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Next Generation Sequencing (NGS) for Identification of Disease Resistant Alleles in Plants

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Pritam Das[1], Tharringwon Marchang Ningshen[1], and Pranab Dutta[2]*

[1]Ph.D. Scholar (Plant Pathology), School of Crop Protection, College of Post Graduate Studies in Agricultural Sciences, Umiam, 793103, Meghalaya, India

[2]Chairman, School of Crop Protection, College of Post Graduate Studies in Agricultural Sciences, Umiam, 793103, Meghalaya, India

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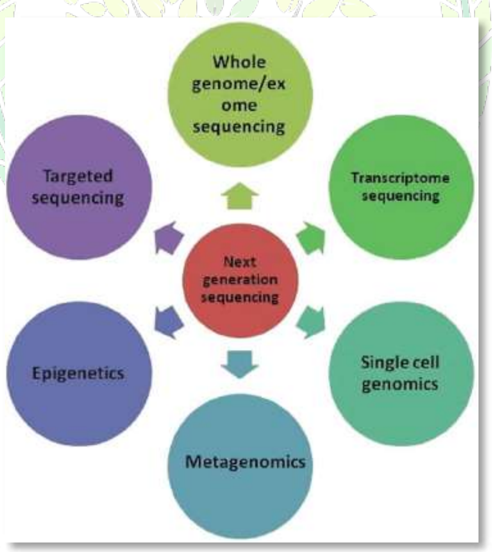
Development of disease resistant crop varieties plays a significant role in overcoming threats caused by plant pathogens. The advent of Next Generation Sequencing (NGS) plays a vital role in the identification of alleles responsible for disease resistance. This technology enables high-throughput, cost-effective, and rapid identification of novel alleles associated with disease resistance in plants. Integration of NGS with marker assisted selection (MAS) and multi-omics breeding programs can enhance the efficiency of breeding programs related to the development of disease resistant cultivars.



With increasing threats related to diseases in crops caused by fungi, bacteria, and viruses, development of disease resistant crop varieties has become increasingly important in the agricultural sector. In plant breeding, identification of disease resistant alleles that confer resistance against pathogens plays a pivotal role. The advent of Next Generation Sequencing (NGS) has revolutionized plant genomics by enabling rapid identification of novel alleles associated with disease resistance in crops.

NGS mainly works based on high-throughput DNA sequencing, which allows millions of DNA fragments to be sequenced simultaneously (Gupta et al., 2020). This technology utilizes several platforms such as Illumina, Thermo Fisher Scientific, and Pacific Biosciences. NGS allows rapid detection of genetic variations and helps in identifying rare and previously unknown alleles. This article explores the role of NGS in identifying novel alleles associated with plant disease resistance and highlights its workflow.

Next Generation Sequencing (NGS) in Identification of Novel Alleles Associated with Plant Disease Resistance

For the identification of disease resistant alleles in plants, NGS technology has played an important role by enabling high-throughput genotyping, whole genome sequencing, and target-based identification of resistance gene analogs. These approaches have played a central role in identifying novel alleles from diverse germplasm, including wild relatives, landraces, and cultivars (Smith, 2025).

The availability of whole genome sequencing (WGS) has shifted fragment-based polymorphism identification to sequence-based SNP identification, thereby improving marker discovery and increasing the number of informative markers. Earlier, WGS based on Sanger sequencing required more time and was limited in discovering specific genes. To overcome this limitation, NGS technologies have become powerful tools that have significantly reduced the cost of WGS and enabled discovery of thousands of markers in a single step (Ray and Satya, 2014).

Thus, NGS has become a powerful tool for identifying several disease resistant genes, as numerous DNA sequence polymorphisms can be detected within a short period of time.

Examples of NGS-Based Disease Resistant Gene Identification in Plants

Based on NGS, several studies have been conducted across the world to identify novel and candidate genes responsible for disease resistance in plants. Zhong et al. (2018) reported a novel gene, RpsHC18, on chromosome 3 that confers resistance against *Phytophthora sojae* in soybean. Two diagnostic markers were developed for RpsHC18 and two NBS-LRR candidate genes were identified.

Using NGS technology such as double-digest restriction site associated DNA sequencing (ddRAD-Seq), a total of 6,514 SNPs were genotyped in an F_2 population derived from wild relatives of tomato. Subsequent genotype-phenotype association studies revealed a 6.8 Mb genomic region on chromosome 6 as a candidate locus for resistance against tomato late blight (Arafa et al., 2017).

Recent advances in NGS have also enabled rapid identification of candidate genes responsible for blackleg resistance in *Brassica napus*. Genome sequencing of *B. rapa* (diploid progenitor of *B. napus*) revealed numerous disease resistance candidate genes, most of which were clustered around the major blackleg resistance locus Rlm4 on chromosome A7 (Tollenaere et al., 2012).

Sequencing-based bulked segregant analysis (Seq-BSA) has identified seven SNPs associated with resistance to Fusarium wilt (FW) and sterility mosaic disease (SMD) in pigeon pea. In silico protein analysis further identified two promising candidate genes, namely *C. cajan_01839* and *C. cajan_03203*, for SMD and FW resistance respectively (Singh et al., 2016).

Targeted NGS has also been used to identify mutations in resistance gene analogs (RGAs) in wild and cultivated beets. Using Ion Torrent sequencing technology, mutations were identified in 21 RGAs. The sequence CCCTCC was identified as a diagnostic marker to differentiate wild and domesticated beets and to assist marker-assisted breeding programs (Stevanato et al., 2017).

Thus, NGS technology has played an instrumental role in identifying resistance genes against several plant pathogens in diverse crops. The detailed findings of the above-mentioned studies are summarized in Table 1.

Table 1: Some of the Key Research Works Carried Out to Identify Disease Resistant Alleles/Markers in Crops Using NGS

Year	Author	Significant Findings
2012	Tollenaere et al.	Next-generation sequencing of *Brassica rapa* revealed numerous disease resistance candidate genes clustered around the blackleg resistance locus Rlm4 on chromosome A7 of *Brassica napus*.
2016	Singh et al.	Seq-BSA identified seven SNPs associated with resistance to Fusarium wilt and sterility mosaic disease in pigeon pea.
2017	Arafa et al.	ddRAD-Seq genotyped 6,514 SNPs in tomato and identified a 6.8 Mb candidate genomic region on chromosome 6 associated with late blight resistance.
2017	Stevanato et al.	Targeted NGS using Ion Torrent identified mutations in 21 resistance gene analogs in beet.
2018	Zhong et al.	Identified soybean resistance gene RpsHC18 on chromosome 3 against *Phytophthora sojae*.

Mechanism of NGS in Identification of Alleles Responsible for Disease Resistance in Plants

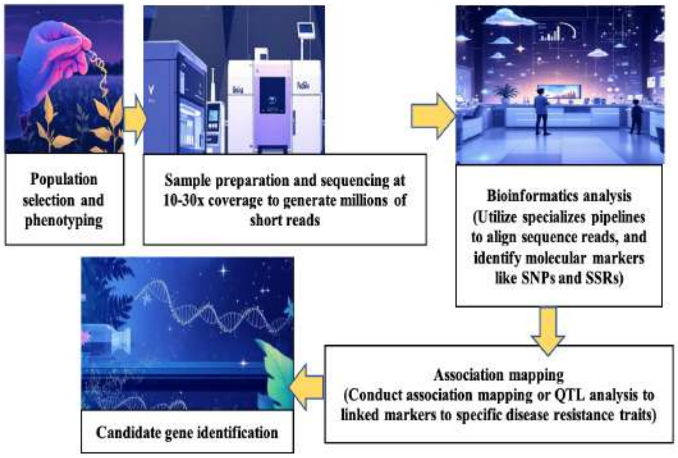


Fig 1: Workflow of NGS for identification of candidate genes responsible for disease resistance in plants.

Conclusion

NGS technology accelerates the discovery of resistant alleles responsible for disease resistance in plants. The combined use of NGS with other advanced genomic tools will further enhance the development of crop varieties resistant to plant diseases. Thus, this technology has great potential to contribute to global food security and sustainable agriculture.

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