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MutMap – A Part Utility of Genome Resequencing

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Abstract
Gene mapping has historically been an intricate, time-consuming, and expensive process that calls for sizable mapping populations and a lot of molecular markers dispersed over all linkage groups. Re-sequencing technologies have made it possible to quickly map genes utilising procedures like SHOREmap, NGM, and MutMap with less time and money invested. The most popular is MutMap since it focuses more on causal SNPs. MutMap+, MutMap-Gap, and QTL-Seq are examples of enhanced and specialised MutMap techniques that have evolved to broaden the scope of applications for the MutMap approach. The Mutmap+ methodology captures traits where the homozygous mutant leads to lethality or sterility. The mutMap-Gap process finds the mutation site in the gap regions of the reference genome, whereas the QTL-Seq method is an upgraded version of MutMap specifically created for mapping quantitative trait loci (QTLs). These techniques are comparable to bulked segregant analysis, frequently used for mapping inherited traits. These methods escape the requirement of genotyping all the individuals of the mapping population and generating high-density linkage maps for mapping the gene for the trait of interest. This article addresses the benefits, established uses of MutMap and its modifications.

Introduction
The genomics era began with the breakthrough discovery of DNA sequencing by Sanger. With automation and refinement of existing DNA sequencing technologies, the time and cost required for whole genome sequencing have reduced significantly, leading to the development of high-quality genomic data. Along with modified sequencing technologies, improvements in bioinformatics tools and algorithms have contributed equally by keeping pace with the enormous amount of data generated by these technologies. Hence, today the data generated from NGS platforms are widely used for several applications, *viz.*, de novo genome sequencing, quantifying expression of genes through RNA-Seq, the discovery of SNPs and other variants, methylation studies, genomic selection, promoter analysis etc. Along with these applications, NGS identifies all those undetected mutations by conventional sequencing methods.

The basis of genetic variation present within the natural population of any species arises due to spontaneous mutations and activation of natural mutagens like transposon, viruses, disease and pest infections, mycotoxins like cycasin, pyrrolizidine alkaloids produced by various fungi, exposure to other chemical mutagens in the form of food, drugs *etc.* In cases where genetic variation is limited in the crossable species, artificial mutagens such as alkylating agents (ethyl methane sulphonate (EMS), acridines dyes, base analogues, X-rays, gamma rays, UV light, fast neutrons *etc*., have been effectively used for generation of

genetic variability. These mutagens create SNPs, InDels or segmental breaks, which alter the observable phenotype.

A set of approaches were designed to expedite the discovery of mutant genes for assessing gene function. These included the SHOREmap (SHOrtREad map), NGM (Next Generation Mapping), MutMap, MutMap+, MutMap-Gap and QTL-Seq methods which could successfully demonstrate mapping of the causal mutation site for the trait of interest. In all of these methods other than QTLSeq, a mutation in the genetic material was created by applying mutagens and the mutant population was screened for the desired phenotype. Then the desired mutant phenotype was crossed with the parent, generating the F1 and F2 population, screened for segregation with the help of SNPs and mapped on the genome, making it a forward genetics approach. These techniques utilize bulked segregant analysis, which normalizes the variations present in the background genome, except for the causal genes, by pooling DNA from multiple individuals to create a bulk.

MutMap

The MutMap method is technically analogous to SHOREmap and NGM as it entails the approach of BSA of mutant progenies obtained in the F2 population. To identify the genes using SHOREmap, and NGM map distantly related mapping lines, which give rise to a highly dense distribution of SNPs between the bulk and the parent. In contrast, the MutMap method relies on the cross between the mutant and its wild type, directly targeting the causal SNPs generated during mutagenesis and responsible for phenotypic behaviour.

- The MutMap scheme was developed by Abe *et al.* (2012) for quick, reliable, and cost-effective mapping of causal SNPs in more than 10 EMS-induced mutant lines of rice.
- Mutations are induced in a homozygous line by a chemical or physical mutagen treatment. A mutant line is then selected and crossed with the parental line. In the F2 generation, plants with the mutant phenotype are selected, and equal amounts of DNA from each selected plant are bulked.
- This DNA bulk is subjected to whole genome sequencing using a next-generation sequencing (NGS) platform with sufficient depth. The depth should be such that the set of reads for a genomic region may be expected to include sequences from almost all the mutant individuals in bulk.
- The short sequence reads are aligned with the parental reference genome, and the genomic positions with SNPs are identified. The mutagen treatment would have induced these SNPs. Each mutant line may be expected to differ from the parent at several (up to around 2,000) SNP loci.
- The numbers of short reads with parental and mutant SNP alleles are scored for each SNP locus, and an SNP index is calculated. The SNP index is the ratio of the number of short reads with the mutant allele at an SNP locus to the total number of short reads covering this SNP locus.
- In case of a recessive mutation, all the F2 individuals with the mutant phenotype will be homozygous for the mutant allele of the concerned gene. As a result, the mutant SNP allele involved in the gene mutation, i.e., the causal SNP allele, will also be homozygous in all these individuals. Therefore, the causal SNP locus will have an SNP index of 1.0.
- In addition, SNP loci tightly linked with the causal SNP locus, but not involved in the gene mutation, will have SNP indices close to one. The remaining SNP loci will have SNP index of ~0.5 since the mutant and parental alleles at these loci will be in nearly 1:1 ratio in the mutant F_2 plants.
- The causal SNP locus can be readily mapped onto the reference parental genome. Thus, the MutMap approach involves whole genome sequencing of a single DNA bulk and

avoids marker development, genotyping of individual plants, and linkage analysis for gene mapping

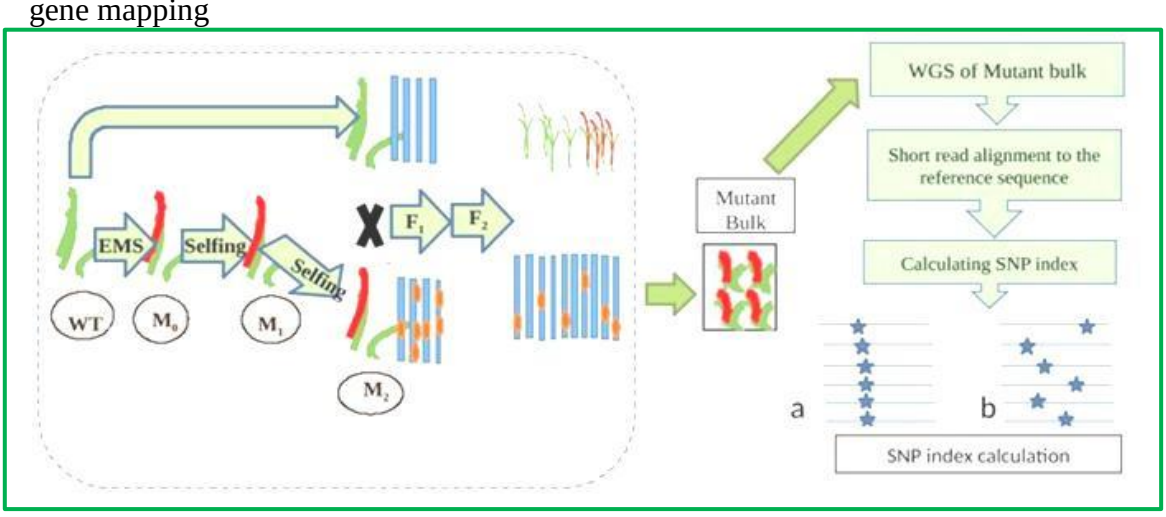


Fig. 1: A basic methodology for MutMap

MutMap-Gap: MutMap-Gap is an extension of MutMap specially designed to identify causative mutation located in the gap region of the reference genome.

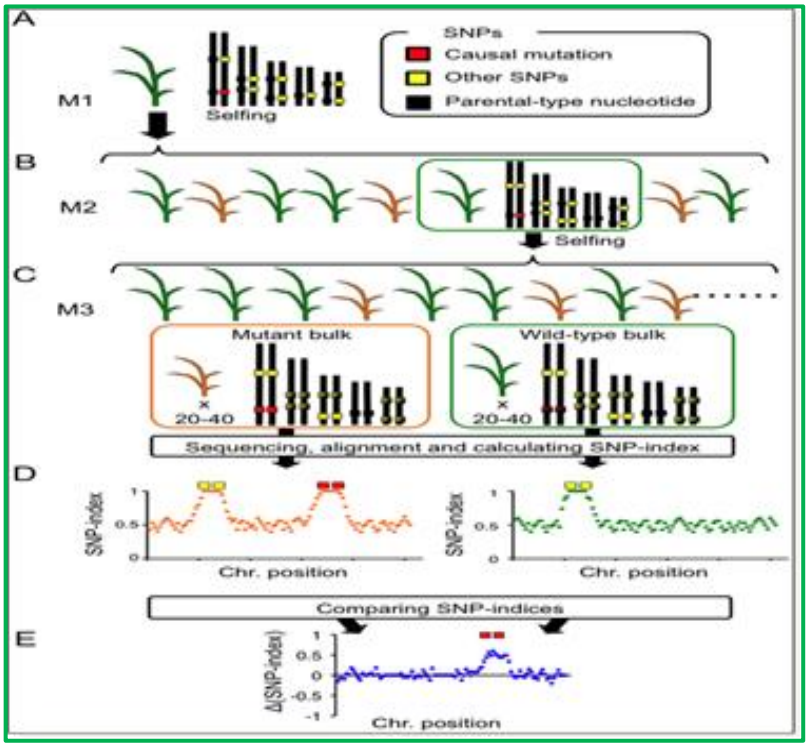
MutMap cannot identify mutations located within parental line-specific genomic regions (gaps) that are missing compared to the 'reference genome'. To identify mutations in such gap regions, an introduction of MutMap-Gap, a combination of MutMap and targeted *de novo* assembly of genomic gap region.

MutMap+ : The mutmap+ strategy is used when the development of the F2 mapping population is impossible due to either the lethality or sterility of the mutant caused by recessive mutations.

- Development of F_2 mapping population is not possible due to either lethality or sterility of the mutant caused by recessive mutations
- Mutant population development and mapping is similar to MutMap except for the following differences:

I. No genetic hybridization is involved and still Mendelian segregation is tracked down.

II. Progeny testing is mandatory as the surviving heterozygous plants of the wild type can be differentiated from the homozygous plants only through progeny testing.



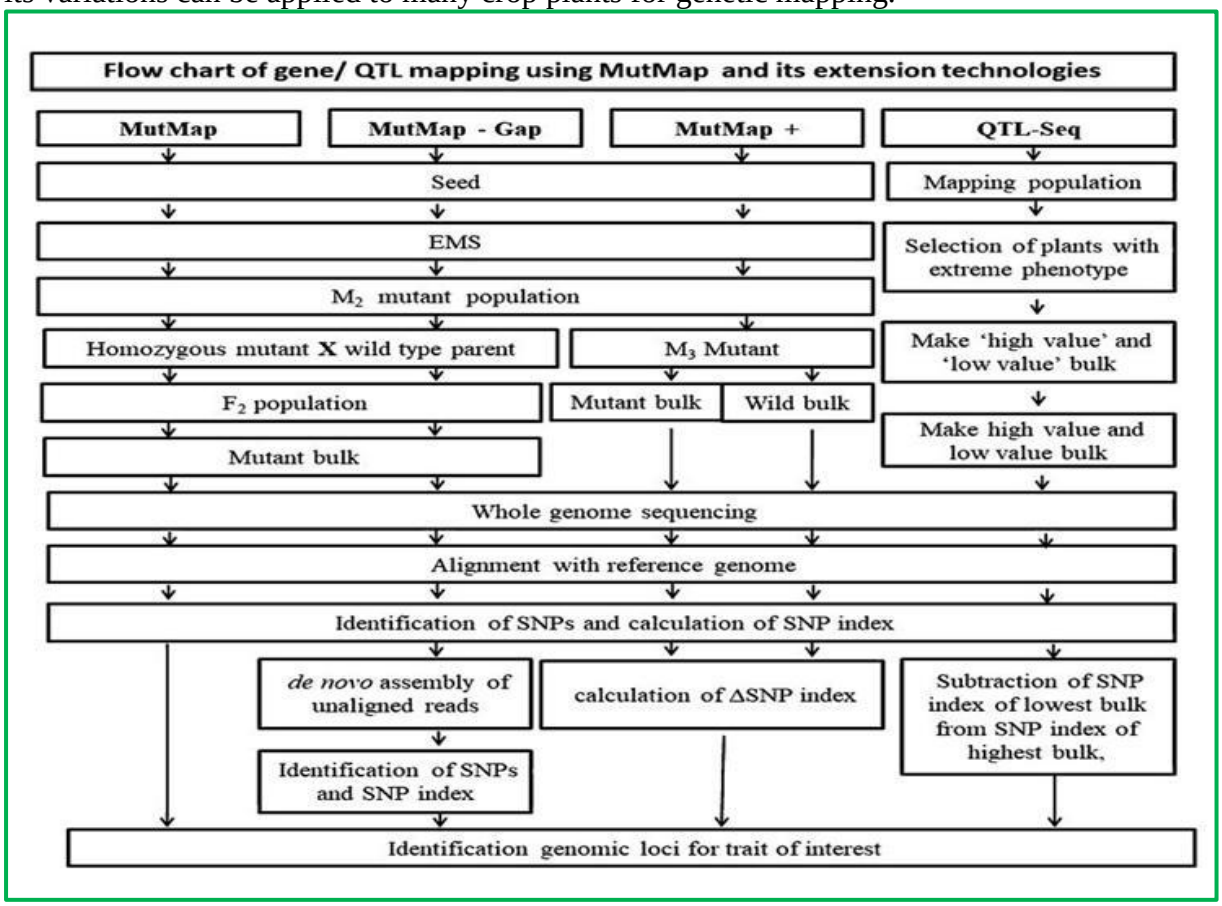
QTL-Seq: QTL-Seq is an extension of BSA and MutMap methodology but with the idea of mapping significant quantitative trait loci (QTL) using the NGS platform. Existing BSA and MutMap methodologies can address only qualitative traits controlled by a single gene that

produces discrete phenotype classes. The majority of the agronomic traits are quantitative and controlled by multiple genes, each with some major or minor effect. Traditionally, QTL mapping is done with the help of genome-wide DNA markers and an appropriate mapping population to tackle such traits, but the whole procedure is time-consuming, labour intensive and costly.

The mutMap technique and its modifications share many common steps, and each methodology offers something new to overcome the limitation of the previous methods. The general flowchart of MutMap and its extension technologies are shown below (Tribhuvan *et al.*, 2018). With the availability of many crop plants' genome sequences, this technique is gaining importance in gene/QTL mapping experiments. Genes governing many essential traits, such as abiotic stress tolerance, disease resistance, seed size and yield *etc*., were successfully mapped using this novel technology.

Conclusion

MutMap methodologies are based on BSA and exploit the power of new-age sequencing technologies to map inherited traits across all plant species where F2 mapping populations can be created at ease. This methodology involves comparatively minimum time and effort but has immense power to identify the causal genes. The potential of this technology has been successfully demonstrated in many plant and crop species, including rice, sorghum, groundnut, soybean, rapeseed, and cucumber. The significant limitations of this technology are the requirement of the draft genome sequence of the crop plants, the inability to handle polygenic inheritance and the complete dependence on induced mutagenesis, which may only sometimes give rise to mutants for every trait of interest. However, with the ever-increasing availability of draft genome sequences across crop species, the use of near-isogenic lines and QTL-Seq technology, all these shortcomings can be resolved, and MutMap technology and its variations can be applied to many crop plants for genetic mapping.



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