetic information present in the form of DNA through sequencing technology has revolutionized and brought in a paradigm shift in various branches of molecular biology and other scientific arenas. In 1977, Fredrich Sanger for the first time expounded the technology to decode the DNA sequence present in an organism which is popularly known as Sanger's sequencing [7]. Sanger's sequencing technique is also termed first-generation sequencing which was based on a chain termination process. Later on, Maxam and Gilbert introduced a more advanced and improved first-generation sequencing arena which was based on enzymatic of particular base sequences [8].

2.2. Sanger's Sequencing

Sanger sequencing is based on the termination of elongation after a particular nucleotide is incorporated and hence that particular nucleotide can be identified. In this technique, chemically modified bases or dNTPs are utilized for the synthesis of a chain of DNA [7]. Individual dNTPs are labelled and after incorporation of a particular dNTP, it further averts the elongation of the DNA or terminates the chain hence also termed as chain termination method. Different DNA fragments are obtained with different sizes. These DNA fragments are separated according to their size in gel electrophoresis and are visualized with a suitable imaging system. The Sanger sequencing technique has been widely utilized and commercialized for around three decades and is even in use currently for very low throughput sequencing [7]. However, despite so many efforts to improve the chain termination technique, it could not improve the speed and

efficiency of this method. It was very difficult to analyze a complex system like a plant genome with this technique. Finally, the method involved high cost, it was much tedious, and also very time-consuming.

2.3. Maxam-Gilbert Sequencing

Maxam and Gilbert developed another first-generation sequencing technique which was based on enzymatic cleavage of nucleotides to deduce the nucleotide sequence. This sequencing technique is also known as the chemical degradation method of DNA sequencing. In this technique, different enzymes are used to produce breaks in single or two nucleotide combinations in four different individual reactions [8]. Different DNA fragments can be separated depending on their sizes using gel electrophoresis. This sequencing technique is considered an advancement over Sanger's sequencing method but at the same time, it is more hazardous as compared to the former as it involves a high level of radioactivity and more toxic chemicals [8].

3. NEXT-GENERATION SEQUENCING TECHNIQUES

The first-generation techniques such as Sanger's were predominant for almost three decades but even after putting so much effort into improving, these did not result in significant advancements in terms of output efficiency and cost reduction [7]. Therefore, there was a requirement for the development of sequencing advances that could overcome such obstacles faced in first generation sequencing arena. With the development of PCR-based markers, the first decade of the 21st century displayed newer advances in DNA sequencing with the use of high throughput sequencing technologies. NGS methods generated millions of copies of DNA fragments by PCR amplification accelerating the overall sequencing process without the use of hazardous chemicals and were less tedious and time-consuming [9]. There are two approaches for next-generation sequencing firstly sequencing by synthesis and then sequencing by ligation. The major sequencing techniques in NGS are 454 pyrosequencing, Illumina solexa, ion torrent, and ABI SOLiD sequencing.

3.1. 454 Sequencing

454 sequencing is based on the detection of ppi molecule, which is released each time a new nucleotide is incorporated into the synthesized DNA strand. This sequencing technique utilizes the approach of sequencing by synthesis. Here DNA samples are first broken down into small fragments randomly and now these fragments are affixed into a bead-like structure surface that possesses several sequences that are complementary to the DNA sequence fragmented. Then each bead is isolated and undergoes PCR for generating millions of copies of DNA sequences fragmented and attached to the beads. Particular light is emitted whenever a specific nucleotide is incorporated. The DNA sequence is deduced by the detection of the emitted light [10]. 454 sequencing generates longer reads as compared to other NGS sequencing techniques hence it is easier for genome assembly and also to map onto the reference genome sequence. On the limitation front, the light signals that are of too high or too low intensity may lead to under or over-estimation of the number of nucleotides incorporated which may generate errors in sequencing [11].

3.2. Illumina Solexa Sequencing

In this sequencing procedure, the DNA sample is fragmented, and at their 5 ' and 3' sites two separate adapters are ligated. Then these adapters are placed on a solid plate that contains densely placed primers having complementary sequences of the DNA sequence fragmented. In the next step, primers are used for amplification by bridge PCR to generate thousands of identical copies of the DNA sequence. This creates a set of sequences that are identical and are derived from the same original sequence to form a cluster. Finally, the sequencing primers are hybridized at the ends of the DNA fragments. Each dNTP used for DNA synthesis will have fluorophores of a specific kind to be attached to them and also will serve as chain terminators thus ensuring the incorporation of a single nucleotide only. Clusters are excited so that a specific signal is emitted for a particular nucleotide and these signals are recorded by a CCD camera and software is used to convert these light signals into nucleotide sequences. After the recording of a signal chain, the terminators are eliminated so that the nucleotide is free for the next round of DNA synthesis. Thus, each time a nucleotide is incorporated, the signal is recorded and finally fluorophore is removed so that the new nucleotide can be incorporated and the cycle goes on [12]. Early generation Illumina sequencers could generate read lengths up to 35 bp but the currently available Illumina sequencers can generate read lengths up to 150 bp along with having high accuracy of over 98%. These sequencers have the advantage of generating paired-end reads in which the sequence of both the ends of DNA cluster can be deduced. The major limitation of this sequencing technique is the requirement of good control over sample loading because overloaded samples cluster overlapping and hence inferior quality of sequencing [13].

3.3. ABI SOLiD Sequencing

Applied Biosystems in 2005 developed and commercialized sequencing technique Supported Oligonucleotide Ligation and Detection, which is also known as SOLiD. Here, similar to the paired-end sequencing, adapters are ligated to both ends affixed on the beads and cloning is done through emulsion PCR. The DNA molecules fixed on the beads are placed on a glass slide having a single acrylamide layer. A specific fluorophore is attached to each of the nucleotides to be used for DNA synthesis and is incorporated and allowed for pairing at the 3'ends of the sample DNA. Then, the output is recorded which is embedded in the form of 16 combinations of two nucleotides at the 3'end formed by utilizing four fluorescent colours. This cycle is repeated removing the first cycle nucleotides and a new set of bases and similar to the first cycle, events are repeated. The output data recorded is to be converted into the sequence of DNA bases and thus the sequence of the DNA samples

can be deduced [14]. The first-generation sequencers could generate very short reads of about 35 bp but the continuous improvements made in the sequencing technique led to an increase in the read lengths to up to 70 bp with a very high accuracy (99.9%). High accuracy is because each nucleotide is read twice while sequencing [15]. The major limitation of the sequencing system is the higher run time, which is required for sequencing, and the relatively shorter reads as compared to other NGS methods generated by sequencing [16].

3.4. Ion Torrent Sequencing

Ion torrent is a similar sequencing platform to 454/Roche but here fluorescently labeled nucleotides are not used, rather a semiconductor chip is utilized to sense the H^+ ions released during DNA synthesis by the polymerase enzyme. Whenever a new nucleotide is incorporated into the chain, one H^+ ion is released that also creates a change in the pH of the solution. This change in pH is detected by the sensor attached to the semiconductor device and then this electronic signal is translated into the nucleotides incorporated in the chain. The nucleotides that are not incorporated are removed by washing prior to the incorporation of the new nucleotide [17]. Ion torrent sequencing generates the longest read lengths among all the NGS methods. It can generate up to 200 bp long lead read lengths with a good throughput of 10 Gb per run. The limitation is the complexity of the interpretation of homopolymer sequences, which leads to an error rate of about 1%. The major limitation of the NGS technology was the generation of shorter read lengths due to which *de novo* assembly is very difficult. Also, the sequencing required longer run time and involved additional equipment costs, which made the entire sequencing process quite expensive [18].

4. THIRD GENERATION SEQUENCING

The second-generation sequencing platforms have brought very significant changes over the FGS methods and are widely commercialized and popular sequencing techniques. However, the requirement of PCR amplification made the processes very time-consuming and also quite expensive. At the same time, the generation of shorter read lengths made genome assembly difficult and very problematic for highly complex genomes with several repetitive sequences. To overcome these challenges of NGS, there was a development of Third Generation sequencing, which did not require PCR amplification thus reducing the time and having low sequencing cost. These TGS methods also generate longer reads over many kbs and thus solve the problem of genome assembly and repetitive sequences of complex genomes [19].

4.1. Pacbios Single Molecule Real-time Technology

Pacific Biosciences developed the first third-generation sequencing platform popularly known as single-molecule real-time technology or SMRT. Here, single DNA molecules are attached to the wells of ten nanometers in diameter by a biotin-streptavidin reaction. Due to the micro size of the wells, the intensity of light is decreased laterally in the wells and the bottom part is well illuminated. DNA sequences are attached to the wells and they contain DNA polymerase with the fluorophore-labeled dNTPs and a single DNA polymerase uses only one fragment of DNA for sequencing. Whenever a nucleotide is incorporated, there is an emission of light signal which is sensed by different sensors. The software programs are used to deduce the DNA sequence with the help of fluorescently labeled nucleotides [20]. The average read length generated by this sequencing platform is about 1 kb but it can generate up to 10 kb of sequencing data. The sample preparation and the overall process are very fast which takes hours only. The major limitation of this sequencing technique is the error rate which can exceed over 10% [21].

4.2. Oxford Nanopore Sequencing

The sequencing technique by Oxford nanopore is based on the change in the signals generated optically or electronically when the nucleotides of a DNA fragment are passed through an extremely small hole also known as a nanopore. These electronic or optical signals are transformed into base sequence data by the software programs. This sequencing technology can also detect the bases undergone methylation to derive the sequence data [22]. This technology can generate longer reads, which is helpful while dealing with complex genomes such as plants, which contain several repetitive sequences and are usually difficult to sequence utilizing the NGS techniques [23].

5. NGS AND ITS IMPLICATIONS IN THE DEVELOPMENT OF CLIMATE-RESILIENT CROPS

Advancements in NGS sequencing led to huge progress in terms of decoding genetic and genomic information of several complex genomic organizations. It resulted in reducing the overall expense and at the same time helped in the assembly of the genomic libraries, which contained repetitive sequences and hence were a challenge for *de novo* assembly. It extended progress in whole genome sequencing by reducing the time taken and the overall cost of sequencing. With the advent of NGS, generating high-quality genetic information that can be aligned to a reference genome sequence became feasible and thus it could greatly help in a finer understanding of the complex genomes like plants and humans [1]. This in turn will greatly facilitate the status of genetic diversity of the concerned crop species and their wild relatives which can be a greatly valuable asset while developing crops with the resilience to climate change.

5.1. Development of Climate-resilient Cereals

Cereals are the staple food crops for most of the world population and thus nearly the entire world population is dependent on cereals for their food security. Hence ensuring the adequate supply of these crops and sustaining the production levels are highly important in this regard. From the viewpoint of reaching such production levels with sustainability, genomic techniques

such as NGS have a key role to play. In recent years, NGS and other genomic approaches have become extremely popular in decoding genetic information related to various agronomic traits associated with crop performance under adverse environments. Several QTLs have been identified regulating such plant responses concerned with its performance under various unpredictable and adverse climatic and biotic challenges. QTLs identified for various such traits include drought tolerance, salinity tolerance, membrane stability under heat stress, crop canopy temperature, rhizospheric architecture, terminal stress tolerance, delayed senescence, and accumulation of water-soluble solutes and their distribution. Genome-wide association analysis of wheat for its performance under heat and drought stress indicated the presence of several locations in the wheat genome to possess QTL hotspots for tolerance against such stresses; chromosomes 2A and 2B were observed to contain such hotspots [24]. Salinity is one of the major problems that severely reduces yield of the cereals such as rice. Various QTLs are identified in rice that possess tolerance under saline conditions and help in maintaining sustainable yield levels. A major QTL conferring salinity tolerance in rice was identified on chromosome 1 termed *saltol,* which controlled various physiological traits to enrich plants with salt tolerance. This QTL was associated with seedling as well as adult plant tolerance and balancing shoot Na+/K+ ratio is the major mechanism through which it provides tolerance [25].

5.2. Development of Climate-resilient Oilseeds and Pulses

Apart from cereals, oilseeds, and pulses are highly important fractions of the human diet providing necessary protein and oil requirements. There are several constraints associated with the maintenance of production levels of these crops and the breeding efforts are yet to be taken in such directions. In these crops, various biotic and abiotic stresses put severe challenges in improving yield levels. Exploiting the available genetic diversity and resources in these crops is very important as in breeding programs, largely those go unnoticed. In the case of crops like chickpeas and pigeon peas, various climatic stresses result in severe loss in terms of maintaining stable grain yields. Such challenges can be overcome only by the dissection of large genetic variation available for exploitation in these crops. There have been several attempts to enrich the germplasm resources in these and utilize them in breeding programs for the development of crop plants that can withstand such future challenges posed by those climatic uncertainties. There have been several attempts to identify genomic regions associated with drought and other abiotic tolerances and transfer the trait to the superior genetic background to utilize it in breeding programs [26-29]. Along with different abiotic stresses, there are several biotic agents which hugely impact the overall production and quality of these crops. Many research programs led to the search for genetic resistance and the deployment of such resistance in crop plants. Such examples include resistance to fusarium wilt in pigeon peas [30], sterility mosaic resistance in pigeon peas [31], and resistance against ascochyta blight and botrytis grey mold in chickpeas [32]. Recently, draft genome sequences of both pigeon peas and chickpeas have been published revealing huge genetic information that can help in gaining overall productivity and also sustaining the requirements of resource-poor areas [33].

5.3. Development of Climate-resilient Fruit and Horticultural Crops

Fruits and other horticultural crops are also a largely important fraction of our overall dietary system, which plays an important role in complementing the total nutritional requirement of a human being. Hence, these crops greatly require the attention of the breeders in terms of developing these crops for climate resilience and protection against such agents of nature, which greatly hamper their production and quality. Sequencing and other genomic techniques have been employed in crops like apples [34], grapes [35], bananas [36], mango [37], and sweet oranges [38]. Further advancements in genomic-assisted breeding with the advent of whole genome sequencing and high-resolution mapping approaches can be very supportive for developing varieties of such crops that will be capable of providing resilience against such agents of climate change. However, even with the development of such technologies, there has not been such huge improvement in terms of developing limes which are climate change-ready in these crops. There is a great scope for the improvement of these crops. Major agronomic crops against various abiotic stresses and their respective QTLs are shown in Table **1** and agronomic crops against various biotic stresses and their respective QTLs are portrayed in Table **2**.

6. BREEDING STRATEGIES BASED ON NGS FOR THE DEVELOPMENT OF CLIMATE-RESILIENT CROPS

NGS and other genomic approaches can be extremely important in the viewpoint of breeding crops with good withstanding capacity under unfavorable and adverse environmental circumstances. The huge untapped genetic diversity for the identification of such alleles which can contribute to the enhanced capacity of plants to perform better under several abiotic and biotic stress conditions can be utilized using these novel genomic techniques. Improving crops for complex genetic traits such as yield techniques like genomic selection can become extremely handy and an extremely vital tool for the complete dissection of such complex traits. However, due to the involvement of huge amounts of costs and being technically challenging, it still could not be employed in large-scale breeding programs and MAS remains the choice of breeders for introgression of QTLs [143]. Although, even after employing so many different technologies it has not been possible to develop a model which can completely help to overcome all the possible challenges posed by the threat of climate change. There is an urgent requirement for the development of tools based

on a multidisciplinary approach that will equip the breeders to select and maintain the lines that will be more compatible with future climate change and also to sustain global food security currently and in the years to come.

Table 1. QTLs identified in major agronomic crops against various abiotic stresses.

S. No.	Crop	Target Trait	Remarks	References
1.	Rice	Salt stress tolerance; Na+/K+ uptake ratio	2 QTLs identified on chromosomes 3 and 6.	[39]
		Salt stress	3 QTLs mapped for days to survive under stress.	[40]
		Heat stress	3 QTLS were detected on chromosomes 1, 4 & 7.	[41]
		Drought stress	3 QTLS were detected on chromosomes 2, 4 & 7.	[42]
		Spikelet fertility under heat stress	A total of 8 QTLs identified under heat stress.	[43]
		salt stress	15 QTLs were found to be putatively associated.	[44]
		Drought stress	13 QTLs were detected to be associated with tolerance.	[45]
		Salt stress	18 QTLs were detected for different traits associated with salt tolerance.	[46]
		Salt stress	72 QTLs identified for salt tolerance.	[47]
		Salt stress	34 QTLs for 10 traits identified.	[48]
		Heat stress	5 QTLs identified	[49]
		Drought stress	21 QTLs were identified using the CIM approach.	[50]
		Heat stress	35 meta-QTLs analyzed.	[51]
		Drought stress	5 meta-QTLs analyzed.	[52]
		Drought stress	28 QTLs were detected on 8 chromosomes.	[53]
2.	Wheat	Drought stress	18 QTLs identified	[54]
		Heat stress	3 QTLs detected on 1B, 3B and 5B.	[55]
		Heat stress	14 QTLs detected.	[56]
		Heat stress	3 QTLs identified.	[57]
		Heat stress	5 QTL regions were found.	[58]
		Drought stress	34 QTLs related to drought tolerance.	[59]
		Heat stress	234 QTLs putatively linked with heat tolerance.	[60]
		Heat and Drought stress	6 stress specific QTLs detected.	[61]
		Heat stress	24 QTLs detected.	[62]
		Salt stress	49 QTLs were mapped.	[63]
		Terminal heat tolerance	26 QTLs identified.	[64]
		Salt stress	19 QTLs were identified	[65]
		Heat and Drought stress	86 MQTLs identified for yield under stress.	[66]
		Salt stress	6 QTLs mapped under salt stress.	[67]
		Salt stress	13 QTLs detected.	[68]
		Drought stress	13 MQTLs localized.	[69]
3.	Chick pea	Salt stress	6 QTLs identified.	[70]
		Salt stress	Cluster of QTLs identified under varied conditions.	[71]
		Salt stress	2 major QTLs identified for yield under salt stress.	[72]
		Salt stress	28 QTLs identified.	[73]
		Salt stress	42 QTLs linked to salinity stress.	[74]
		Heat Stress	Different QTLs identified across environments.	[75]
		Heat stress	2 QTLs detected.	[76]
		Heat stress	28 and 23 QTLs mapped under different conditions.	[77]
4.	Soybean	Drought stress	10 QTLs associated with drought stress.	[78]
		Drought stress	6 QTLs detected.	[79]
		Drought stress	23 QTLs were detected for drought stress.	[80]
		Drought stress	10 QTLs detected.	[81]
		Salt stress	2 novel QTLs identified.	[82]
5.	Tomato	Salt stress	12 interactive QTLs mapped.	[83]
		Salt stress	5 QTLs identified.	[84]
		Salt stress	5 QTLs identified.	[85]
		Salt stress	6 major QTLs detected.	[86]

Table 2. QTLs identified in major agronomic crops against various biotic stresses.

S. No.	Crop	Target Trait	Remarks	References
1.	Rice	Brown plant hopper	2 QTLs identified.	[87]
		Rice blast resistance	4 QTLs detected.	[88]
		Sheath blight	2 QTLs identified.	[89]
		Sheath blight	6 QTLs identified.	[90]
		Brown spot resistance	3 QTLs identified.	[91]
		Green leaf hopper	One major QTL and 3 minor QTLs identified.	[92]
		Leaf folder	5 QTLs were identified.	[93]
		Rice blast resistance	6 QTLs mapped.	[94]
		Rice root knot nematode	11 QTLs detected.	[95]
		Rice root knot nematode	2 QTLs identified.	[96]
2.	Wheat	Fusarium blight resistance	2 QTLs identified.	[97]
		Powdery mildew resistance	3 and 5 QTLs detected in two different populations.	[98]
		Karnal bunt resistance	2 novel and one earlier reported QTLs mapped.	[99]
		Stripe rust	4 to 6 QTLs reported in different populations.	[100]
		Flag smut	5 QTLs identified.	[101]
		Leaf rust	35 meta-QTLs located on 17 chromosomes	[102]
		Loose smut	3 major QTLs detected.	[103]
		Orange wheat blossom midge	2 novel QTLs detected.	[104]
		Hessian fly	Identified 2 novel QTLs.	[105]
		Stripe rust	Identification of 61 meta-QTLs.	[106]
		Cereal cyst nematode	A total of 19 QTLs were detected.	[107]
3.	Maize	Head smut	13 QTLs identified.	[108]
		Downy mildew	Detection of 6 QTLs.	[109]
		Southern leaf blight	4 SLB resistance QTLs identified.	[110]
		Grey leaf spot	26 QTLs detected.	[111]
		Head smut	Fine mapping of a major QTL.	[112]
		Shoot fly	29 QTLs detected.	[113]
		Sorghum downy mildew	5 QTLs detected.	[114]
		Gray leaf spot	30 QTLs identified.	[115]
		Corn leaf aphid	One QTL at chromosome 4 other at 6 were identified.	[116]
		Head smut	2 QTLs identified.	[117]
		White spot	6 QTLs localized.	[118]
4.	Sorghum	Green bug resistance	9 QTLs detected.	[119]
		Ergot resistance	Identified 9 QTLs.	[120]
		Shoot fly resistance	25 QTLs detected.	[121]
		Green bug resistance	4 major QTLs identified.	[122]
		Rust resistance	64 QTLs putatively located.	[123]
		Target leaf spot	2 genomic regions observed to show resistance.	[124]
5.	Pearl millet	Downy mildew resistance	2 QTLs detected.	[125]
		Downy mildew resistance	One major QTL identified.	[126]
		Blast resistance	2 QTLs detected.	[127]
		Downy mildew resistance	53 loci with DM resistance observed.	[128]
6.	Cotton	Verticillium wilt resistance	2 QTLs detected.	[129]
		Bacterial blight	Identified 2 QTLs.	[130]
		Fusarium wilt	3 QTLs detected.	[131]
		Fusarium wilt	5 QTLs localized.	[132]
7.	Green gram	Powdery mildew	2 QTLs identified.	[133]
		Yellow mosaic	5 QTLs detected.	[134]
		Bruchid and bean bug resistance	2 QTLs for bruchid resistance and one for bean bug detected.	[135]
8.	Black gram	Bruchid resistance	2 and 6 different QTLs identified.	[136]
		Bruchid resistance	3 QTLs identified.	[137]
		Yellow mosaic	2 major QTLs identified.	[138]

(Table 2) contd.....

S. No.	Crop	Target Trait	Remarks	References
9.	Groundnut	Leafspot	11 QTLs identified in three environments.	[139]
		Leafspot	6 QTLs identified.	[140]
		Late leaf spot	9 candidate genes spanning over 14 intronic and 3 SNPs.	[141]
		Leaf spot	2 major QTLs one on chromosome 3A and the other on 4B detected.	[142]

6.1. Reproductive Traits and Drought Tolerance

The temperature in conjunction with photoperiod plays a significant role in the development of a crop, especially in the case of floral initiation and transition of a plant from vegetative to the reproductive stage. With the events of global warming and climate change, there has been a constant rise in the overall temperature but without having a change in the photoperiod levels of the crop. Many crop plants are positively regulated through the increased temperature, which results in their overall additional growth and physical development but the reproductive advancement is not accompanied by a similar pace; also it leads to less accumulation of photosynthates as the plants get less time for photosynthesis as compared to normal circumstances. To fetch further information in this regard, there is the development of various germplasm resources, which include populations like NILs (Near Isogenic Lines), MAGIC (Multi Parent Advanced Generation Intercrosses), NAM (Nested Association Mapping) populations, which can be very helpful in the mapping of genes and QTLs related to such traits, which will enable plants to withstand the adverse aspects of drought by modification of various physiological mechanisms [143]. For drought tolerance, there are multi-scientific approaches that are used to improve overall plant response to moisture-scarce conditions and also to increase water use efficiency. With the advent of modern sequencing and the genomic arena, there is an emphasis on more QTL identification and QTL use in breeding programs to support the development of new lines with enhanced drought tolerance. In cereals like wheat and maize, there have been extensive studies showing various root characteristics and the QTLs associated with such traits, which showed positive responses concerning drought and moisture stress environments [144, 145].

6.2. Development of Tolerance to Salinity and Water Logging

In case of problem soils such as saline and sodic soils, water logging is one of the major challenges to be faced in terms of breeding for higher productive ability and sustainability of production. There have been several reports on the presence of significant genetic variability in various crops for water logging and submergence tolerance and also different physiological mechanisms of such tolerances. Even in the case of crop like rice, which remains for a considerable period under water logging, there is a requirement of tolerance to water logging especially during germination and seedling establishment stage because hypoxia effects can result in a severe reduction in the crop stand resulting in large scale loss in attainable economic yield. Various QTLs identified in rice can provide enhanced level performance under submergence and hypoxic conditions. One such example is *sub1* allele, which is located on chromosome 9 popular for enriching tolerance levels of the majority of the mega rice varieties against submergence and water logging conditions [146]. It has been found that the allele encodes an ethylene response factor, which is involved in the determination of submergence tolerance [147]. *Sub1* allele showed no such penalty in terms of productivity when grown under non-flooded areas [148] but resulted in significant improvement in productivity and quality under submerged conditions when compared to non-*sub1* cultivars [149]. Apart from *sub1*, there have been different QTLs reported in the case of rice which were responsible for enhanced submergence tolerance in the crop [150, 151]. Increased levels of salinity in soils due to unwarranted use of poor-quality water for irrigation and poor drainage is also one of the major problem areas reducing the quality and the productivity of important crops. In many cases, salt tolerance is governed by minor genes along with the maternal effect and in some cases, a partial dominance effect was also observed. Thus, breeding for such traits becomes quite complex and involves the incorporation of other associated factors, which provides superior performance under saline conditions. Just like submergence tolerance, the best example of enhanced performance under salinity is provided in the case of rice by *saltol* allele located on chromosome 1 [152]. This QTL has been introgressed in many popular rice varieties to incorporate enhanced salinity tolerance [153] and *saltol* introgressed lines also have resulted in a lesser loss in the yield levels under salinity for years of evaluation as compared to non-*saltol* lines [154]. There are several other genes also identified, which are associated with increased performance of plants and lesser yield loss under salinity. Further research for identifying and incorporating such genes in different salt-sensitive crops is required for sustainable production of crops under such problem soils. The advent of technologies like NGS for targeting individual QTLs for incorporation and development of effective markers to aid in the selection process can be greatly helpful to develop cultivars with enhanced levels of productivity under salinity and other obstacles for improving crop productivity.

6.3. Development of Tolerance against Biotic Agents

Global warming and overall changes in the global climate not only increase the risk of high losses through abiotic stresses but also lead to increased pests infestation and diseases due to changes in temperature, rainfall patterns, and other associated environmental variables [155]. It also hampers plants' internal ability to tolerate

infestations of diseases and pests reducing the immune response of the plant. Studies have indicated that there was over a 40% increase in the case of rapeseed when the external temperature was increased by 5 °C, which showed that the increased temperature levels influenced the quantum of disease infestation in a particular crop [156]. The results of the earlier experiment suggest that there is a noticeable variation in the effectiveness of various R genes of the host plants when the plant and the pathogen are put under some change or variation in the environmental variable such as temperature in this case. The reason behind this may be the differential selection pressure on the pathogen and the R gene in changing environments resulting in a change of their effectivity. Further advancements in the available knowledge of host plant interactions under different variable environmental circumstances will be very significant in developing climate-resilient crops. Advancements in sequencing techniques and genotyping assays may be greatly useful for the development of strategies that will allow breeders to understand the effect of climate change on the infestation of various diseases and insect pests in different crops and the change that is brought by the interaction of various components of climatic variables with pests, pathogens, and genes present in the crops for resistance against such agents.

7. UTILIZING NOVEL GENOMIC TOOLS FOR THE DEVELOPMENT OF CLIMATE-READY CROPS

Sequencing technologies have revolutionized crop development, enabling the creation of climate-ready varieties. By deciphering the genetic code, novel genomic tools offer insights into traits crucial for climate resilience. High-throughput sequencing facilitates the identification of genes associated with stress tolerance and adaptation. Leveraging this knowledge, breeders can employ precise breeding strategies to develop crops resilient to climate change. These advancements underscore the pivotal role of sequencing in the creation of resilient agricultural systems, essential for ensuring food security in a changing climate.

7.1. Molecular Markers for Genomic assisted Breeding

The 1980s saw the start of the age of molecular marker development and applications using genomic data. A decade after this milestone in plant genomics research, PCR-based DNA markers were developed. Since then, the uses of several molecular markers in diverse facets of plant molecular breeding and genomics have been documented [157]. With targeted or randomly chosen oligonucleotide primers, the PCR process realistically amplifies particular DNA sequences from genomic DNA sections. Molecular markers are among the most helpful instruments for plant improvement research that are now accessible. The majority of these markers are polymorphic nucleic acids in individuals or populations [158]. Point mutations in oligonucleotide priming sites result in genotypes with differing pools of fragments. In plant breeding experiments utilizing genomic data, the molecular marker techniques that are often employed are RFLP, AFLP, RAPD, SCAR, SSR, CpSSR, IRAP, REMAP, ISSR, RAMP, SSCP, SAMPL, SRAP, CAPS, EST, SNP, DArT, STS, RBIP, and IPBS [159]. It is practically impossible to find a perfect molecular marker technique that satisfies every need and does not present any difficulties when used. Therefore, while choosing the right DNA marker approaches to enable the attainment of a certain set of research objectives, it is always vital to examine a few key variables [157]. The understanding of the set objective, the degree of expected genetic variation and data to be generated from the study samples, the sample size to be worked with, the accessibility of probes or primer sets, the availability of the necessary facilities and technical ability, time constraints, and financial considerations all play a major role in the decision of which marker technique to use [160]. However, a sizable number of plant molecular breeding projects have lately used these new, sophisticated molecular marker techniques to accomplish a variety of study goals. The creation of molecular markers that are more effective for the genomic analysis of economically significant crops has been the main focus of molecular marker research throughout the years. Conversely, not much funding has been allocated to the development of molecular markers for the genomic analysis of underutilized crops that are not economically relevant [159]. As a result, sequence information or data to support primer creation is still sadly lacking in the majority of underutilized crops. Thus, several DNA marker approaches remain unsuitable for use in such crops. However, it is anticipated that as the cost of DNA sequencing drops dramatically and the cost of developing molecular markers falls, these crops will also be covered shortly [159]. This introduction to molecular marker techniques will deepen our understanding and make it easier to apply DNA marker approaches to plant breeding in a way that promotes sustainable agricultural output and usage.

7.2. Transcriptome Sequencing

Many omics techniques, including transcriptomics, proteomics, metabolomics, and genomics, have been created since the start of the post-genomic period. Transcriptomics is the second most ancient and widely applied of these methods [161]. Studies on transcriptomics concentrate on the transcriptome. Over the past 20 years, genomic sequence databases have grown significantly because of their high throughput, increasing accuracy, and cost efficiency [162]. Molecular biology still faces significant challenges in the intricate mapping of a genome to many phenotypes, tissues, developmental stages, and environmental influences. Not only is a deeper comprehension of gene control transcripts and expression challenging, but it is also the fundamental cause of the issue. Numerous species have been the subject of considerable transcriptomics research, which offers vital insights into the structure, expression, and control of genes [163]. Because sequencing technology has advanced so quickly in recent years, transcriptomics research has expanded greatly [164]. Recent advances in sequencing

technology have allowed transcriptome study methodologies to advance from basic DNA microarray platforms to RNA-Seq technology [165]. With its high sensitivity, high throughput, and effectiveness, it can assess a whole transcriptome without the need for a genomic reference sequence, among its many benefits. In molecular biology, biotechnology, and bioinformatics, RNA-Seq technology is a widely used sequencing method [166]. Numerous model plants have been used to test this technique, including *Rehmannia glutinosa* [167], *Calotropis gigantea* [168], *Polygonum cuspidatum* [169], *Zea mays* [170, 171], and *Rehmannia glutinosa* [167]. The total amount of RNA molecules, including messenger RNA (mRNA) and non-coding RNA (nc-RNA), transcribed from a certain tissue or cell at a given functional or developmental stage is known as the transcriptome. Since they precisely control the transfer of genetic information from DNA to protein, they are known as "bridges" [172]. In contrast, non-coding RNA affects gene expression, protein synthesis, and several physiological activities on multiple levels [173]. Thus, transcriptomics studies improve the knowledge about the operations of tissues, cells, and organisms. A relatively recent technique that quantifies the transcriptome's total biological quantities is called RNA-Seq. This makes it easier to analyze the transcriptome [174]. In summary, the development of omics methods, transcriptomics in particular, has transformed molecular biology by offering a profound understanding of gene expression, regulation, and function. One of the oldest and most used omics techniques, transcriptomics, has been essential to comprehend the intricacy of the transcriptome in different species. Transcriptomics research has grown to an unprecedented extent due to the rapid advances in sequencing technology, especially the switch from DNA microarrays to RNA-Seq [165]. This advancement has made it easier to conduct thorough investigations of RNA molecules, including messenger RNA (mRNA) and non-coding RNA (ncRNA), which has helped to clarify their functions as important regulators of gene expression and biological processes [174]. Transcriptomics will remain crucial in the future for deciphering the complex relationships between genotype and phenotype, which will further advance our comprehension of biological systems at the molecular level.

7.3. Epigenome Sequencing

The control of gene expression and the maintenance of genomic integrity depend heavily on epigenetic changes. One of the main systems of epigenetic regulation, DNA methylation affects the growth, development, stress tolerance, and adaptation of all living things, including plants [175]. Understanding the mechanisms behind these processes and creating ways to increase agricultural plants' production and stress tolerance depend heavily on the detection of DNA methylation marks. Cytosine methylation takes place in plants at symmetric CG, asymmetric CHH, and symmetric CHG sites, where H can be any nucleotide other than G. Small interfering RNAs (siRNAs) are responsible for directing de novo methylation in CG, CHG, and CHH contexts. This process is primarily driven by domains rearranged methyltransferase1 (DRM1) and 2 (DRM2) [176, 177]. The control of gene expression, as well as the silencing and reactivation of TEs, are linked to the relevance of cytosine methylation for plant evolution [178]. TE amplifications depend on non-CG methylation, as evidenced by the fact that whereas CHG methylation varies and is frequently correlated with genome size, CHH methylation is generally conserved across plant species on a whole genome level [179]. Nonetheless, methylation pattern analysis techniques are quite varied and have advanced significantly in recent years. NGS and sequencing-based DNA methylation mapping have made it possible to do genome-wide methylation profiling at single-nucleotide resolution, displacing earlier chromatographic approaches [180]. A common method for characterizing the genome and assessing differential DNA methylation is genome-wide DNA methylation analysis [181, 181]. Frommer *et al*. [182] first described bisulfite DNA sequencing, which opened the door for the next generation of NGS techniques known as whole-genome bisulfite sequencing (WGBS), which allows for high-throughput investigation of DNA methylation. The basis of the locus-specific bisulfite sequencing approach is the conversion of cytosines in single-stranded DNA to uracils by sodium bisulfite, which is followed by PCR amplification of certain loci within the changed DNA, their cloning, and Sanger sequencing. Since reduced representation bisulfite sequencing (RRBS) only looks at a representative portion of the genome and produces DNA methylation profiles with single-nucleotide precision, it is a more affordable option than whole genome bisulfite sequencing (WGBS) [183]. This method, which was first created to investigate mammals, targets CG islands and sequences them in several phases. To put it briefly, the CCGG sequence is recognized by the enzyme MspI, which is insensitive to methylation and cuts genomic DNA into tiny pieces with CG dinucleotides at the ends. After selecting and isolating CG-rich segments, end repair, A-tailing, ligation to methylated adapters, bisulfite conversion, PCR amplification, and end sequencing are the subsequent stages. Targeted BS, also known as Methylation Capture Sequencing (MC-seq), is a less expensive option to WGBS that uses BS to gather DNA methylation data [184]. Because targeted NGS is associated with bisulfite treatment, it may detect DNA methylation at single-nucleotide resolution [184]. Targeted NGS is intended to focus on certain genomic areas of interest [185]. WGS and MC-seq are comparable in that both methods need target enrichment using hybridization capture with biotinylated oligonucleotide probes in order to capture certain areas during sample preparation. Target enrichment for methylomic areas of interest may be achieved precisely with this approach, which is then followed by bisulfite treatment. The Methyl DNA Immunoprecipitation (MeDIP) method, which uses a 5mC antibody for methylation analysis and may be combined with array detection (MeDIP-chip) or sequencing (MeDIP-seq), is one of the few alternatives to bisulfite treatment. This technique produces data that apply to different crops [186] and is

consistent with WGBS [187]. In summary, enhancing plant productivity and stress resilience requires a thorough understanding of the critical function that DNA methylation plays in the control of gene expression and genomic integrity. Next-generation sequencing and bisulfite sequencing are two recent developments in methylation pattern research that provide strong methods for thorough DNA methylation profiling. In addition, single-nucleotide resolution insights into methylation dynamics can be obtained by methods such as reduced representation bisulfite sequencing and whole-genome bisulfite sequencing.

7.4. Genome Sequencing for Identification of Genome Editing

Site-directed nuclease systems (SDN) 1, 2, and 3 are the three types of nuclease-based genome editing methods [188, 189]. Applications of SDN1 rely on non-homologous end-joining (NHEJ), an endogenous process that is the most widely used method in plants to repair double-strand DNA breaks. Random point mutations typically arise in the repaired locus because NHEJ is an error-prone process [190]. If a template sequence is available, the cell may use homology-directed repair (HDR), an alternative repair method [191]. The application will be classified as SDN2 if this repair template is similar to the autochthonous sequence except for one or a few nucleotides [188]. This mechanism will be classified as SDN3 if longer DNA sequences, which may be of extra, allelic, or foreign origin, are integrated into the target genome in a site-specific manner [188]. By using a synthetic single-stranded oligonucleotide complementary to the target sequence, oligonucleotide-directed mutagenesis (ODM) introduces precise, site-specific modifications of one or more nucleotides by the cellular mismatch repair mechanism without the need for the introduction of a nuclease [192]. In sequencing-based identification of genome editing events, particularly those induced by site-directed nuclease systems (SDN), various strategies are employed to detect the alterations introduced at targeted loci. For SDN1 applications, where NHEJ is predominantly utilized for repair, sequencing analysis typically reveals random point mutations at the repaired locus due to the error-prone nature of NHEJ [193]. When a repair template resembling the endogenous sequence, with minor differences, is provided, the repair mechanism shifts towards homology-directed repair (HDR), leading to precise nucleotide alterations. This scenario characterizes SDN2 events, distinguishable through sequencing by the specific nature of the introduced changes [188]. On the other hand, SDN3 classification occurs when longer DNA sequences from various sources are integrated site-specifically into the genome, which can be identified through sequencing by the presence of foreign or allelic sequences at the target site [188]. Additionally, oligonucleotide-directed mutagenesis (ODM) can induce precise modifications without nucleases, leveraging the cellular mismatch repair mechanism. Sequencing analysis in ODM typically reveals specific alterations consistent with the introduced oligonucleotide sequence, allowing for the identification of site-specific modifications [192]. Therefore, sequencing serves as a critical tool in discerning the nature and precision of genome editing events induced by different nuclease-based and non-nuclease-based methodologies.

7.5. Sequencing of Plant Microbial Community

A platform that makes it possible to extract DNA sequence data directly from environmental materials has been made possible by NGS [194]. Numerous applications are said to be possible with these data, some of which include comparing the microbiota found in healthy and diseased individuals [195] by studying the biodiversity of the ecosystem [196], studying DNA evolution [197], and analyzing gut DNA fragments [198]. The many taxa that are accessible as environmental samples will be presented with certainty with a comparison of the sequencing data with an expanding standard reference library of identifiable species. Through the use of DNA clustering and annotation using phylogenetic and alignment approaches, recent advances in computational tools have improved the study of biodiversity across geography and time [196]. Ecological study is currently focused on exploiting enormous amounts of sequence data due to parallel advances in the number and breadth of data gathered using NGS platforms. With this method, PCR-attributed error and bias findings have been reduced, and run times have been greatly shortened. Recently, a multitude of platforms have been identified, each having pros and cons. The aforementioned platforms employ unique templates for their production and utilize diverse chemistries for the detection of sequencing signals [199, 200]. In conclusion, NGS enables researchers to analyze and make use of enormous volumes of sequence data, advancing study in the fields of ecology and evolution. Through the use of NGS platforms and computational tools, scientists may effectively investigate the intricate workings of complex biological systems, therefore augmenting our comprehension of ecosystem dynamics, biodiversity, and evolutionary processes.

CONCLUSION AND THE WAY FORWARD

With the advent of technologies like next-generation sequencing and genomic-assisted breeding, there are prodigious opportunities in the direction of developing climate resilience crops with sustainable production levels. Yet, there has not been great progress in terms of turning such opportunities into reality. The major limitation in this regard has been the feasibility of utilizing such technologies everywhere and also the expense associated with that. However, the technologies associated with genomics-assisted breeding are evolving such rapidly that they can become much more accessible and cost-effective in due course of time. Improvements in the development of efficient molecular markers along with increasing knowledge about various metabolites and other responses against different biotic and climatic difficulties will further accelerate the breeding programme. A multidisciplinary approach integrating components of various fields such as biotechnology, bioinformatics,

proteomics and metabolomics, physiology, and molecular biology could become highly useful in the complete understanding of mechanisms of plant response as well as the development of a particular phenotype in such adverse circumstances. Global warming and climatic fluctuations are a serious threat to the future sustainability of agricultural production. There is a serious concern regarding the maintenance of productivity levels developing such crop cultivars which will be able to withstand such fluctuating environmental circumstances. Hence, there is a requirement to evolve such technologies, which will assist breeders in incorporating genetic tolerance against various agents of biotic and abiotic stresses. Recent advancements in sequencing and other genomic technologies can become greatly useful for the development of such resilient varieties breaking the traditional barriers of hybridization. These technologies can further help in the identification of key genes and metabolites involved in the regulation of plant responses under various adverse environments. Although it is nearly impossible to predict the exact influence of climate change on the crop production scenario, continuous improvements in genomics and genomics-assisted breeding techniques can contribute significantly to minimizing the quantum of the negative impacts on crop plants.

LIST OF ABBREVIATIONS

PCR	=	Polymerase Chain Reaction
SDN	=	Site Directed Nuclease
HDR	=	Homology-Directed Repair
ODM	=	Oligonucleotide-Directed Mutagenesis

CONSENT FOR PUBLICATION

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