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Next Generation Sequencing: An Effective Tool for Crop Improvement

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ABSTRACT

Next generation sequencing a recent phenomenon has become quite popular in crop breeding programmes. Plant breeding involves continuous efforts to modify or alter genetic architecture of crop plants for betterment to mankind. Traditional plant breeding approaches involved phenotypic selection, hybridisation, multilocation trials etc., but with the application of reverse genetics it is possible to develop desirable varieties in a short span of time. Different types of molecular markers have been developed and are being used since 3 decades now. In large breeding populations it becomes very difficult for genotyping as it requires a lot of time and money. With the start of Next generation sequencing it is now quite easier to sequence a large amount of genome in less time and with reduction in costs. This article highlights the key concepts, advantages, procedure and application of NGS technologies in crop improvement.

INTRODCUTION

DNA sequencing a process that determines the order of nucleotides in DNA, or in other words it helps to determine the nucleic acid sequence in DNA. Any method that is involved in knowing the order of the four nucleotide bases viz. Adenine, guanine, cytosine and thymine will come under a DNA sequencing technology. It is very important to know about the genome of an organism as it is a very basic requirement for biological research to be conducted in numerous applied fields like biotechnology, plant breeding, virology etc. DNA sequencing technology has brought a revolution in the field of genomics and genetics, it itself is a part of massive advanced revolution that are coming up every day. The traditional Sanger based sequencing has been quite popular for a range of applications. However the vast amount of DNA and expressed gene sequence data of today is the result of next or second generation sequencing technologies. With reference to Sanger sequencing NGS technologies produce shorter reads and are also prone to a larger rates of error but it has gained high popularity due to its ability to produce huge quantities of data in a very short period of time with comparatively lesser cost. With the everyday increasing population crop productivity is very necessary because of hunger and malnutrition that are faced by almost every country in the world (Godfray *et al.*, 2010).

The development in plant breeding and agricultural biotechnology can prove to be a key tool for crop improvement to feed such a large population. Next generation sequencing the advanced form of sequencing is very effective tool for developing new molecular markers and identify genes of importance. (Edwards and Batley, 2010). With the help of next generation sequencing technologies it is possible to resequencing the whole genome of plants more

effectively than ever before. It is now possible to sequence hundreds or even thousands of related genomes to evaluate genetic diversity within or among the germplasm tools. The identification of genetic variations among large populations has become so easy and precise with the use of NGS. The recent sequencing technologies include Illumina / Solexa sequencing, Roche 454 sequencing, SOLID sequencing, Ion torrent: Proton/PGM sequencing**.**

Advantages of Next Generation Sequencing (NGS) in Crop Improvement

i. Identification and isolation of genes that are associated with QTLs responsible for agronomic traits.
ii. Detection of numerous DNA Polymorphism for marker development
iii. Development of molecular markers for linkage mapping
iv. High throughput genotyping for screening large breeding populations
v. Transcriptome profiling
vi. Association mapping
vii. Wide hybridisation and introgression of alien genes.
viii. Increased accuracy and precision, simple and cost effective

Disadvantages of Next generation sequencing

i. Less cost effective for sequencing low number of targets (1-20)
ii. Time consuming for sequencing low number of targets.

How is Next generation sequencing performed?

NGS involves three well known steps – library preparation, sequencing and data analysis. The workflow behind NGS involves fragmenting DNA/RNA into multiple pieces, ligating with adapters, sequencing the libraries, and reassembling them to form a genomic sequence. It is similar to capillary electrophoresis but in NGS millions of fragments are sequenced parallel which saves time and cost. (Fig. 1)

- ***Library preparation***- DNA/RNA samples are prepared to make them compatible to sequencers. This is done by fragmenting DNA and ligating them with specialized adapters at both ends.
- ***Sequencing***- The libraries are loaded into a flow cell and placed on the sequencer. The DNA clusters are amplified into millions of copies of single stranded DNA. Chemically modified nucleotide added with a fluorescent tag bind to the DNA template through natural complementarity. The tag indicated which nucleotide has been added. After reading the forward strand the reverse strand is read.
- ***Data analysis***- After sequencing the instrument software identifies nucleotides. In recent times certain apps are use to analyse NGS data without bioinformatics training.

Applications of Next Generation sequencing in crop improvement

Marker development

Some progress has been received in developing 2000-3000 novel SSR markers each for chickpea, pigeon pea and groundnut. (Varshney *et al.2012*). With the help of Next generation sequencing and high throughput genotyping methods it became possible. Based on 454/FLX and

Illumina transcript reads, transcriptome assemblies have been developed for chickpea (44,845 TACs) and pigeon pea (21,434 TACs). These Illumina reads with corresponding transcriptome assemblies provided >10,000 SNPs each in chickpea and pigeon pea. These resources made it easier to construct genetic maps in the three legumes. After analysis of phenotypic and genotypic data candidate markers for drought tolerance related root traits in chickpea, resistance to foliar diseases in groundnut and resistance to sterility mosaic disease in pigeon pea have been provided.

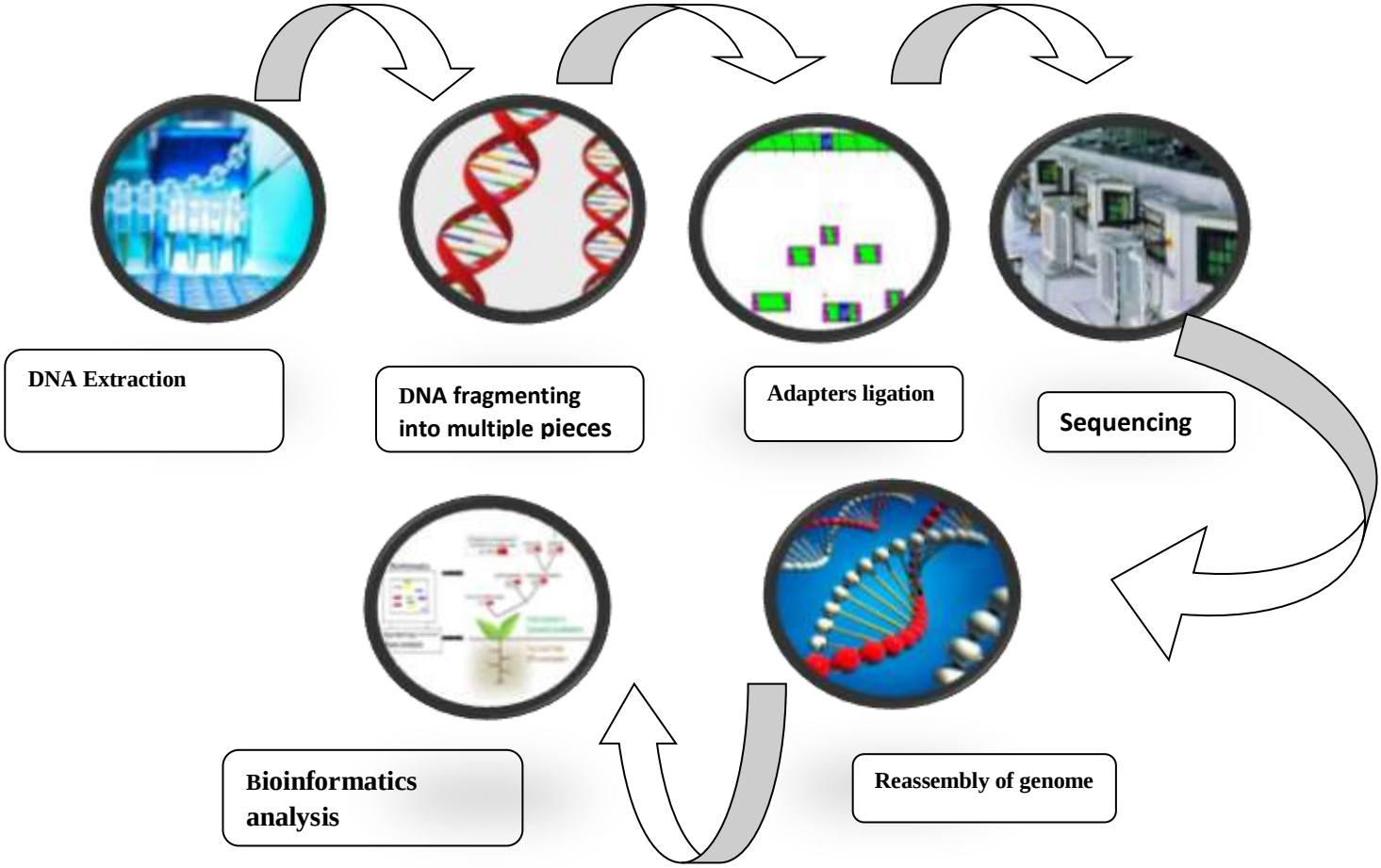

Fig.1. Workflow in next generation sequencing

Impact of bio stimulants on row crops

Row crops like corn, soybean, rice, wheat, sunflower, cotton and rapeseed are most important crops. It becomes necessary to improve the quality and yield of such crops. With this aim the impact of bio stimulants was studied on row crops. With the help of high throughput phenotyping evaluation of a set of three foliar bio stimulant prototypes applied on corn and soybean was done. This made possible to screen out the best prototypes. Next generation sequencing allowed us to characterise the molecular action of the selected prototype. After analysis it showed that genes involved in nitrogen fixation in soybean and genes involved in maltose synthesis, sugar transport, hormone metabolism and phloem loading were upregulated in corn.

Next generation sequencing vs. Sanger sequencing

The concepts behind Sanger as well as NGS are similar, in both of them DNA polymerase adds fluorescent nucleotides one by one into a developing template strand. Each nucleotide is recognised with its fluorescent tag. The main difference between both the methods is the sequencing volume. The Sanger's method can sequence only a single DNA fragment at a time, whereas NGS can sequence millions of fragments simultaneously in each run. This helps in sequencing thousands of genes at one time. NGS provides the advantage of deep sequencing with which one can identify novel genes or variations.

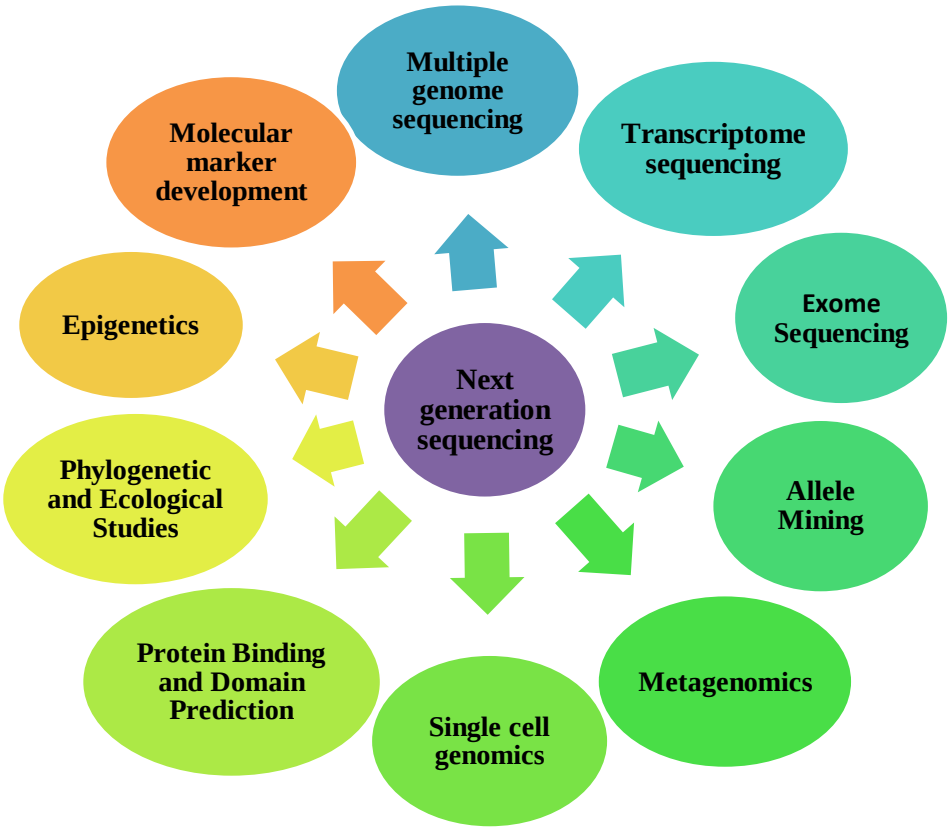

Fig. 1. Applications of NGS in crop improvement

CONCLUSION

Therefore it is quite evident that with the use of next generation sequencing along with high throughput genotyping/phenotyping and integration of different omics approaches it is possible to identify novel genes as well as it helps in opening new perspectives in the discovery, evaluation and development of innovative yet sustainable solutions to meet the increasing demands of crop production. NGS not only allows us to study the entire crop genome but also provides us with the option of modifying according to the goals of the plant breeder. Thus it proves to be an effective tool in crop improvement.

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