Accelerating Salt-Tolerant Brassica Development through MutMap

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The food and nutritional security of the burgeoning world population faces several formidable challenges such as land degradation, freshwater scarcity and climate change. Available evidence shows that these problems are likely to aggravate in the future. It is thus imperative to augment the productivity of existing agricultural lands as well as to bring the abandoned lands under crop production in a socially acceptable and economically viable manner. Current food production and distribution systems have not been able to meet the food and nutritional requirements of a large part of the global population. The world population is expected to exceed 9 billion by 2050, which will require a 57% increase in food production (FAO, 2021). The situation is particularly grim in many underdeveloped and developing countries where problems of salinity-induced land and water degradation have also risen substantially in the last few decades. Salinization of soil is becoming a major agricultural problem worldwide, especially in arid and semi-arid regions. Over the years, we haven't paid much attention to soil salinization, but as the availability of arable land decreases and land use changes, the problem is becoming more serious. Soil salinization harms soil properties and plant physiology. About one billion hectares of arid and semi-arid areas of the world are currently barren due to salinity or water scarcity, while 20% of the irrigated area is affected by secondary salinization (Dagar and Minhas, 2016). Globally, salinization is causing a loss of about 2,000 hectares of arable land daily, contributing to a 1-2% loss of agricultural soil yearly (Zaman et al., 2018). The problem of soil salinity is expected to worsen in the future due to increasing water tables, over-irrigation, flooding, silting, and seepage. Soil salinity is a major factor that decreases agricultural productivity and poses a challenge to the capacity of agriculture to sustain a growing population. This puts food security at risk and makes it challenging to achieve the UN-SDGs by 2030. Researchers are investigating ways to develop sustainable techniques to meet future demands, and integrating different techniques could be key to conserving soil degradation and preventing soil salinity. While progress has been made to increase the productivity of saline lands, we need to fine-tune our research strategies to address emerging challenges and draw a roadmap for future investigation in this domain for harnessing salt affected soils for higher yield and sustainability around the globe.

Presently, existing variability is exhausted from modern cultivars hence, there is a need to enhance genetic gain. The genetic gain equation depended on four factors that affect plant breeding progress: the degree of phenotypic standard deviation present in a crop population (σp), the probability that a trait will be inherited from parents to offspring (heritability, h^2), the proportion of the population selected as parents for the next generation (selection intensity, i) and 'T' is the time required to complete a breeding procedure. The model of genetic gain (Moose and Mumm, 2008) is

$$\Delta G = \frac{\sigma_P \times h^2 \times i}{T}$$

Here, ΔG increased by an enhanced value of σp, h^2, and i and reducing the Time. The phenotypic variability is caused by natural variation, spontaneous and induced mutation. Induced mutation is produced by mutagens like physical, chemical, and biological mutagens. Physical mutagens are gamma rays, X-rays, UV rays, and fast neutrons, a biological mutagen is T-DNA, whereas, chemical mutagens are ethyl methane sulfonate (EMS), methyl methane sulfonate (MMS), alkylating agent, and base analogue. The mutation caused base insertion, deletion, and substitution in DNA which is called gene mutation. It adds rare alleles to the gene pool and increases variability in low-variation crops. ΔG provides a powerful framework for crop improvement i.e., molecular plant breeding offers enhanced ΔG. Traditionally gene mapping is a complex, time-consuming and costly affair requiring large mapping populations and abundant molecular markers spread across the entire linkage group.

To exploit whole genome sequencing, and resequencing in crop breeding, recently MutMap called a forward genetic approach based on high-throughput NGS technologies being used. MutMap is a new way of gene identification for accelerated crop improvement. MutMap was proposed by Abe et al. (2012) for mapping a monogenic recessive gene in Oryza sativa cv. Hitomebore mutagenized with EMS to identify genomic loci responsible for the change in leaf colour from dark green to pale green. MutMap is based on whole-genome resequencing (WGRS) of pooled DNA from a segregating population i.e., F2, of plants that show an agronomically important phenotype. In short, it is the method of rapid gene isolation using a cross of the mutant to wild-type parental line and based on bulk segregant analysis (BSA) of mutant progenies obtained in the F2 population.

The MutMap concerning *Brassica spp*.

A Brassica cultivar with a reference genome sequence is mutagenized by the use of EMS or any other mutagen. The M1 of the mutagenized plant is self-pollinated and brought to the M2 or later mutant generation to ensure that the mutant gene becomes homozygous. Identify agronomically important mutant phenotype in M2 or later generation. This mutant is crossed with the wild type of the same cultivar used for mutagenesis. The resulting F1 plant is self-pollinated to produce F2 progeny (>100) which is segregating for the mutant and wild type phenotype. DNA of F2 progeny which shows mutant phenotype are bulked and subjected to WGS followed by alignment to the reference sequence. All the nucleotide changes that occur in the mutant by mutagenesis are identified as SNPs and indels between mutant and wild-type cultivars. SNPs with sequence reads composed only of mutant sequences (SNP index of 1) are closely linked to the causal SNP for the mutant phenotype. SNP index is the ratio of mutant SNPs and the total number of SNPs governing the trait. SNP index should be equal to 1 near the causal gene and 0.5 for the unlinked loci. SNP indices can be scanned across the whole Brassica genome to find the loci containing SNP index 1, harbouring the gene governing the mutant phenotype (Fig. 1.).

In addition to MutMap, several techniques can be used to identify genes in a crop population for a target trait i.e., MutMap+, MutMap-Gap, SHOREmap, NGM, and QTL-Seq. MutMap+ is useful for the mutant, which is not amenable for crossing to their wild type because it directly exploits M3 for sequencing instead of backcrossing with wild-type plants. Similar to QTL-Seq, M3 lines mutant and wild-type bulks are formed and subjected to WGRS like QTL-seq and with the help of SNP index, genomic loci responsible for target trait are deduced (Feik et al., 2013).

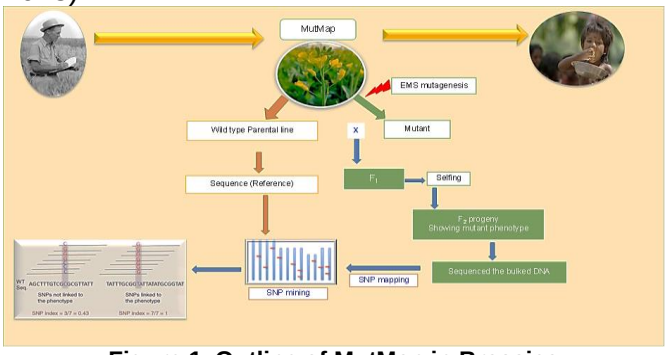

Figure 1. Outline of MutMap in Brassica

MutMap-Gap combines the previously reported MutMap method with targeted gap filling by de novo assembly (Takagi et al., 2013). The principle of this technique is a WGS-based method for the identification of the causative nucleotide changes for a given mutant phenotype in a genomic region that is missing from the reference genome.

Applications of MutMap in *Brassica* spp.

MutMap is a technique utilized in crops such as rice, wheat, and Brassica sp. to identify genes that are responsible for a specific phenotype. Although limited genes have been identified in Brassica sp., they are listed in the Table. It is important to explore this technique for other agronomically important traits in Brassica spp., particularly for biotic and abiotic stress tolerance.

S.N.	*Brassica* sp.	Mutant	Gene	Genetic control	Mutagen	Role	Reference
1.	Chinese cabbage (*Brassica campestris* L.)	*cer1*	*Brcer1*	Single recessive nuclear gene	EMS	Wax deficiency leads to bright green and Glossy phenotype increasing their commercial value	Liu *et al.*, 2021; Wang *et al.*, 2017
		BrFLC2	*Brebm*	Recessive nuclear gene		Early-bolting phenotype	Fu *et al.*, 2021
		ebm3	*Brebm*	Single recessive nuclear gene		Early-bolting-Curly leaves	Tan *et al.*, 2021
		nym1	*Brnym1*	Recessive gene		Stay-green phenotype	Wang *et al.*, 2020
2.	Cabbage (*B. oleracea* L. var. capitata)	*CGL-3*	*BoGL-3*	Single dominant nuclear gene	Spontaneous mutation	Wax-less plant	Dong *et al.*, 2019
3.	Oilseed rape (*B. napus*)	*G7*	*Bna.IAA7.C05*	Dominant gene	Somatic mutation	Dwarf plant	Cheng *et al.*, 2019
4.	Purple cai-tai (*B. rapa* L. var. purpurea)	*BrCER4*	*Bra011487 and Bra011470*	Two genes	-	Glossy phenotype	Wang *et al.*, 2019

Advantages and limitations of MutMap

MutMap is a method that can identify causal mutations and create high-resolution mapping without the need for a large mapping population or linkage map information. It also requires less time and labour compared to other methods that rely on natural variation and exploit-induced mutation. However, MutMap does depend on induced

mutation, which is not always successful for all traits. Additionally, it requires a draft genome sequence of the crop and is unable to measure polygenic inheritance.

Future Prospectus

The use of advanced molecular techniques has created new opportunities for plant breeding and crop improvement. MutMap is a tool that allows for the direct identification of causal mutations or very high-resolution mapping. This is particularly useful for identifying important traits such as yield, resistance to pests and diseases, and tolerance to environmental stresses. By reducing the time required for these tasks, MutMap improves the efficiency of marker-assisted selection. This, in turn, helps to ensure food security in the future and increases overall genetic gain.

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