

SUGARCANE GENETIC IMPROVEMENT THROUGH MUTATION BREEDING AND WITH OTHER MOLECULAR APPROACHES

Ali Raza^{1*}, Ali Hassan¹, Shaheen Abbas¹, Muhammad Noshrewan¹

¹Department of Plant Breeding & Genetics (PBG), Faculty of Agriculture and Environment (FA&E), The Islamia University of Bahawalpur (IUB), Pakistan

*Corresponding author e-mail: alizraza005@gmail.com

ABSTRACT Sugarcane (*Saccharum* spp. complex) is a tropical and subtropical crop grown for sugar and biofuel worldwide. With the extensive genetic sampling of sugarcane genotypes using conventional methods, researchers approached the use of molecular techniques including mutation breeding, CRISPR-Cas9 Genome editing, Targeting Induced Local Lesions In Genomes (TILLING), and Genome Wide Association Mapping (GWAS) for its improvement. Mutant genotypes with enhanced juice quality, yield, sucrose percentage, resistance to fungal diseases and tolerance to drought and salinity have been discovered using Ethyl Methane sulfonate (EMS), gamma, and X-rays. However, mutation has few limitations because of unpredictable induced mutations, and large resources needed for mutagenesis, screening, and field trials but still mutagenesis offers an optimistic future if more potential mutagens, effective methods of their application, and techniques for screening of superior mutant progeny are developed. This review will discuss the basis of mutation and sugarcane yield, mutation strategies used in sugarcane breeding, evaluate their successes, find restrictions, and prospects for increasing sugarcane production through mutant breeding.

Keywords: Sugarcane; Mutation Breeding; Ethyl Methane Sulfonate; Gamma Rays

INTRODUCTION

Sugarcane is an important crop in the worldwide agricultural landscape belonging to the Gramineae family having high biomass productivity and serving both as a key source of sugar and an excellent candidate for biofuel production. Despite its importance, traditional sugarcane breeding confronts various obstacles. The crop's complicated polyploid DNA, low fertility, and extended breeding cycle all limit quick genetic improvements (Aitken, 2022). For food crops that are asexually propagated, the use of mutation breeding with other biotechnological techniques offers a crucial way to overcome these hurdles and generate more genetic variability for crop improvement (Bado, Yamba, Sesay, Laimer, & Forster, 2018). Yield is a polygenic trait, affected by gene expression of different traits like photosynthesis efficiency, resistance to insect pests, and tolerance to abiotic stresses like cold, heat, temperature, and drought. The discovery of favorable mutant genes resulted in the improvement of these characters in sugarcane with considerable success. By inducing genetic mutations, either through physical or chemical means, mutation breeding can create novel genes. Mutant strains, M8457 and M8721, with improved yield, more sucrose content, higher water tissue content, abundant chlorophyll content, and salinity tolerant have been obtained with gamma rays exposure

(Purankar, Nikam, Devarumath, & Penna, 2022). But due to certain adverse effects of physical or chemical mutagens on plant growth, this method has some limitations.

Antioxidants, compounds with the capacity to postpone or prevent oxidative stress. Notably, the antioxidant action of whole grain flour surpasses that of refined flour, with wheat bran standing out for its impressive 76.47% antioxidant activity (Leváková and Lacko-Bartošová, 2017). The cultivation of cereals, particularly wheat, has a long tradition in Europe, where it accounts for more than half of the total cereal production in the European Union (Horvat et al., 2020). Scientific evidence suggests that whole grains, as commonly consumed in the United States and Europe, reduce the risk of chronic diseases, including cancer and heart disease (Miller et al., 2000).

Mutation Breeding Fundamentals: After a comprehensive examination of traditional breeding methods, efforts focused on mutation breeding to introduce genetic variability in crops. Both physical and chemical mutagens have been used to develop improved cultivars and hybrids in different field crops including sugarcane. Physical mutagens cause non-specific mutations that penetrate deeply and allow for large-scale mutagenesis with stable, heritable consequences (Yali & Mitiku, 2022). Chemical

mutagens, on the other hand, give focused mutation induction with ease of application and high mutation rates.

Sugarcane yield improvement by induced mutations can be done by the improvement of existing sugarcane varieties through mutations and combining the radiation technology with conventional breeding programs. But care should be practiced while applying the mutagens because reduced mutagen dose may not produce the desired level of mutations. Increased dose of physical and chemical mutagens in other crops like tomato (EMS, colchicine etc.) is harmful as it will cause the increased cell death, stunted plant growth, reduced chlorophyll content and other detrimental effects on plant growth and development (Ahmed et al., 2017). Similarly, mutagen application duration should also be considered.

Historical perspective: Sugarcane has been cultivated since 5000 BC, although breeding only started in the nineteenth century to strengthen its genetic makeup and first interspecific hybrid cultivar, Co 205, was released in 1918 (Ram, Hemaprabha, Singh, & Appunu, 2022). Method of precision breeding, CRISPR-Cas9 Genome editing, helped the researchers to precisely alter any gene either to switch off or replace a gene with a better version in sugarcane.

With the advancement in molecular genetics, mutation induced varieties showcases enhanced juice quality, yield, sucrose percentage, and resistance for fungal diseases. Another study conducted for integrated pest management selected mutants, particularly **MutA** of NCo376 and **Mut1** and **Mut23** of N41 showed promise for future field studies to control both Fusarium stem rot and pest *E. saccharina* (Mahlanza, Rutherford, Snyman, & Watt, 2015). Recently, resistant soma-clones were found but had inferior yield traits, suggesting the need to utilize them as parents in hybridization programs with commercial varieties to develop agronomically superior Yellow Leaf Disease (YLD)-resistant sugarcane varieties (Kona et al., 2019). TILLING is now being used to find the mutants conferring disease resistance, stress tolerance, improved yield, and complex genome mystery in sugarcane (Mohapatra, Majhi, Mondal, & Samantara, 2023). Considerable improvements in sugarcane yield have been achieved but more enhancement is needed to meet the raising sugar demand for growing population. Present sugarcane farming depends heavily on biotechnological techniques and mutation breeding, which promotes refinements in productivity, resilience, and disease resistance to create a more sustainable agricultural.

Genetic basis of sugarcane yield:

Sugarcane is a C4 plant having higher yield potential like maize in contrast to other field crops, rice, and wheat having a C3 pathway of carbon fixation. Genetic factors, such as those about growth rate, photosynthetic efficiency, stress responses, and sucrose metabolism, affect sugarcane production. The genetic diversity of sugarcane germplasm provides a huge gene bank that can be used to create new cultivars with enhanced yield characteristics. Sugarcane is a hybrid of *Saccharum officinarum* and *Saccharum spontaneum*, characterized by its large genome

and high polyploid and aneuploid properties. This genetic complexity and low fertility under natural conditions make traditional breeding challenging, but breeders can also use the wide genetic base that polyploidy offers to select and improve features through mutation breeding (Budeguer et al., 2021). Introducing more genetic variability and genes through mutation breeding that confer disease resistance and improved agronomic qualities, the genetic base of sugarcane can be expanded by in vitro culture-induced mutations, which increases the yield potential of sugarcane.

Mutation breeding techniques: Both physical and chemical mutagenic techniques have been used in improving the cane yield. Breeding material exposed to mutagens can give the desirable sugarcane clones with the resistance to various biotic and abiotic factors in in-vitro selection. Sugarcane has been mutated using physical mutagens like radiation. The usage of UV, X, and gamma radiation can alter DNA and result in genetic mutations that might not happen naturally, so sugarcane cultivars with improved disease resistance, higher yields, and greater stress tolerance have been produced. Combining somaclonal variation with irradiation can isolate desirable mutant lines, while genetically stable mutants confirmed in field-testing can be useful for sugarcane improvement. Mutation in sugarcane callus irradiated with gamma rays can bring variations in various morphological characters and in vitro selection with polyethylene glycol (PEG) of these mutants resulted in isolation for drought tolerance in sugarcane (Hartati, Suhesti, & Yuniyati, 2022). Recently, Whole Genome Sequencing (WGS) analyses of irradiation-induced mutants has improved our understanding of mutation frequency and spectrum which also aids in selecting radiation type and conditions for generating specific mutations (Jo & Kim, 2019). Chemical mutagens like Ethyl Methyl Sulfonate (EMS) are used in mutant breeding to induce point mutations in DNA, resulting in significant phenotypic alterations in sugarcane characteristics like internode number, girth, and sucrose content. EMS-induced mutations have been shown using PCR analysis to enhance sugarcane production and improve its quality, as the mutant progeny show higher sucrose levels than their parents ((Dalvi, Tawar, Suprasanna, Dixit, & Prasad, 2021). Researchers have successfully employed CRISPR-Cas9 to target various alleles of the magnesium chelatase gene in sugarcane, which is crucial for chlorophyll biosynthesis and may lessen the demand for nitrogen fertilizer (Eid, Mohan, Sanchez, Wang, & Altpeter, 2021). Mutation breeding techniques and other molecular techniques are pivotal innovations in sugarcane cultivation due to their ability to introduce novel genetic diversity and accelerate the development of superior varieties.

Challenges and Future Directions: Mutation breeding has been a transformative force in crop improvement, including sugarcane. However, it faces several challenges and limitations. Sugarcane is a highly heterozygous polyploid with complex genome having 100-130 chromosomes, vary according to cultivated clones, resulting in difficult management of the genetic variation as compared to diploid species. Further the

effectiveness of mutation breeding in sugarcane is limited due to complex polygenic characteristics like yield and stress resistance, uncertain induced mutations, and large resources required for mutagenesis, screening, and field trials.

Future directions for improving sugarcane yield through mutation breeding involve integrating mutagenesis with conventional selection, genomics, phenomics, advanced molecular breeding tools, germplasm use, and collaborative research. Sugarcane proteomics is expected to reach the next level by genome sequencing resulting in improved sugarcane yield (Barnabas, Ramadass, Amalraj, Palaniyandi, & Rasappa, 2015). All these approaches aim to address current challenges in sugarcane breeding and unlock significant yield improvements through innovative mutation breeding strategies.

CONCLUSION

The transformative potential of mutation breeding and other associated molecular techniques like CRISPR-Cas9 has significantly accelerated the breeding cycle leading to improved cane yield, agronomic characters, and resistance to various biotic and abiotic factors. However, limitations in the application of various mutagens with varying effects offer some hurdles to utilizing their full potential. However, with the increasing global challenges in agriculture, mutation breeding has the potential for crop improvement including sugarcane to ensure higher production and better performance in different climatic conditions.

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