**REVIEW ARTICLE**

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

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**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.

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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 21$^{st}$ 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 4) 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 methodo-logies 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 chromato-graphic 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

Not applicable.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

## REFERENCES

[1] Maity A, Paul D, Lamichaney A, *et al.* Climate change impacts on seed production and quality: Current knowledge, implications, and mitigation strategies. Seed Sci Technol 2023; 51(1): 65-96.
http://dx.doi.org/10.15258/sst.2023.51.1.07

[2] Dutta S, Singh AK, Mondal BP, Paul D, Patra K. Digital inclusion of the farming sector using drone technology. Human-Robot Interaction - Perspectives and Applications. IntechOpen 2023; p. 16.

[3] Jurgilevich A, Käyhkö J, Räsänen A, *et al.* Factors influencing vulnerability to climate change-related health impacts in cities – A conceptual framework. Environ Int 2023; 173: 107837.
http://dx.doi.org/10.1016/j.envint.2023.107837 PMID: 36921561

[4] Das A, Rout BM, Datta S, Singh S, Munshi AD, Dey SS. Spinach (Spinacia oleracea L.) breeding: From classical to genomics-

centric approach. Smart Plant Breeding for Vegetable Crops in Post-genomics Era. Singapore: Springer Nature Singapore 2023; pp. 117-42.
http://dx.doi.org/10.1007/978-981-19-5367-5_6

[5] Das A, Singh S, Islam Z, *et al.* Current progress in genetic and genomics-aided breeding for stress resistance in cucumber (*Cucumis sativus* L.). Sci Hortic 2022; 300: 111059.
http://dx.doi.org/10.1016/j.scienta.2022.111059

[6] Chen L, Wang Q, Tang M, *et al.* QTL mapping and identification of candidate genes for heat tolerance at the flowering stage in rice. Front Genet 2021; 11: 621871.
http://dx.doi.org/10.3389/fgene.2020.621871 PMID: 33552136

[7] Tsiatis AC, Norris-Kirby A, Rich RG, *et al.* Comparison of Sanger sequencing, pyrosequencing, and melting curve analysis for the detection of KRAS mutations: Diagnostic and clinical implications. J Mol Diagn 2010; 12(4): 425-32.
http://dx.doi.org/10.2353/jmoldx.2010.090188 PMID: 20431034

[8] aMaxam AM, Gilbert W. A new method for sequencing DNA. Proc Natl Acad Sci U S A 1977; 74(2): 560-4.
http://dx.doi.org/10.1073/pnas.74.2.560 ; bMaxam AM, Gilbert W. From Sanger sequencing to genome databases and beyond. Bio techniques 2019; 66(2): 60-3.
http://dx.doi.org/10.1073/pnas.74.2.560

[9] Dahui Q. Next-generation sequencing and its clinical application. Cancer Biol Med 2019; 16(1): 4-10.
http://dx.doi.org/10.20892/j.issn.2095-3941.2018.0055 PMID: 31119042

[10] Rothberg JM, Leamon JH. The development and impact of 454 sequencing. Nat Biotechnol 2008; 26(10): 1117-24.
http://dx.doi.org/10.1038/nbt1485 PMID: 18846085

[11] Ellegren H. Comparative genomics and the study of evolution by natural selection. Mol Ecol 2008; 17(21): 4586-96.
http://dx.doi.org/10.1111/j.1365-294X.2008.03954.x PMID: 19140982

[12] Bronner IF, Quail MA, Turner DJ, Swerdlow H. Improved protocols for illumina sequencing. Curr Protoc Hum Genet 2013; 79(1): 18-2.
http://dx.doi.org/10.1002/0471142905.hg1802s79 PMID: 26270174

[13] Kircher M, Heyn P, Kelso J. Addressing challenges in the production and analysis of illumina sequencing data. BMC Genomics 2011; 12(1): 382.
http://dx.doi.org/10.1186/1471-2164-12-382 PMID: 21801405

[14] Ondov BD, Varadarajan A, Passalacqua KD, Bergman NH. Efficient mapping of applied biosystems solid sequence data to a reference genome for functional genomic applications. Bioinformatics 2008; 24(23): 2776-7.
http://dx.doi.org/10.1093/bioinformatics/btn512 PMID: 18842598

[15] Harismendy O, Ng PC, Strausberg RL, *et al.* Evaluation of next generation sequencing platforms for population targeted sequencing studies. Genome Biol 2009; 10(3): R32.
http://dx.doi.org/10.1186/gb-2009-10-3-r32 PMID: 19327155

[16] Bao S, Jiang R, Kwan W, Wang B, Ma X, Song YQ. Evaluation of next-generation sequencing software in mapping and assembly. J Hum Genet 2011; 56(6): 406-14.
http://dx.doi.org/10.1038/jhg.2011.43 PMID: 21525877

[17] Merriman B, Rothberg JM. Progress in ion torrent semiconductor chip based sequencing. Electrophoresis 2012; 33(23): 3397-417.
http://dx.doi.org/10.1002/elps.201200424 PMID: 23208921

[18] Lahens NF, Ricciotti E, Smirnova O, *et al.* A comparison of Illumina and Ion Torrent sequencing platforms in the context of differential gene expression. BMC Genomics 2017; 18(1): 602.
http://dx.doi.org/10.1186/s12864-017-4011-0 PMID: 28797240

[19] Bleidorn C. Third generation sequencing: Technology and its potential impact on evolutionary biodiversity research. Syst Biodivers 2016; 14(1): 1-8.
http://dx.doi.org/10.1080/14772000.2015.1099575

[20] McCarthy A. Third generation DNA sequencing: Pacific biosciences' single molecule real time technology. Chem Biol 2010; 17(7): 675-6.

http://dx.doi.org/10.1016/j.chembiol.2010.07.004 PMID: 20659677

[21]   Ardui S, Ameur A, Vermeesch JR, Hestand MS. Single molecule real-time (SMRT) sequencing comes of age: Applications and utilities for medical diagnostics. Nucleic Acids Res 2018; 46(5): 2159-68.
http://dx.doi.org/10.1093/nar/gky066 PMID: 29401301

[22]   Jain M, Olsen HE, Paten B, Akeson M. The Oxford Nanopore MinION: delivery of nanopore sequencing to the genomics community. Genome Biol 2016; 17: 1-11.

[23]   Goodwin S, Gurtowski J, Ethe-Sayers S, Deshpande P, Schatz MC, McCombie WR. Oxford Nanopore sequencing, hybrid error correction, and de novo assembly of a eukaryotic genome. Genome Res 2015; 25(11): 1750-6.
http://dx.doi.org/10.1101/gr.191395.115 PMID: 26447147

[24]   Sukumaran S, Reynolds MP, Sansaloni C. Genome-wide association analyses identify QTL hotspots for yield and component traits in durum wheat grown under yield potential, drought, and heat stress environments. Front Plant Sci 2018; 9: 81.
http://dx.doi.org/10.3389/fpls.2018.00081 PMID: 29467776

[25]   Thomson MJ, de Ocampo M, Egdane J, *et al*. Characterizing the Saltol quantitative trait locus for salinity tolerance in rice. Rice 2010; 3(2-3): 148-60.
http://dx.doi.org/10.1007/s12284-010-9053-8

[26]   Varshney RK, Gaur PM, Chamarthi SK, *et al*. Fast-track introgression of "QTL-hotspot" for root traits and other drought tolerance traits in JG 11, an elite and leading variety of chickpea. Plant Genome 2013; 6(3): plantgenome2013.07.0022.
http://dx.doi.org/10.3835/plantgenome2013.07.0022

[27]   Bharadwaj C, Tripathi S, Soren KR, *et al*. Introgression of " *QTL-hotspot* " region enhances drought tolerance and grain yield in three elite chickpea cultivars. Plant Genome 2021; 14(1): e20076.
http://dx.doi.org/10.1002/tpg2.20076 PMID: 33480153

[28]   Bhat JA, Deshmukh R, Zhao T, *et al*. Harnessing high-throughput phenotyping and genotyping for enhanced drought tolerance in crop plants. J Biotechnol 2020; 324: 248-60.
http://dx.doi.org/10.1016/j.jbiotec.2020.11.010 PMID: 33186658

[29]   Cui Y, Zhang W, Lin X, Xu S, Xu J, Li Z. Simultaneous improvement and genetic dissection of drought tolerance using selected breeding populations of rice. Front Plant Sci 2018; 9: 320.
http://dx.doi.org/10.3389/fpls.2018.00320 PMID: 29599789

[30]   Saxena RK, Singh VK, Kale SM, *et al*. Construction of genotyping-by-sequencing based high-density genetic maps and QTL mapping for fusarium wilt resistance in pigeonpea. Sci Rep 2017; 7(1): 1911.
http://dx.doi.org/10.1038/s41598-017-01537-2 PMID: 28507291

[31]   Gnanesh BN, Bohra A, Sharma M, *et al*. Genetic mapping and quantitative trait locus analysis of resistance to sterility mosaic disease in pigeonpea [*Cajanus cajan* (L.) Millsp.]. Field Crops Res 2011; 123(2): 53-61. [Cajanus cajan (L.) Millsp.].
http://dx.doi.org/10.1016/j.fcr.2011.04.011

[32]   Chandrashekharaiah PS, Paul V, Kushwaha S, Sanyal D, Dasgupta S. Biotechnological approaches for enhancing stress tolerance in legumes. *Sustainable Agriculture Reviews 51*. Legume Agriculture and Biotechnology 2021; 2: 247-93.

[33]   Varshney RK, Chen W, Li Y, *et al*. Draft genome sequence of pigeonpea (Cajanus cajan), an orphan legume crop of resource-poor farmers. Nat Biotechnol 2012; 30(1): 83-9.
http://dx.doi.org/10.1038/nbt.2022 PMID: 22057054

[34]   Zhang L, Hu J, Han X, *et al*. A high-quality apple genome assembly reveals the association of a retrotransposon and red fruit colour. Nat Commun 2019; 10(1): 1494.
http://dx.doi.org/10.1038/s41467-019-09518-x PMID: 30940818

[35]   Velasco R, Zharkikh A, Troggio M, *et al*. A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. PLoS One 2007; 2(12): e1326.
http://dx.doi.org/10.1371/journal.pone.0001326 PMID: 18094749

[36]   D'Hont A, Denoeud F, Aury JM, *et al*. The banana (Musa acuminata) genome and the evolution of monocotyledonous

plants. Nature 2012; 488(7410): 213-7.
http://dx.doi.org/10.1038/nature11241 PMID: 22801500

[37]   Singh N K, Mahato A K, Jayaswal P K. The genome sequence and transcriptome studies in mango (Mangifera indica L.). The Mango Genome 2021; 165-86.

[38]   Xu Q, Chen LL, Ruan X, *et al*. The draft genome of sweet orange (Citrus sinensis). Nat Genet 2013; 45(1): 59-66.
http://dx.doi.org/10.1038/ng.2472 PMID: 23179022

[39]   Koyama ML, Levesley A, Koebner RMD, Flowers TJ, Yeo AR. Quantitative trait loci for component physiological traits determining salt tolerance in rice. Plant Physiol 2001; 125(1): 406-22.
http://dx.doi.org/10.1104/pp.125.1.406 PMID: 11154348

[40]   Lin HX, Zhu MZ, Yano M, *et al*. QTLs for Na+ and K+ uptake of the shoots and roots controlling rice salt tolerance. Theor Appl Genet 2004; 108(2): 253-60.
http://dx.doi.org/10.1007/s00122-003-1421-y PMID: 14513218

[41]   Chang-lan ZHU, Ying-hui XIAO, Chun-ming WANG, Ling JIANG, Hu-Qu Z, Jian-min WAN. Mapping QTL for heat-tolerance at grain filling stage in rice. Rice Sci 2005; 12(1): 33.

[42]   Kato Y, Hirotsu S, Nemoto K, Yamagishi J. Identification of QTLs controlling rice drought tolerance at seedling stage in hydroponic culture. Euphytica 2008; 160(3): 423-30.
http://dx.doi.org/10.1007/s10681-007-9605-1

[43]   Jagadish SVK, Cairns J, Lafitte R, Wheeler TR, Price AH, Craufurd PQ. Genetic analysis of heat tolerance at anthesis in rice. Crop Sci 2010; 50(5): 1633-41.
http://dx.doi.org/10.2135/cropsci2009.09.0516

[44]   Tian L, Tan L, Liu F, Cai H, Sun C. Identification of quantitative trait loci associated with salt tolerance at seedling stage from Oryza rufipogon. J Genet Genomics 2011; 38(12): 593-601.
http://dx.doi.org/10.1016/j.jgg.2011.11.005 PMID: 22196402

[45]   Mardani Z, Rabiei B, Sabouri H, Sabouri A. Mapping of QTLs for germination characteristics under non-stress and drought stress in rice. Rice Sci 2013; 20(6): 391-9.
http://dx.doi.org/10.1016/S1672-6308(13)60150-X

[46]   Qiu X, Yuan Z, Liu H, *et al*. Identification of salt tolerance-improving quantitative trait loci alleles from a salt-susceptible rice breeding line by introgression breeding. Plant Breed 2015; 134(6): 653-60.
http://dx.doi.org/10.1111/pbr.12321

[47]   Gimhani DR, Gregorio GB, Kottearachchi NS, Samarasinghe WLG. SNP-based discovery of salinity-tolerant QTLs in a bi-parental population of rice (Oryza sativa). Mol Genet Genomics 2016; 291(6): 2081-99.
http://dx.doi.org/10.1007/s00438-016-1241-9 PMID: 27535768

[48]   Rahman MA, Bimpong IK, Bizimana JB, *et al*. Mapping QTLs using a novel source of salinity tolerance from Hasawi and their interaction with environments in rice. Rice (N Y) 2017; 10(1): 47.
http://dx.doi.org/10.1186/s12284-017-0186-x PMID: 29098463

[49]   Shanmugavadivel P, Sv AM, Prakash C, *et al*. High resolution mapping of QTLs for heat tolerance in rice using a 5K SNP array. Rice 2017; 10(1): 1-11.
PMID: 28078486

[50]   Sabar M, Shabir G, Shah SM, Aslam K, Naveed SA, Arif M. Identification and mapping of QTLs associated with drought tolerance traits in rice by a cross between Super Basmati and IR55419-04. Breed Sci 2019; 69(1): 169-78.
http://dx.doi.org/10.1270/jsbbs.18068 PMID: 31086495

[51]   Raza Q, Riaz A, Bashir K, Sabar M. Reproductive tissues-specific meta-QTLs and candidate genes for development of heat-tolerant rice cultivars. Plant Mol Biol 2020; 104(1-2): 97-112.
http://dx.doi.org/10.1007/s11103-020-01027-6 PMID: 32643113

[52]   Selamat N, Nadarajah KK. Meta-analysis of quantitative traits loci (QTL) identified in drought response in rice (Oryza sativa L.). Plants 2021; 10(4): 716.
http://dx.doi.org/10.3390/plants10040716 PMID: 33917162

[53]   Huang S, Liu M, Chen G, *et al*. Favorable QTLs from Oryza longistaminata improve rice drought resistance. BMC Plant Biol 2022; 22(1): 136.

http://dx.doi.org/10.1186/s12870-022-03516-w PMID: 35321642

[54] Mathews KL, Malosetti M, Chapman S, *et al.* Multi-environment QTL mixed models for drought stress adaptation in wheat. Theor Appl Genet 2008; 117(7): 1077-91.
http://dx.doi.org/10.1007/s00122-008-0846-8 PMID: 18696042

[55] Mohammadi VA, Zali AA, Bihamta MR. Mapping QTLs for heat tolerance in wheat. J Agric Sci Technol 2008; 2008(10): 261-7.

[56] Esten Mason R, Mondal S, Beecher FW, Hays DB. Genetic loci linking improved heat tolerance in wheat (*Triticum aestivum* L.) to lower leaf and spike temperatures under controlled conditions. Euphytica 2011; 180(2): 181-94.
http://dx.doi.org/10.1007/s10681-011-0349-6

[57] Paliwal R, Röder MS, Kumar U, Srivastava JP, Joshi AK. QTL mapping of terminal heat tolerance in hexaploid wheat (*T. aestivum* L.). Theor Appl Genet 2012; 125(3): 561-75.
http://dx.doi.org/10.1007/s00122-012-1853-3 PMID: 22476874

[58] Talukder SK, Babar MA, Vijayalakshmi K, *et al.* Mapping QTL for the traits associated with heat tolerance in wheat (*Triticum aestivum* L.). BMC Genet 2014; 15(1): 97.
http://dx.doi.org/10.1186/s12863-014-0097-4 PMID: 25384418

[59] Zhang H, Cui F, Wang H. Detection of quantitative trait loci (QTLs) for seedling traits and drought tolerance in wheat using three related recombinant inbred line (RIL) populations. Euphytica 2014; 196(3): 313-30.
http://dx.doi.org/10.1007/s10681-013-1035-7

[60] Acuña-Galindo MA, Mason RE, Subramanian NK, Hays DB. Meta-analysis of wheat QTL regions associated with adaptation to drought and heat stress. Crop Sci 2015; 55(2): 477-92.
http://dx.doi.org/10.2135/cropsci2013.11.0793

[61] Tahmasebi S, Heidari B, Pakniyat H, McIntyre CL. Mapping QTLs associated with agronomic and physiological traits under terminal drought and heat stress conditions in wheat ( *Triticum aestivum* L.). Genome 2017; 60(1): 26-45.
http://dx.doi.org/10.1139/gen-2016-0017 PMID: 27996306

[62] Bhusal N, Sarial AK, Sharma P, Sareen S. Mapping QTLs for grain yield components in wheat under heat stress. PLoS One 2017; 12(12): e0189594.
http://dx.doi.org/10.1371/journal.pone.0189594 PMID: 29261718

[63] Hussain B, Lucas SJ, Ozturk L, Budak H. Mapping QTLs conferring salt tolerance and micronutrient concentrations at seedling stage in wheat. Sci Rep 2017; 7(1): 15662.
http://dx.doi.org/10.1038/s41598-017-15726-6 PMID: 28127051

[64] Hassan FSC, Solouki M, Fakheri BA, Nezhad NM, Masoudi B. Mapping QTLs for physiological and biochemical traits related to grain yield under control and terminal heat stress conditions in bread wheat (*Triticum aestivum* L.). Physiol Mol Biol Plants 2018; 24(6): 1231-43.
http://dx.doi.org/10.1007/s12298-018-0590-8 PMID: 30425437

[65] Ren Y, Xu Y, Teng W, Li B, Lin T. QTLs for seedling traits under salinity stress in hexaploid wheat. Cienc Rural 2018; 48(3): 48.
http://dx.doi.org/10.1590/0103-8478cr20170446

[66] Liu H, Mullan D, Zhang C, *et al.* Major genomic regions responsible for wheat yield and its components as revealed by meta-QTL and genotype–phenotype association analyses. Planta 2020; 252(4): 65.
http://dx.doi.org/10.1007/s00425-020-03466-3 PMID: 32970252

[67] Asif MA, Garcia M, Tilbrook J, *et al.* Identification of salt tolerance QTL in a wheat RIL mapping population using destructive and non-destructive phenotyping. Funct Plant Biol 2021; 48(2): 131-40.
http://dx.doi.org/10.1071/FP20167 PMID: 32835651

[68] Amoah NKA, Akromah R, Kena AW, Manneh B, Dieng I, Bimpong IK. Mapping QTLs for tolerance to salt stress at the early seedling stage in rice (Oryza sativa L.) using a newly identified donor 'Madina Koyo'. Euphytica 2020; 216(10): 156.
http://dx.doi.org/10.1007/s10681-020-02689-5

[69] Kumar A, Saripalli G, Jan I, *et al.* Meta-QTL analysis and identification of candidate genes for drought tolerance in bread wheat (*Triticum aestivum* L.). Physiol Mol Biol Plants 2020; 26(8): 1713-25.

[70] Flowers TJ, Gaur PM, Gowda CLL, *et al.* Salt sensitivity in chickpea. Plant Cell Environ 2010; 33(4): 490-509.
http://dx.doi.org/10.1111/j.1365-3040.2009.02051.x PMID: 19843257

[71] Vadez V, Krishnamurthy L, Thudi M, *et al.* Assessment of ICCV 2 × JG 62 chickpea progenies shows sensitivity of reproduction to salt stress and reveals QTL for seed yield and yield components. Mol Breed 2012; 30(1): 9-21.
http://dx.doi.org/10.1007/s11032-011-9594-6

[72] Pushpavalli R, Krishnamurthy L, Thudi M, *et al.* Two key genomic regions harbour QTLs for salinity tolerance in ICCV 2 × JG 11 derived chickpea (Cicer arietinum L.) recombinant inbred lines. BMC Plant Biol 2015; 15(1): 124.
http://dx.doi.org/10.1186/s12870-015-0491-8 PMID: 25994494

[73] Soren KR, Madugula P, Kumar N, *et al.* Genetic dissection and identification of candidate genes for salinity tolerance using Axiom® CicerSNP array in chickpea. Int J Mol Sci 2020; 21(14): 5058.
http://dx.doi.org/10.3390/ijms21145058 PMID: 32709160

[74] Atieno J, Colmer TD, Taylor J, *et al.* Novel salinity tolerance loci in chickpea identified in glasshouse and field environments. Front Plant Sci 2021; 12: 667910.
http://dx.doi.org/10.3389/fpls.2021.667910 PMID: 33995463

[75] Jha U C, Bohra A, Nayyar H, *et al.* Breeding and genomics approaches for improving productivity gains in chickpea under changing climate. Genomic Designing of Climate-Smart Pulse Crops 2019; 135-64.

[76] Jha UC, Kole PC, Singh NP. QTL mapping for heat stress tolerance in chickpea (*Cicer arietinum* L.). Legume Research-An International Journal 2021; 44(4): 382-7.

[77] Kushwah A, Bhatia D, Singh I, *et al.* Identification of stable heat tolerance QTLs using inter-specific recombinant inbred line population derived from GPF 2 and ILWC 292. PLoS One 2021; 16(8): e0254957.
http://dx.doi.org/10.1371/journal.pone.0254957 PMID: 34370732

[78] Du W, Wang M, Fu S, Yu D. Mapping QTLs for seed yield and drought susceptibility index in soybean (*Glycine max* L.) across different environments. J Genet Genomics 2009; 36(12): 721-31.
http://dx.doi.org/10.1016/S1673-8527(08)60165-4 PMID: 20129399

[79] Yang W, Wang M, Yue A, *et al.* QTLs and epistasis for drought-tolerant physiological index in soybean ( *Glycine max* L.) across different environments. Caryologia 2014; 67(1): 72-8.
http://dx.doi.org/10.1080/00087114.2014.892278

[80] Ren H, Han J, Wang X, *et al.* QTL mapping of drought tolerance traits in soybean with SLAF sequencing. Crop J 2020; 8(6): 977-89.
http://dx.doi.org/10.1016/j.cj.2020.04.004

[81] Dhungana SK, Park JH, Oh JH, *et al.* Quantitative trait locus mapping for drought tolerance in soybean recombinant inbred line population. Plants 2021; 10(9): 1816.
http://dx.doi.org/10.3390/plants10091816 PMID: 34579348

[82] Cho KH, Kim MY, Kwon H, Yang X, Lee SH. Novel QTL identification and candidate gene analysis for enhancing salt tolerance in soybean (Glycine max (L.) Merr.). Plant Sci 2021; 313: 111085.
http://dx.doi.org/10.1016/j.plantsci.2021.111085 PMID: 34763870

[83] Foolad MR, Chen FQ, Lin GY. RFLP mapping of QTLs conferring salt tolerance during germination in an interspecific cross of tomato. Theor Appl Genet 1998; 97(7): 1133-44.
http://dx.doi.org/10.1007/s001220051002

[84] Foolad MR, Chen FQ. RFLP mapping of QTLs conferring salt tolerance during the vegetative stage in tomato. Theor Appl Genet 1999; 99(1-2): 235-43.
http://dx.doi.org/10.1007/s001220051229

[85] Foolad MR, Zhang LP, Lin GY. Identification and validation of QTLs for salt tolerance during vegetative growth in tomato by selective genotyping. Genome 2001; 44(3): 444-54.
http://dx.doi.org/10.1139/g01-030 PMID: 11444704

[86]   Li J, Liu L, Bai Y, *et al.* Seedling salt tolerance in tomato. Euphytica 2011; 178(3): 403-14.
http://dx.doi.org/10.1007/s10681-010-0321-x

[87]   Alam SN, Cohen MB. Detection and analysis of QTLs for resistance to the brown planthopper, Nilaparvata lugens, in a doubled-haploid rice population. Theor Appl Genet 1998; 97(8): 1370-9.
http://dx.doi.org/10.1007/s001220051031

[88]   Fukuoka S, Okuno K. QTL analysis and mapping of pi21, a recessive gene for field resistance to rice blast in Japanese upland rice. Theor Appl Genet 2001; 103(2-3): 185-90.
http://dx.doi.org/10.1007/s001220100611

[89]   Sato H, Ideta O, Ando I, *et al.* Mapping QTLs for sheath blight resistance in the rice line WSS2. Breed Sci 2004; 54(3): 265-71.
http://dx.doi.org/10.1270/jsbbs.54.265

[90]   Pinson SRM, Capdevielle FM, Oard JH. Confirming QTLs and finding additional loci conditioning sheath blight resistance in rice using recombinant inbred lines. Crop Sci 2005; 45(2): 503-10.
http://dx.doi.org/10.2135/cropsci2005.0503

[91]   Dudhare MS, Jadhav PV, Mishra SK. Molecular mapping of QTLs for resistance to brown spot disease in rice. J Plant Dis Sci 2008; 3(1): 21-3.

[92]   Fujita D, Doi K, Yoshimura A, Yasui H. A major QTL for resistance to green rice leafhopper (Nephotettix cincticeps Uhler) derived from African rice (*Oryza glaberrima* Steud.). Breed Sci 2010; 60(4): 336-41.
http://dx.doi.org/10.1270/jsbbs.60.336

[93]   Rao Y, Dong G, Zeng D, *et al.* Genetic analysis of leaffolder resistance in rice. J Genet Genomics 2010; 37(5): 325-31.
http://dx.doi.org/10.1016/S1673-8527(09)60050-3    PMID: 20513633

[94]   Zhang Y, Yang J, Shan Z, *et al.* Substitution mapping of QTLs for blast resistance with SSSLs in rice (Oryza sativa L.). Euphytica 2012; 184(1): 141-50.
http://dx.doi.org/10.1007/s10681-011-0601-0

[95]   Jena M, Mohapatra SL, Panda RS, Mohanty SK, Thatoi HN, Sahu SC. Genetic loci associated with root-knot nematode resistance in rice cv. Ramakrishna. ORYZA-An International Journal on Rice 2013; 50(2): 132-9.

[96]   Galeng-Lawilao J, Kumar A, De Waele D. QTL mapping for resistance to and tolerance for the rice root-knot nematode, Meloidogyne graminicola. BMC Genet 2018; 19(1): 53.
http://dx.doi.org/10.1186/s12863-018-0656-1  PMID: 30081817

[97]   Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW, Frohberg RC. RFLP mapping of QTL for Fusarium head blight resistance in wheat. Crop Sci 1999; 39(3): 805-11.
http://dx.doi.org/10.2135/cropsci1999.0011183X003900030032x

[98]   Chantret N, Mingeot D, Sourdille P, Bernard M, Jacquemin JM, Doussinault G. A major QTL for powdery mildew resistance is stable over time and at two development stages in winter wheat. Theor Appl Genet 2001; 103(6-7): 962-71.
http://dx.doi.org/10.1007/s001220100645

[99]   Singh S, Sharma I, Sehgal SK, *et al.* Molecular mapping of QTLs for Karnal bunt resistance in two recombinant inbred populations of bread wheat. Theor Appl Genet 2007; 116(1): 147-54.
http://dx.doi.org/10.1007/s00122-007-0654-6  PMID: 17952401

[100]  Bariana HS, Bansal UK, Schmidt A, *et al.* Molecular mapping of adult plant stripe rust resistance in wheat and identification of pyramided QTL genotypes. Euphytica 2010; 176(2): 251-60.
http://dx.doi.org/10.1007/s10681-010-0240-x

[101]  Toor AK, Bariana HS. Flag smut of wheat-pathogen biology and host resistance. Disease resistance in wheat. Wallingford, UK: CABI 2012; pp. 295-303.
http://dx.doi.org/10.1079/9781845938185.0295

[102]  Soriano JM, Royo C. Dissecting the genetic architecture of leaf rust resistance in wheat by QTL meta-analysis. Phytopathology 2015; 105(12): 1585-93.
http://dx.doi.org/10.1094/PHYTO-05-15-0130-R  PMID: 26571424

[103]  Kumar S, Knox RE, Singh AK, *et al.* High-density genetic mapping of a major QTL for resistance to multiple races of loose smut in a tetraploid wheat cross. PLoS One 2018; 13(2): e0192261.
http://dx.doi.org/10.1371/journal.pone.0192261  PMID: 29485999

[104]  Zhang L, Geng M, Zhang Z, *et al.* Molecular mapping of major QTL conferring resistance to orange wheat blossom midge (Sitodiplosis mosellana) in Chinese wheat varieties with selective populations. Theor Appl Genet 2020; 133(2): 491-502.
http://dx.doi.org/10.1007/s00122-019-03480-4  PMID: 31773176

[105]  Zhao L, Abdelsalam NR, Xu Y, *et al.* Identification of two novel Hessian fly resistance genes H35 and H36 in a hard winter wheat line SD06165. Theor Appl Genet 2020; 133(8): 2343-53.
http://dx.doi.org/10.1007/s00122-020-03602-3  PMID: 32436021

[106]  Jan I, Saripalli G, Kumar K, *et al.* Meta-QTLs and candidate genes for stripe rust resistance in wheat. Sci Rep 2021; 11(1): 22923.
http://dx.doi.org/10.1038/s41598-021-02049-w  PMID: 34824302

[107]  Pundir S, Sharma R, Kumar D, *et al.* QTL mapping for resistance against cereal cyst nematode (Heterodera avenae Woll.) in wheat (*Triticum aestivum* L.). Sci Rep 2022; 12(1): 9586.
http://dx.doi.org/10.1038/s41598-022-12988-7  PMID: 35688926

[108]  Welz HG, Xia XC, Bassetti P, Melchinger AE, Lübberstedt T. QTLs for resistance to Setosphaeria turcica in an early maturing Dent×Flint maize population. Theor Appl Genet 1999; 99(3-4): 649-55.
http://dx.doi.org/10.1007/s001220051280  PMID: 22665201

[109]  George MLC, Prasanna BM, Rathore RS, *et al.* Identification of QTLs conferring resistance to downy mildews of maize in Asia. Theor Appl Genet 2003; 107(3): 544-51.
http://dx.doi.org/10.1007/s00122-003-1280-6  PMID: 12759731

[110]  Balint-Kurti PJ, Zwonitzer JC, Wisser RJ, *et al.* Precise mapping of quantitative trait loci for resistance to southern leaf blight, caused by Cochliobolus heterostrophus race O, and flowering time using advanced intercross maize lines. Genetics 2007; 176(1): 645-57.
http://dx.doi.org/10.1534/genetics.106.067892  PMID: 17339203

[111]  Shi L, Li X, Hao Z, *et al.* Comparative QTL mapping of resistance to gray leaf spot in maize based on bioinformatics. Agric Sci China 2007; 6(12): 1411-9.
http://dx.doi.org/10.1016/S1671-2927(08)60002-4

[112]  Chen Y, Chao Q, Tan G, *et al.* Identification and fine-mapping of a major QTL conferring resistance against head smut in maize. Theor Appl Genet 2008; 117(8): 1241-52.
http://dx.doi.org/10.1007/s00122-008-0858-4  PMID: 18762906

[113]  Satish K, Srinivas G, Madhusudhana R, *et al.* Identification of quantitative trait loci for resistance to shoot fly in sorghum [*Sorghum bicolor* (L.) Moench]. Theor Appl Genet 2009; 119(8): 1425-39.
http://dx.doi.org/10.1007/s00122-009-1145-8  PMID: 19763534

[114]  Jampatong C, Jampatong S, Jompuk C, *et al.* Mapping of QTL affecting resistance against sorghum downy mildew (Peronosclerospora sorghi) in maize (Zea mays L). Maydica 2013; 58(2): 119-26.

[115]  Berger DK, Carstens M, Korsman JN, *et al.* Mapping QTL conferring resistance in maize to gray leaf spot disease caused by Cercospora zeina. BMC Genet 2014; 15(1): 60.
http://dx.doi.org/10.1186/1471-2156-15-60  PMID: 24885661

[116]  Betsiashvili M, Ahern KR, Jander G. Additive effects of two quantitative trait loci that confer Rhopalosiphum maidis (corn leaf aphid) resistance in maize inbred line Mo17. J Exp Bot 2015; 66(2): 571-8.
http://dx.doi.org/10.1093/jxb/eru379  PMID: 25249072

[117]  Li Y, Wu X, Jaqueth J, *et al.* The identification of two head smut resistance-related QTL in maize by the joint approach of linkage mapping and association analysis. PLoS One 2015; 10(12): e0145549.
http://dx.doi.org/10.1371/journal.pone.0145549  PMID: 26689370

[118]  Kistner MB, Galiano-Carneiro AL, Kessel B, Presterl T, Miedaner T. Multi-parental QTL mapping of resistance to white spot of maize (*ZEA MAYS* ) in southern Brazil and relationship to QTLs of other foliar diseases. Plant Breed 2021; 140(5): 801-11.
http://dx.doi.org/10.1111/pbr.12964

[119]  Agrama H, Widle G, Reese J, Campbell L, Tuinstra M. Genetic mapping of QTLs associated with greenbug resistance and

tolerance in Sorghum bicolor. Theor Appl Genet 2002; 104(8): 1373-8.
http://dx.doi.org/10.1007/s00122-002-0923-3 PMID: 12582593

[120]   Parh DK, Jordan DR, Aitken EAB, *et al.* QTL analysis of ergot resistance in sorghum. Theor Appl Genet 2008; 117(3): 369-82.
http://dx.doi.org/10.1007/s00122-008-0781-8 PMID: 18481043

[121]   Aruna C, Bhagwat VR, Madhusudhana R, *et al.* Identification and validation of genomic regions that affect shoot fly resistance in sorghum [*Sorghum bicolor* (L.) Moench]. Theor Appl Genet 2011; 122(8): 1617-30.
http://dx.doi.org/10.1007/s00122-011-1559-y PMID: 21387095

[122]   Punnuri S, Huang Y, Steets J, Wu Y. Developing new markers and QTL mapping for greenbug resistance in sorghum [*Sorghum bicolor* (L.) Moench]. Euphytica 2013; 191(2): 191-203. [*Sorghum bicolor* (L.) Moench].
http://dx.doi.org/10.1007/s10681-012-0755-4

[123]   Wang X, Mace E, Hunt C, *et al.* Two distinct classes of QTL determine rust resistance in sorghum. BMC Plant Biol 2014; 14(1): 366.
http://dx.doi.org/10.1186/s12870-014-0366-4 PMID: 25551674

[124]   Kimball J, Cui Y, Chen D, *et al.* Identification of QTL for Target Leaf Spot resistance in Sorghum bicolor and investigation of relationships between disease resistance and variation in the MAMP response. Sci Rep 2019; 9(1): 18285.
http://dx.doi.org/10.1038/s41598-019-54802-x PMID: 31797989

[125]   Jones ES, Breese WA, Liu CJ, Singh SD, Shaw DS, Witcombe JR. Mapping quantitative trait loci for resistance to downy mildew in pearl millet: Field and glasshouse screens detect the same QTL. Crop Sci 2002; 42(4): 1316-23.
http://dx.doi.org/10.2135/cropsci2002.1316

[126]   Jones ES, Liu CJ, Gale MD, Hash CT, Witcombe JR. Mapping quantitative trait loci for downy mildew resistance in pearl millet. Theor Appl Genet 1995; 91(3): 448-56.
http://dx.doi.org/10.1007/BF00222972 PMID: 24169834

[127]   Maganlal S J, Sanghani A O, Kothari V V, Raval S S, Kahodariya J H, Ramani H R. The SSR based linkage map construction and identification of QTLs for blast (Pyricularia grisea) resistance in pearl millet (*Pennisetum glaucum* (L.) r. br.). . J Pharmacogn Phytochem 2018; 7(2): 3057-64.

[128]   Chelpuri D, Sharma R, Durga KK, *et al.* Mapping quantitative trait loci (QTLs) associated with resistance to major pathotype-isolates of pearl millet downy mildew pathogen. Eur J Plant Pathol 2019; 154(4): 983-94.
http://dx.doi.org/10.1007/s10658-019-01718-x

[129]   Cui Y, Ge Q, Zhao P, Chen W, Sang X, Zhao Y. Rapid mining of candidate genes for verticillium wilt resistance in cotton based on BSA-Seq analysis. Front Plant Sci 2016; 2021(12): 1664-462X.

[130]   Elassbli H, Abdelraheem A, Zhu Y, *et al.* Evaluation and genome-wide association study of resistance to bacterial blight race 18 in U.S. Upland cotton germplasm. Mol Genet Genomics 2021; 296(3): 719-29.
http://dx.doi.org/10.1007/s00438-021-01779-w PMID: 33779828

[131]   Han W, Zhao J, Deng X, Gu A, Li D, Wang Y. Quantitative trait locus mapping and identification of candidate genes for resistance to fusarium wilt race 7 using a resequencing-based high density genetic bin map in a recombinant inbred line population of Gossypium barbadense. Front Plant Sci 2022; 13: 88.

[132]   Zhang J, Abdelraheem A, Ma J, *et al.* Mapping of dynamic QTLs for resistance to Fusarium wilt (Fusarium oxysporum f. sp. vasinfectum) race 4 in a backcross inbred line population of Upland cotton. Mol Genet Genomics 2022; 297(2): 319-32.
http://dx.doi.org/10.1007/s00438-021-01846-2 PMID: 35020076

[133]   Kasettranan W, Somta P, Srinives P. Mapping of quantitative trait loci controlling powdery mildew resistance in Mungbean (Vigna radiata (L.) Wilczek). J Crop Sci Biotechnol 2010; 13(3): 155-61.
http://dx.doi.org/10.1007/s12892-010-0052-z

[134]   Kitsanachandee R, Somta P, Chatchawankanpanich O, *et al.* Detection of quantitative trait loci for mungbean yellow mosaic India virus (MYMIV) resistance in mungbean (<i>Vigna radiata</i> (L.) Wilczek) in India and Pakistan. Breed Sci 2013;

63(4): 367-73.
http://dx.doi.org/10.1270/jsbbs.63.367 PMID: 24399908

[135]   Hong MG, Kim KH, Ku JH, *et al.* Inheritance and quantitative trait loci analysis of resistance genes to bruchid and bean bug in Mungbean ( *Vigna radiata* L. Wilczek). Plant Breed Biotechnol 2015; 3(1): 39-46.
http://dx.doi.org/10.9787/PBB.2015.3.1.039

[136]   Soufframanien J, Gupta SK, Gopalakrishna T. Identification of quantitative trait loci for bruchid (Callosobruchus maculatus) resistance in black gram [*Vigna mungo* (L.) Hepper]. Euphytica 2010; 176(3): 349-56. [Vigna mungo (L.) Hepper].
http://dx.doi.org/10.1007/s10681-010-0210-3

[137]   Somta P, Chen J, Yundaeng C, *et al.* Development of an SNP-based high-density linkage map and QTL analysis for bruchid (Callosobruchus maculatus F.) resistance in black gram (*Vigna mungo* (L.) Hepper). Sci Rep 2019; 9(1): 3930.
http://dx.doi.org/10.1038/s41598-019-40669-5 PMID: 30850726

[138]   Vadivel K, Manivannan N, Mahalingam A, Satya VK, Vanniarajan C, Ragul S. Identificationand Validation of Quantitative Trait Loci of Mungbean Yellow MosaicVirus Disease Resistance in Blackgram. Legume Res 2021. [*Vigna mungo* (L). Hepper].
http://dx.doi.org/10.18805/LR-4459

[139]   Khedikar YP, Gowda MVC, Sarvamangala C, Patgar KV, Upadhyaya HD, Varshney RK. A QTL study on late leaf spot and rust revealed one major QTL for molecular breeding for rust resistance in groundnut (*Arachis hypogaea* L.). Theor Appl Genet 2010; 121(5): 971-84.
http://dx.doi.org/10.1007/s00122-010-1366-x PMID: 20526757

[140]   Liang Y, Baring M, Wang S, Septiningsih EM. Mapping QTLs for leafspot resistance in peanut using SNP-based next-generation sequencing markers. Plant Breed Biotechnol 2017; 5(2): 115-22.
http://dx.doi.org/10.9787/PBB.2017.5.2.115

[141]   Pandey MK, Khan AW, Singh VK, *et al.* QTL -seq approach identified genomic regions and diagnostic markers for rust and late leaf spot resistance in groundnut (*A rachis hypogaea*L. ). Plant Biotechnol J 2017; 15(8): 927-41.
http://dx.doi.org/10.1111/pbi.12686 PMID: 28028892

[142]   Han S, Yuan M, Clevenger JP, *et al.* A SNP-based linkage map revealed QTLs for resistance to early and late leaf spot diseases in peanut (Arachis hypogaea L.). Front Plant Sci 2018; 9: 1012.
http://dx.doi.org/10.3389/fpls.2018.01012 PMID: 30042783

[143]   Singh BD, Singh AK, Singh BD, Singh AK. Mapping populations. Marker-assisted plant breeding: Principles and practices 2015; 125-50.

[144]   Alahmad S, El Hassouni K, Bassi FM, *et al.* A major root architecture QTL responding to water limitation in durum wheat. Front Plant Sci 2019; 10(19): 436.
http://dx.doi.org/10.3389/fpls.2019.00436 PMID: 31024600

[145]   Rufo R, Salvi S, Royo C, Soriano J. Exploring the genetic architecture of root-related traits in mediterranean bread wheat landraces by genome-wide association analysis. Agronomy 2020; 10(5): 613.
http://dx.doi.org/10.3390/agronomy10050613

[146]   Septiningsih EM, Pamplona AM, Sanchez DL, *et al.* Development of submergence-tolerant rice cultivars: The Sub1 locus and beyond. Ann Bot 2009; 103(2): 151-60.
http://dx.doi.org/10.1093/aob/mcn206

[147]   Xu K, Xu X, Fukao T, *et al.* Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. Nature 2006; 442(7103): 705-8.
http://dx.doi.org/10.1038/nature04920 PMID: 16900200

[148]   Dar M H, Zaidi N W, Waza S A, Verulkar S B, Ahmed T, Singh P K. No yield penalty under favorable conditions paving the way for successful adoption of flood tolerant rice. Sci Rep 2018; 8(1): 1-7. PMID: 29311619

[149]   Singh R, Singh Y, Xalaxo S, *et al.* From QTL to variety-harnessing the benefits of QTLs for drought, flood and salt tolerance in mega rice varieties of India through a multi-institutional network. Plant Sci 2016; 242: 278-87.
http://dx.doi.org/10.1016/j.plantsci.2015.08.008 PMID: 26566845

[150] Angaji SA. Mapping QTLs for submergence tolerance during germination in rice. Afr J Biotechnol 2008; 7(15)

[151] Gonzaga ZJC, Carandang J, Singh A, Collard BCY, Thomson MJ, Septiningsih EM. Mapping QTLs for submergence tolerance in rice using a population fixed for SUB1A tolerant allele. Mol Breed 2017; 37(4): 47.
http://dx.doi.org/10.1007/s11032-017-0637-5

[152] Islam MR, Gregorio GB, Salam MA, Collard BCY, Singh RK, Hassan L. Validation of SalTol linked markers and haplotype diversity on chromosome 1 of rice. Mol Plant Breed 2012; 3(10): 103-14.
http://dx.doi.org/10.5376/mpb.2012.03.0010

[153] Krishnamurthy SL, Pundir P, Warraich AS, *et al.* Introgressed saltol QTL lines improves the salinity tolerance in rice at seedling stage. Front Plant Sci 2020; 11: 833.
http://dx.doi.org/10.3389/fpls.2020.00833 PMID: 32595689

[154] Bimpong IK, Manneh B, Sock M, *et al.* Improving salt tolerance of lowland rice cultivar 'Rassi' through marker-aided backcross breeding in West Africa. Plant Sci 2016; 242: 288-99.
http://dx.doi.org/10.1016/j.plantsci.2015.09.020 PMID: 26566846

[155] Boonekamp PM. Are plant diseases too much ignored in the climate change debate? Eur J Plant Pathol 2012; 133(1): 291-4.
http://dx.doi.org/10.1007/s10658-011-9934-8

[156] Huang YJ, Pirie EJ, Evans N, Delourme R, King GJ, Fitt BDL. Quantitative resistance to symptomless growth of *Leptosphaeria maculans* (phoma stem canker) in *Brassica napus* (oilseed rape). Plant Pathol 2009; 58(2): 314-23.
http://dx.doi.org/10.1111/j.1365-3059.2008.01957.x

[157] Nadeem MA, Nawaz MA, Shahid MQ, *et al.* DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnol Biotechnol Equip 2018; 32(2): 261-85.
http://dx.doi.org/10.1080/13102818.2017.1400401

[158] Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 2005; 142(1-2): 169-96.
http://dx.doi.org/10.1007/s10681-005-1681-5

[159] Amiteye S. Basic concepts and methodologies of DNA marker systems in plant molecular breeding. Heliyon 2021; 7(10): e08093.
http://dx.doi.org/10.1016/j.heliyon.2021.e08093 PMID: 34765757

[160] Anne C. Choosing the right molecular genetic markers for studying biodiversity: From molecular evolution to practical aspects. Genetica 2006; 127(1-3): 101-20.
http://dx.doi.org/10.1007/s10709-005-2485-1 PMID: 16850217

[161] Lockhart DJ, Winzeler EA. Genomics, gene expression and DNA arrays. Nature 2000; 405(6788): 827-36.
http://dx.doi.org/10.1038/35015701 PMID: 10866209

[162] Karsch-Mizrachi I, Takagi T, Cochrane G. The international nucleotide sequence database collaboration. Nucleic Acids Res 2018; 46(D1): D48-51.
http://dx.doi.org/10.1093/nar/gkx1097 PMID: 29190397

[163] Lowe R, Shirley N, Bleackley M, Dolan S, Shafee T. Transcriptomics technologies. PLOS Comput Biol 2017; 13(5): e1005457.
http://dx.doi.org/10.1371/journal.pcbi.1005457 PMID: 28545146

[164] Abdel-Ghany SE, Hamilton M, Jacobi JL, *et al.* A survey of the sorghum transcriptome using single-molecule long reads. Nat Commun 2016; 7(1): 11706.
http://dx.doi.org/10.1038/ncomms11706 PMID: 27339290

[165] Mironova VV, Weinholdt C, Grosse I. RNA-seq data analysis for studying abiotic stress in horticultural plants. Abiotic Stress Biology in Horticultural Plants 2015; pp. 197-220.
http://dx.doi.org/10.1007/978-4-431-55251-2_14

[166] Hong M, Tao S, Zhang L, *et al.* RNA sequencing: New technologies and applications in cancer research. J Hematol Oncol 2020; 13(1): 166.
http://dx.doi.org/10.1186/s13045-020-01005-x PMID: 33276803

[167] Ma L, Dong C, Song C, *et al. De novo* genome assembly of the potent medicinal plant *Rehmannia glutinosa* using nanopore technology. Comput Struct Biotechnol J 2021; 19: 3954-63.
http://dx.doi.org/10.1016/j.csbj.2021.07.006 PMID: 34377362

[168] Hoopes GM, Hamilton JP, Kim J, *et al.* Genome assembly and annotation of the medicinal plant *Calotropis gigantea*, a producer of anticancer and antimalarial cardenolides. *G3: Genes, Genomes.* G3 2018; 8(2): 385-91.
http://dx.doi.org/10.1534/g3.117.300331 PMID: 29237703

[169] Wang X, Hu H, Wu Z, *et al.* Tissue-specific transcriptome analyses reveal candidate genes for stilbene, flavonoid and anthraquinone biosynthesis in the medicinal plant *Polygonum cuspidatum.* BMC Genomics 2021; 22(1): 353.
http://dx.doi.org/10.1186/s12864-021-07658-3 PMID: 34000984

[170] Xu W, Li R, Zhang N, Ma F, Jiao Y, Wang Z. Transcriptome profiling of Vitis amurensis, an extremely cold-tolerant Chinese wild Vitis species, reveals candidate genes and events that potentially connected to cold stress. Plant Mol Biol 2014; 86(4-5): 527-41.
http://dx.doi.org/10.1007/s11103-014-0245-2 PMID: 25190283

[171] Xu X, Crow M, Rice BR, *et al.* Single-cell RNA sequencing of developing maize ears facilitates functional analysis and trait candidate gene discovery. Dev Cell 2021; 56(4): 557-568.e6.
http://dx.doi.org/10.1016/j.devcel.2020.12.015 PMID: 33400914

[172] Costa V, Angelini C, De Feis I, Ciccodicola A. Uncovering the complexity of transcriptomes with RNA-Seq. J Biomed Biotechnol 2010; 2010: 1-19.
http://dx.doi.org/10.1155/2010/853916 PMID: 20625424

[173] Kumar S, Razzaq SK, Vo AD, Gautam M, Li H. Identifying fusion transcripts using next generation sequencing. Wiley Interdiscip Rev RNA 2016; 7(6): 811-23.
http://dx.doi.org/10.1002/wrna.1382 PMID: 27485475

[174] Wang Z, Gerstein M, Snyder M. RNA-Seq: A revolutionary tool for transcriptomics. Nat Rev Genet 2009; 10(1): 57-63.
http://dx.doi.org/10.1038/nrg2484 PMID: 19015660

[175] Cao X, Jacobsen SE. Role of the arabidopsis DRM methyltransferases in de novo DNA methylation and gene silencing. Curr Biol 2002; 12(13): 1138-44.
http://dx.doi.org/10.1016/S0960-9822(02)00925-9 PMID: 12121623

[176] Law JA, Jacobsen SE. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat Rev Genet 2010; 11(3): 204-20.
http://dx.doi.org/10.1038/nrg2719 PMID: 20142834

[177] He XJ, Ma ZY, Liu ZW. Non-coding RNA transcription and RNA-directed DNA methylation in Arabidopsis. Mol Plant 2014; 7(9): 1406-14.
http://dx.doi.org/10.1093/mp/ssu075 PMID: 24966349

[178] Madlung A, Tyagi AP, Watson B, *et al.* Genomic changes in synthetic Arabidopsis polyploids. Plant J 2005; 41(2): 221-30.
http://dx.doi.org/10.1111/j.1365-313X.2004.02297.x PMID: 15634199

[179] Takuno S, Ran JH, Gaut BS. Evolutionary patterns of genic DNA methylation vary across land plants. Nat Plants 2016; 2(2): 15222.
http://dx.doi.org/10.1038/nplants.2015.222 PMID: 27249194

[180] Harrison A, Parle-McDermott A. DNA methylation: A timeline of methods and applications. Front Genet 2011; 2: 74.
http://dx.doi.org/10.3389/fgene.2011.00074 PMID: 22303369

[181] Beck D, Ben Maamar M, Skinner MK. Genome-wide CpG density and DNA methylation analysis method (MeDIP, RRBS, and WGBS) comparisons. Epigenetics 2022; 17(5): 518-30.
http://dx.doi.org/10.1080/15592294.2021.1924970 PMID: 33975521

[182] Frommer M, McDonald LE, Millar DS, *et al.* A genomic sequencing protocol that yields a positive display of 5-methylcytosine residues in individual DNA strands. Proc Natl Acad Sci USA 1992; 89(5): 1827-31.
http://dx.doi.org/10.1073/pnas.89.5.1827 PMID: 1542678

[183] Gu H, Smith ZD, Bock C, Boyle P, Gnirke A, Meissner A. Preparation of reduced representation bisulfite sequencing libraries for genome-scale DNA methylation profiling. Nat Protoc

2011; 6(4): 468-81.
http://dx.doi.org/10.1038/nprot.2010.190 PMID: 21412275

[184] Morselli M, Farrell C, Rubbi L, Fehling HL, Henkhaus R, Pellegrini M. Targeted bisulfite sequencing for biomarker discovery. Methods 2021; 187: 13-27.
http://dx.doi.org/10.1016/j.ymeth.2020.07.006 PMID: 32755621

[185] Kozarewa I, Armisen J, Gardner AF, Slatko BE, Hendrickson CL. Overview of target enrichment strategies. Curr Protoc Mol Biol 2015; 112(1): 21.1-, 23.
http://dx.doi.org/10.1002/0471142727.mb0721s112 PMID: 26423591

[186] Lafon-Placette C, Faivre-Rampant P, Delaunay A, Street N, Brignolas F, Maury S. Methylome of DN ase I sensitive chromatin in*P opulus trichocarpa* shoot apical meristematic cells: A simplified approach revealing characteristics of gene-body DNA methylation in open chromatin state. New Phytol 2013; 197(2): 416-30.
http://dx.doi.org/10.1111/nph.12026 PMID: 23253333

[187] Wardenaar R, Liu H, Colot V, Colomé-Tatché M, Johannes F. Evaluation of MeDIP-chip in the context of whole-genome bisulfite sequencing (WGBS-seq) in Arabidopsis. Tiling Arrays Methods in Molecular Biology. Totowa, NJ.: Humana Press 2013; pp. 203-24.

[188] Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function. EFSA J 2012; 10(10): 2943.

[189] Podevin N, Davies HV, Hartung F, Nogué F, Casacuberta JM. Site-directed nucleases: A paradigm shift in predictable, knowledge-based plant breeding. Trends Biotechnol 2013; 31(6): 375-83.
http://dx.doi.org/10.1016/j.tibtech.2013.03.004 PMID: 23601269

[190] Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell 2014; 157(6): 1262-78.
http://dx.doi.org/10.1016/j.cell.2014.05.010 PMID: 24906146

[191] Sonoda E, Hochegger H, Saberi A, Taniguchi Y, Takeda S. Differential usage of non-homologous end-joining and homologous recombination in double strand break repair. DNA Repair 2006; 5(9-10): 1021-9.
http://dx.doi.org/10.1016/j.dnarep.2006.05.022 PMID: 16807135

[192] Mohanta TK, Bashir T, Hashem A, Abd Allah EF, Bae H. Genome editing tools in plants. Genes 2017; 8(12): 399.
http://dx.doi.org/10.3390/genes8120399 PMID: 29257124

[193] Bortesi L, Fischer R. The CRISPR/Cas9 system for plant genome editing and beyond. Biotechnol Adv 2015; 33(1): 41-52.
http://dx.doi.org/10.1016/j.biotechadv.2014.12.006 PMID: 25536441

[194] Fadiji AE, Babalola OO. Metagenomics methods for the study of plant-associated microbial communities: A review. J Microbiol Methods 2020; 170: 105860.
http://dx.doi.org/10.1016/j.mimet.2020.105860 PMID: 32027927

[195] Andersson AF, Lindberg M, Jakobsson H, Bäckhed F, Nyrén P, Engstrand L. Comparative analysis of human gut microbiota by barcoded pyrosequencing. PLoS One 2008; 3(7): e2836.
http://dx.doi.org/10.1371/journal.pone.0002836 PMID: 18665274

[196] Hajibabaei M, Shokralla S, Zhou X, Singer GAC, Baird DJ. Environmental barcoding: A next-generation sequencing approach for biomonitoring applications using river benthos. PLoS One 2011; 6(4): e17497.
http://dx.doi.org/10.1371/journal.pone.0017497 PMID: 21533287

[197] Boessenkool S, Epp LS, Haile J, *et al.* Blocking human contaminant DNA during PCR allows amplification of rare mammal species from sedimentary ancient DNA. Mol Ecol 2012; 21(8): 1806-15.
http://dx.doi.org/10.1111/j.1365-294X.2011.05306.x PMID: 21988749

[198] Deagle B, Kirkwood R, Jarman SN. Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. Mol Ecol 2009; 18(9): 2022-38.
http://dx.doi.org/10.1111/j.1365-294X.2009.04158.x PMID: 19317847

[199] Mardis ER. Next-generation sequencing platforms. Annu Rev Anal Chem 2013; 6(1): 287-303.
http://dx.doi.org/10.1146/annurev-anchem-062012-092628 PMID: 23560931

[200] van Dijk EL, Auger H, Jaszczyszyn Y, Thermes C. Ten years of next-generation sequencing technology. Trends Genet 2014; 30(9): 418-26.
http://dx.doi.org/10.1016/j.tig.2014.07.001 PMID: 25108476