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In-vitro mutagenesis Approaches for Flowering Control in Sugarcane – A Review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Sugarcane (*Saccharum officinarum* L.) is one of the important commercial crops grown globally as a source of raw material for wide range of sugar and bio-energy industries. Crop being a member of family Poaceae, terminates its growth by flowering (arrowing). The process of flowering is highly

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complex process and is mediated by temperature, photoperiod, humidity, altitude and latitude besides crop genetic makeup. Though flowering is crucial for crop improvement programmes, uncontrolled flowering under commercial cultivation poses a serious problem for sugarcane farmers and millers with a considerable loss in yield and quality. Therefore, in order to device sugarcane genotype with no flowering habit enormous efforts have been made through various approaches, among them *mutagenesis* is one such approach wherein propagation materials will be exposed to chemicals or radiation in order to bring desirable traits. This technique looks to be the prominent crop improvement strategy for sugarcane. Hence, in this paper review we brought together the earlier work done in the field of mutation breeding in devising non-flowering sugarcane genotypes and their impacts on commercial sugarcane production.

Keywords: Sugarcane; yield and quality; flowering genes; mutagenesis; PCR.

1. INTRODUCTION

Sugarcane (*Saccharum* spp.) is considered as one of the most important crops cultivated in the tropics and subtropical areas around the globe. It belongs to the genus *Saccharum* of the family Poaceae. The genus comprises six species that include *Saccharum spontaneum*, *Saccharum officinarum*, *Saccharum robustum*, *Saccharum edule*, *Saccharum barberi* and *Saccharum sinensis* [1]. Botanically, sugarcane belongs to the Andropogonae tribe of the family: Gramineae, order: Glumiflorae, class: Monocotyledoneae, subdivision: Angiospermae, division: *Embryophyta siphonogama*. The subtribe is Sacharae and the genus *Saccharum*, derived from the Sanskrit "sarkara = white sugar". It was believed that, the plant reached the Mediterranean region from India. Physiologically sugarcane belongs to C_4 plant commercially propagated through stems cuttings. The genus *Saccharum* composed of six species which includes wild species, *Saccharum spontaneum L.* and *Saccharum robustum*; cultivated species, *Saccharum officinarum L, Saccharum barberi, Saccharum sinense* and *Saccharum edule*. However, *Saccharum officinarum L* is the widely cultivated cane all over the world which was evolved by hybridization between *S. officinarum* and *S. spontaneum*. The first artificial interspecific hybrids between these two species were created to overcome disease outbreaks and were followed by repeated backcrossing using *S. officinarum* as the recurrent female parent to restore high sucrose content; this process was popularly termed as nobilization of cane.

At present, sugarcane is cultivated in more than 20 million hectares in tropical and subtropical regions of the world, and approximately 1.3 billion metric tons of crushable stems are produced. Top ten producers and consumers of sugar are depicted in the table (Table 1). For hundreds of years, sugarcane has served as a source of sugar and represents an important renewable biofuel source, which is seen as a global commodity and important energy source. Besides being purposely used to produce sugar, sugarcane accounts for almost two thirds of the world's production and has lately gained more attention due to increased ethanol production [2]. Sugarcane is not only vital for sugar production but also for its by-products are used as industrial raw materials for fuel, chemicals, bio-fertilisers, paper and pulp production Bio-refining is a common process through which more products are produced supplementing those already available for use. Through increased bio ethanol production and consumption globally, it is highly anticipated that the need for more sugarcane production is key since sugarcane can produce approximately 4000-6000 litre or l /ha of ethanol [3]. Sugarcane bagasse is the major waste product generated by sugar mills after extraction of the sucrose from cane juice and is largely used for energy cogeneration at the sugar mill and bagasse can be used as animal feed production thus increasing the overall efficiency of the crop system. Different products are processed out of bagasse which includes paper production, used in bread as a dietary fibre, as a wood substitute in the production of wood composite, as well as in carbon fibres synthesis [4]. Enzymatic and hydrolytic processes are considered to allow the bagasse carbon units from cellulose and hemicelluloses and this can be used for ethanol production which makes us sugarcane a very useful versatile efficient crop for energy production [5]. Sucrose is the principle food product but different research has proved that sucrose is a raw material for production of higher value products to include some natural pharmaceutical compounds [6]. Several foods, drinks and dishes around the globe have been derived from sugarcane and its products under

different local names in different countries to include syrup, ganne ka rass, guarab, sayur nganten, cachaça, rum, falernum, jaggery, panela, molasses, rapadura, rock candy among others. Bagasse and molasses the main by-products of sugarcane are documented to have various applied uses. Molasses are composed of 60% of sucrose and inert sugars, 13% inorganic salts. Recently, sugarcane importance globally had increased as a result of the important industrial raw material attached to it in order to produce sugar and allied industries producing alcohol, acetic acid, butanol, paper, plywood, industrial enzymes and animal feed [7].

2. CHALLENGES FOR SUGARCANE PRODUCTION

The sugarcane production is greatly affected by biotic and abiotic stresses (Table 2). Several scientists reported that different environmental factors limit crop productivity and destroy biomass and termed these factors as stress or disturbance [8]. Abiotic stress lead to crop loss worldwide, reduce average yields among most crop plants by more than 50%. These environmental factors were the most known stress conditions that affected crop production and productivity which included low temperature, drought, and high salinity. The biggest percentage of crop loss, limited plant growth and crop productivity were attributed to environmental stresses [9,10].

Abiotic stress drought is one of the most deleterious abiotic stresses was found to affect crop productivity worldwide [11]. Sugarcane being useful source of sugar and ethanol, it had a relatively high water-demanding capacity compared to other crops since its growth is direct related to water deficit [12]. An estimation by Kingston (1994) showed that sugarcane can yield between 8–12-ton cane per mL of irrigation water therefore water deficit was seen as a cause of 60% productivity losses [13-16]. This was one of the reasons why sugarcane production was mainly done in regions which receive a considerable amount of rainfall so that sugarcane can receive enough water for growth and development [17], but in areas with limited rainfall sugarcane production must be supplemented or full irrigation [18].

Table 1. Top 10 Sugar producing countries (Million Metric tonnes) during 2021-22 and 2022-23

Sl. No.	**Country**	**Production 2021-22**	**2022-23**
1.	Brazil	35,450	36,370
2.	India	36,880	35,800
3.	EU	16,479	16,255
4.	Thailand	10,157	10,500
5.	China	9,600	10,000
6.	United States	8,287	8,201
7.	Pakistan	7,140	7,180
8.	Russia	6,000	6,500
9.	Mexico	6,556	6,360
10.	Australia	4,120	4,450
Total world production		180,348	182,891
Sl. No.	**Country**	2021-22	2022-23
1.	India	29,000	29,500
2.	European Union	17,000	17,000
3.	China	14,800	15,800
4.	United States	11,313	11,295
5.	Brazil	9,500	9,800
6.	Indonesia	7,600	7,900
7.	Russia	6,350	6,140
8.	Pakistan	6,000	6,100
9.	Mexico	4,342	4,160
10.	Egypt	3,430	3,485
	Total consumption World	173,240	178,843

(USDA, 2022) (Source: https://apps.fas.usda.gov/psdonline/circulars/sugar.pdf)

Table 2. Biotic and abiotic stress constraints of sugarcane

Abiotic stresses	Biotic stresses
➢ Drought/ moisture stress ➢ Waterlogging stress ➢ High temperature/ heat stress ➢ Salinity/ alkalinity stress ➢ Low temperature/ cold stress ➢ Plant nutrients stress ➢ Shortage of water ➢ Flooding during rainy season	➢ Weeds ➢ Pests like sugarcane borer, white fly, white wooly aphid, ➢ Insects like sugarcane borer, scales, white fly, white wooly aphid, mille bugs and white grub ➢ Bacterial diseases like Gumming disease, Leaf scald, Mottled stripe, Ratoon stunting disease, Red stripe ➢ Fungal diseases as red rot, smut, wilt, rust, Pokka boeng, grassy shoot disease viral diseases like sugarcane

The complexity of sugarcane's response to stresses like water deficit further coupled with the limitations of available molecular tools and strategies to identify and exploiting the large genes effects and alleles associated with selection traits for developing drought tolerant varieties suitable for commercial crop production conditions. Genetic engineering of sugarcane to produce drought tolerant genotypes was still a major challenge [19-21].

Sugarcane is highly sensitivity to salinity because of being a glycophyte which is exhibited at different growth stages. In different glycophytes like sugarcane, the main ionic stress in relation with high salinity is due to sodium toxicity which leads to ion imbalance or disequilibrium, hyper ionic and hyper-osmotic stress, which eventually disorganizes the overall metabolic activities and causing plant death [22].

The phenotypic symptoms of abiotic stresses exhibited by sugarcane includes stunted growth, poor tillering and root growth, necrotic leaves with scorched tips and margins, reduction in internodal length and girth of cane, impaired cane quality with reduced juice purities, jaggery and sugar processing challenges and these were attributed mainly to soil salinity stress among others [23].

A four-year study recently conducted by the World Bank (http://www.sriindia.net) showed that due to climate change, there will be a 30% reduction in sugarcane production worldwide. Sugarcane being known for its well documented economic importance but being a highly heterozygous, polyploidy and frequently aneuploidy nature with a complex genome affected by poor fertility, and the long breeding/selection cycle its genetics has not been mainly considered by scientists and researchers compared to other crops.

Jalaja et al. (2008) studied CoC 671 a sugarcane variety in Maharashtra; despite being an early maturing variety with high sugar content, it showed signs of declining which is attributed to its genetic degeneration [24]. This variety was found to be susceptible to many diseases and pests which have led to lower yield and sugar content thus conventional breeding methods failed to improve this variety.

2.1 Flowering in Sugarcane

Flowering in sugarcane is being a complex physiological process comprising of different developmental stages which require different physiological and environmental conditions. Flowering in sugarcane is genetically determined trait and it is influenced by a number of plant and environmental factors which include photoperiod, temperature, moisture and nutrition [25]. Productivity of cane and sugar is heavily affected by flowering. Flowering as a genetic trait is of great importance for breeding but uncontrolled flowering in commercial fields poses a serious challenge to both farmers and millers due to loss of cane and sugar yield.

Development of a sugarcane variety with stable performance and good traits is the biggest challenge faced by sugarcane breeders. On exposure to different environmental conditions particularly in tropical regions, sugarcane automatically responds by flowering. The heterozygous and polyploid nature of sugarcane determines the flowering pattern of sugarcane varieties thus plenty of challenges at hand to sugarcane breeders and researchers to name: poor fertility, non-synchrony and non-flowering of specific genotypes. Sugarcane has a unique

character of accumulating 50 per cent sucrose content in the stalk compared to its dry weight [26-30], which later transforms into increase in sugar content thus increasing the sugar yield and grower profitability. Through plant breeding there is a possibility of improving yields though successful synchronised production of inflorescences required for crossing plants remains the biggest constraint.

Most of the genome sequence information related to sugarcane can now be accessed [31]. Based on the available sequence information, sugarcane being a hybrid possessing a complex polyploidy and aneuploidy genome could be used to identify different gene homologues. Different flowering pathway genes have been studied in sorghum which is the closest diploid relative to sugarcane with a complete genome sequence. These genes identified in the flowering pathway could be assigned to different groups like photoperiod perception, internal clock cycle and floral induction, in relation to the function it plays in other species.

2.1.1 Effect of flowering on sugarcane yield

Flowering in sugarcane (Fig. 1) causes losses both in the yield of cane (field loss) and in the percentage of recoverable sugar (quality loss). Determination of sugar yield losses had been done by the use of several methods [32,33]. Flowering can be reduced by photoperiodic control in sugarcane as it is one of the few methods of preventing flowering without affecting the growth of sugarcane. Flowering in sugarcane lowers cane tonnage as this is directly related to the percentage of flowering and age of the crop at flowering. Non-flowered sugarcane stalks had an increased fresh weight compared to flowered sugarcane stalks, but according to Singh (1980) after 35 to 45 days of inflorescence emergence in two sugarcane varieties namely Co-1158 and Co-740 flowered canes had more weight compared to the non-flowered canes [34,35].

Sucrose content was heavily affected by flowering; highly observed in periods of high flowering percentage. The flowering lowered the quality and purity of sugarcane juice mainly when sugarcane was harvested after four or more months of maturity [36]. It was observed that sucrose per cent juice in flowered stalks was significantly higher during early season but it gradually increased in the later season in the non-flowered stalks [35,37]. Suppressing the flowering through photoperiodic treatment showed that there was no significant difference in sucrose content available between flowered and non- flowered canes [38]. Long (1976) indicated that as sugarcane crop became aged there was also a steep rise in fibre content of varieties, which was contrary to Rao (1977) and Nuss (1989) who observed no difference in fibre content of both flowered and non-flowered canes [39,40,41].

One of the major limitations of the sugarcane variety VCF 0517 using in our research studies is flowering affecting the productivity of cane and sugar of VCF 0517 variety. Despite extensive breeding efforts in India, till date there is no non-flowering genotype developed in order to reduce the losses imposed due to flowering in different sugarcane varieties.

Fig. 1. Sugarcane vareity with flowering and non-flowering

Van Vloten (1910) calculated sugar yield of both flowering and non-flowering culms in Java and recorded a reduction of 0.04-0.05 mT ha^{-1} for each 1% flowering which was also reported by Hes (1951) [36,42]. Using stools of culms as experimental plots in Barbados, Rao (1977) described stool yields as a function of percent flowering. He further calculated a potential sugar loss of 0.05 mT $ha^{-1}$for each 1% flowering. Further, through conventional hybridization, sugarcane genetic improvement could be done but was hindered by intricate flowering behaviour of sugarcane. This could be due to perennial and highly heterozygous nature of the sugarcane followed with a long juvenile period which limits the speed of improvement using traditional methods [40,43].

2.1.2 Genes involved in regulation of sugarcane flowering

Biological rhythm is the process by which gene expression level increases and decreases at a constant basis during a 24h cycle and this is controlled by endogenous (internal biological circadian clock) or exogenous (external) stimuli [44,45]. Circadian rhythms are termed as endogenous rhythms that cycle over a period of time close to 24h. An example of exogenous rhythms includes the synchronisation to the length of day and night, which is also termed as diurnal rhythm but on exposure to constant light or dark conditions such rhythms stop to persist [44,46,47]. Light/dark spells don't affect some genes but these genes respond to changes in photosynthetic compounds and other internal rhythms.

Higgins et al. (2010) clarified that small percentage of genes were unique to short day or long day plants. Light perception and duration of light could change the amplitude and timing of expression of the endogenous circadian clock genes. Floral induction resulted from changes in expression level of flowering pathway genes which could be due to alteration in expression of the endogenous circadian clock genes [48,49].

Coelho et al. (2013) while working on one Brazilian sugarcane cultivar in his findings showed as many as 2–8 homologues for GI, TF1L-like, CO, EHD1, GHD7 and FT. These different homologues were expressed differently in some tissues at various developmental stages as analysed from the SUCEST database [50]. Higgins et al. (2010) and Murphy et al. (2011) 50 illustrated and documented the genetic control of photoperiod induced flowering in different species namely Arabidopsis, rice, sorghum and Brachypodium [48,51]. These findings showed a high degree of conservation within flowering pathway thus providing a breakthrough to understand sugarcane flowering pathway.

Fifteen sugarcane homologous genes were identified and published which were divided into two groups' *viz*., photoperiod perception and floral induction (Tables 3 & 4). Sugarcane gene homologues and sequences that enable floral induction previously have been interpreted from already published work and aligned with different sequence databases like Sugarcane v0.1 G Browse and the sorghum genome [51-54]. In the sorghum database Phytozome, nine genes more were identified and functionally annotated to play vital roles in flowering pathway [55], and the sorghum sequences aligned against the sugarcane sequence database [54].

2.1.3 Strategies for flowering inhibition in sugarcane

Flowering is the major constrain in the sugarcane that will affect the yield and quality of the cane and sugar. There is a need for adopting various strategies in order reduce the flowering without affecting other agronomical parameters of the sugarcane crop.

Research studies in Hawaii have shown that sugarcane being grown as a two-year crop, flowering may happen twice during overall crop cycle. There was an observable sucrose yield loss in cases where flowering happened more than six months before harvest [36,58]. Therefore, there was a need to test different methods and ways that can either reduce or prevent flowering and among them the use of plant growth regulators (PGRs) was applicable at a commercial basis.

Different compounds were tested for their ability to inhibit flowering in sugarcane whereby only six compounds advanced to field-scale testing namely maleic hydrazide (MH-30; 1, 2-dihydro3, 6-pyridazinedione), monuron [CMU; 3-(p-chlorophenyl)-1,1-dimethylurea], diuron [DCMU; 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea], paraquat (Gramoxone; 1,1'-dimethyl-4, 4'-bipyridinium salt), diquat [Reglone; 6,7-dihydrodipyridol (1,2-a:2', I'-c) pyrazinediium salt], and ethephon (Ethrel; 2-chloroethylphosphonic acid). The U.S.

Table 3. Genes responsible for positive regulation of flowering in sugarcane

No.	Gene name	Gene function
1.	CO (Constans)	- Induction of the expression of the flowering-time gene FT - Encode a protein with two zinc fingers loosely related to those of GATA transcription factors.
2.	GI(Gigantea)	- Encode a putative membrane protein - Act as the gating factors to regulate the signal transduction of a photoreceptor
3.	FT-Flowering Locus T	- An early target of CO
4.	PRR37(Ma1) – Pseudo-Response Regulator 37/Maturity Gene 1,	- Controls photoperiodic flowering. - Encodes PHYB, and the nearly complete photoperiod insensitivity.
5.	PRR's–Pseudo-Response Regulator's, PRR1– Pseudo-Response Regulator 1,	- Inhibits expression of Circadian Clock Associated 1 (CCA1) and Late Elongated Hypocotyl (LHY).
6.	AP1 – APETALA 1,	- Floral meristem identity gene - specifies carpel and stamen identity in the flower,
7.	LEAFY	- Floral initiation is determined to a large degree by the level of LEAFY expression. - Directly activates at least one of the later genes, AP1 - Encodes transcriptional regulator that promotes the transition to flowering. - Involved in floral meristem development. - LFY is involved in the regulation of AP3 expression.
8.	SOC1 – Suppressor Of Over expression Of Constans 1	- Overexpression causes early flowering and suppresses the effect of mutations

[Source: 48, 56, 57]

Environmental Protection Agency registered diquat commercially which was used in Hawaii for 15 years but the major challenge was it was less efficient in reducing flowering (50 per cent) and caused damages to crop like desiccating leaves but now it is no longer used in Hawaiian sugarcane to control flowering. In 1988, Environmental Protection Agency also registered Ethephon as a good compound to use during flower initiation season [58,59].

In 1981, ethephon tests started and positive results indicated that it had the ability to act as a potential flower control agent [60,61]. Findings from two yield field trials indicated that there was a 15 per cent reduction in flowering and this led to an increase in sugar yield by a volume of 3.7 metric tonnes per hectare (mT ha^{-1}). These results were not conclusive as it was recommended that more studies should be done to assess the effect of ethephon on a commercial scale.

Sugarcane crops were treated with ethephon prior to the time of harvest to increase the sugar yield by increasing the sucrose percentage. Sugar producers in Hawaii termed this process as "rip" as the application of ethephon was aimed at ripening cane which showed positive and consistent results [62]. Osgood et al. (1983) clarified that application of ethephon during early crop stages did not induce the rip effect therefore increase in sugar gains during field trials was directly related to increase in cane tonnage which was partially attributed to reduction in flowering [61].

2.2 Induction of Mutation to Sugarcane Explants

Mutation breeding, morphology, cytogenetics, biotechnology and molecular biology are regarded as relevant conventional breeding methods in the field of plant breeding. Mutation occurs due to changes in the base sequence of genes which are induced either spontaneously or artificially both in seed and vegetative propagated crops. Brunner (1995) noted there is a possibility of generating desirable traits which are not easily expressed in nature or lost due to evolution [63]. Through mutation breeding more than 3100 mutant cultivated varieties have

been developed in about 190 plant species and this has significantly impacted crop improvement.

Somaclonal variation can be classified either as genetic or epigenetic changes to include polyploidy, aneuploidy, mutation (point) and new insertions of (retro) transposons [64, 65]. Different physiological changes occurred during *in vitro* conditions were the causes of some phenotypic variability therefore developed plantlets under normal field conditions revert to their parent type. Plantlets developed from aneuploidy cells may show different genetic behaviour since aneuploids may have lower or higher number of chromosome. Through use of direct regeneration method available, the existing genetic heterogeneity present within cells in the form of different cytotypes can easily be exploited [66,67] noted that direct regeneration of plants from explants was an effective and efficient way of reducing soma-clonal variation but as well indicated some shortcomings that may present chance of soma-clone in the regenerated population.

According to James (2004) the genetics of sugarcane crop make it complex. Sugarcane is an old crop which has evolved for over 1015 years through conventional breeding, selection cycle and vegetative propagation of resulting cultivars [68]. In order to diversify the genetic pool and potential of sugarcane, different important desirable traits have been introduced by use of soma-clonal variation as a result of *in vitro* culture or mutagenic treatments [69]. Soma-clonal variation refers to any kind of genetic or epigenetic variation detected in plants derived from cell cultures regardless of the morphogenic route or explant used [64,70].

Protoclonal, gametoclonal and mericlonal variation terminologies were used to describe variants got from protoplasts, anthers and meristem culture. Different reports indicated that soma-clonal variations are induced by culture media in two ways: exposure to growth regulators and length of time in culture and these resulted in generation of desirable traits in sugarcane [71].

Table 4. Genes responsible for negative regulation of flowering in sugarcane

Sl. No.	Gene name	Gene function
1.	Late Elongated Hypocotyl (LHY)	- Components of the oscillator - Involved in the photoperiodic induction of flowering. - Encode highly similar single Myb domain DNA binding proteins - Inhibitors of the evening complex (EC)
2.	ELF3 (early flowering	- Functions in both hypocotyl inhibition and flowering time
3.	PHYC (phytochrome C)	
4.	CCA1 (circadian clock associated)	- Functions in both hypocotyl inhibition and flowering time. - Inhibitors of the evening complex (EC) - Encodes a MYB-related transcription factor involved in the phytochrome induction of a light-harvesting chlorophyll a/b-protein (Lhcb) gene.
5.	FLC – FLOWERING LOCUS C,	- Plays a central role in vernalisation, and much of this response involves reductions in FLC mRNA. - FLC represses flowering, this occurs through repressing the flowering time genes SOC1 and FT
6.	PHYB (Phytochrome B)	- Regulation of hypocotyl elongation - Mediate light inhibition of hypocotyl elongation in red light. - Plays an inhibitory role in floral initiation
7.	TOC1 – TIME OF CHOLOPHYLL A/B BINDING PROTEIN 1,	- Does not function as a classic response regulator. - Cycles in light/dark cycles with a peak in the evening and shows a circadian rhythm

[Source: 48, 56, 57]

As reported by the scientists, all the possible causes of soma-clonal variation include karyotype changes, cryptic changes associated with chromosome rearrangement, transposable elements, somatic gene rearrangements, gene amplification and depletion, somatic crossing over and sister-chromatid exchanges. It was also revealed that the choice of morphogenic route coupled with indirect somatic embryogenesis has a great influence on the frequency of soma-clonal variation and results into sugarcane plants that have varying number of chromosomes and agronomic characteristics [64, 72].

Somaclonal variation being a random event therefore critical selection and identification of desirable soma-clones can easily be done by adding a selective agent for example incorporation of a fungal culture filtrate under *in vitro* conditions or through field-based screening of regenerated plantlets (69). Under *in vitro* conditions, callus cultures are exposed to physical and chemical mutagens as this induces soma-clonal variations whereas increases the frequency of occurrence of variations. Researchers reported that induced mutagenesis poses the ability to elicit beneficial and functional modifications in cultivars [73]. In order to increase Somaclonal variations in sugarcane both physical and chemical mutagens have been used successfully [69, 73-77].

Different agronomic characteristics and features in crops to include salinity and drought tolerance have been successfully improved through both chemical and radiation-mediated in vitro mutagenesis and selection [78]. The major advantage of mutation breeding in vegetatively propagated crops is its ability to improve one or a few important traits of a given cultivar, without alternating the entire genetic makeup. A direct crop improvement through use of hybridization, mutation breeding, and tissue culture approaches in plant breeding was reported [79]. However, some undesirable soma-clonal variations often generated during tissue culture cycles are shown in many important field crops namely: Zea mays [80], *Oryza sativa* [81], *Triticum aestivum* [82], and *Saccharum spp*. Complex.

Sugarcane embryogenic cultures were treated with different doses of gamma radiations (10–50 Gy), challenged with different levels of NaCl (42.8 to 256.7 mM) [83]. Plantlets, regenerated from irradiated calli of sugarcane cultivars CoC671, Co 86,032, and Co 94,012, were planted in the field and agronomically desirable variants were identified for cane yield and sucrose concentration. A new sugarcane variety ‘Guifu 98-296’ which is a drought-prone upland of Guangxi Province, China by use of mutation breeding was developed [84]. A high sugar yield was observed in two mutants and this was attributed to increase in stalk length, stalk number, and stalk diameter. Khan et al. (2007) used gamma rays to treat vegetative sugarcane cuttings in order to improve yields without any negative impact on other agronomic traits [75]. Single bud setts of sugarcane variety CoJ 64 was used to induce variation for cane number, cane girth, and red rot resistance [85].

Based on the above research results we have initiated the induction of mutagenesis in elite sugarcane variety VCF0517 with the objectives of developing non-flowering sugarcane genotype is under progress.

2.2.1 Physical mutagenesis

Mutations can be induced in different parts of a plant by use physical mutagens which are both ionizing and non-ionizing radiations which include: X-rays and gamma rays which are commonly used for sugarcane mutagenesis. Radiations are the most commonly used mutagens to develop mutant varieties and some researcher reported that 64% of radiation induced mutant varieties were through use of gamma rays as the major mutagen [86]. Out of 313 mutant varieties developed, only 169 mutant varieties were obtained by using gamma rays [87].

When buds of a striped sugarcane variety were exposed to X-ray treatment, a sharp increase in colour range of stripes as well as differences in flowering propensity were observed [88]. During *in vitro* studies, a total of 164 resistant lines were selected but when exposed to field trials for two years it was found out that only 8 lines were resistant against red rot disease [89].

Morphologically distinguished mutant clones from the original sugarcane variety Co 449 which was exposed to 500r, 1000r, 2000r, 3000r, 4000r, 5000r and 10000r of gamma radiation from a 60Co source. It was observed that a single plant each from the vegetative progeny of buds subjected to treatment doses of 500r and 3000r were found to be resistant while all the others (an average of about 1500 for each dosage) were susceptible [90].

Stimulation of growth with the application of low X-ray doses where applied in main shoot in some of the sugarcane varieties was reported. They also discovered that sugarcane variety Bo 14 contained stripped chimera and chimera with copious ivory markings in Bo17. There was no significant difference between morphological characters and juice quality of these clones and normal plants. Variety Bo 32 was discovered to be susceptible to *P. tucumanensis* and its chimera showed a marked reduction in size and brix values [91].

Increased sugar content was observed upon exposure of sugarcane varieties to 1500r to 2000r [92]. Through the use gamma radiation, valuable sugarcane mutants of these two varieties (Co 527 and Co 663) have been obtained [93].

Mutants which are highly resistant to red rot were obtained after irradiation with gamma rays of cane setts of variety Co 997 at dose rate of 2000rad. The resultant mutant was almost the same in relation to distinguishable features as the original non-irradiated stock [94]. In a study of four sugarcane varieties in three generations (M1, M2, M3), the varieties were irradiated with Gamma rays and X-rays at different doses 1, 2, 3, 4 and 5kr. This made it possible to isolate an important glabrous leaf sheath mutant from varieties Co 419 and Co 527 which was true to type in the M3 generation [91].

The mutants obtained were resistant to *P. tucumanensis* from different sugarcane varieties namely Co 997, Co449, Co527 and Co312 by application of both irradiation and chemical mutagenic treatment [95]. Isolated Co 997-24-1 red rot resistant mutant which had similar characteristic features to Co 997, a susceptible variety in terms of quantity of cane and sugar yield, and amount of sucrose content in sugarcane juice.

In 1974, Bari got buds from four high yielding sugarcane varieties to include: BL 4, BL 19, CoL 54 and L 116 and treated them with gamma rays at a dose rate of 2, 3 and 4 KR dose managed to obtain some mutants which were resistant to red rot and mosaic virus [96]. Haq et al. (1974) obtained nine mutants of Co 633 and six of Co 527 and these mutants showed different levels of resistance to *G. tucumanensis* but Co 527-85 mutant showed resistance in both field and laboratory trials [97].

Triton and Apollo cutting sub-clones of sugarcane were exposed to gamma rays at a dose rate of 3 and 5 KR, observed a significant increase in sugar content and lower fibre. Fiji disease virus resistant lines were developed through soma-clonal variation under *in vitro* conditions, selected lines managed to retain the high yield as characterized by the parents [98,99].

Variability can be induced in a high frequency in sugarcane following sett irradiation. Studies based on grand growth period like bud radio-sensitivity estimation due to acute gamma rays' treatment effects at dose rate of 1.0, 2.0, 3.0, 4.0, 5.0, 7.5, 10.0 kr, on sugarcane (*Saccharum spp*.) clone Co 547 setts showed that cane yield decreased significantly from 3·0kr and this was attributed to low plant survival at maturity. Different parameters were considered for selection of variants to include: increased internode thickness, higher tiller number and rind colour changes. Therefore, the treatment dose rate was limited to not more than 3.0kr in order to maintain a positive high yield, optimum sugar recovery and population size [100].

The induction of sugarcane mutations and morphological variations at the ninth month stage in VM1 generation was reported [101]. Through application of mutations in Co series varieties mainly mutagenic treatment of single bud setts to obtain various traits like dwarfness, non-flowering, high sugar content, glabrous leaves, increased growth rate, increased cane yield and resistance to *G. tucumanensis* [102].

Different sugarcane varieties treated with 2Kr of gamma rays and obtained mutant of Co 740 with erect leaves, other mutants of Co 419 that had increased stem diameter, individual cane weight and sucrose percentage. The mutant had showed higher shoot population which yielded more number of millable canes without affecting the economic characters like sucrose content and juice purity [40, 103].

The effect of different doses of gamma irradiation treatments sugarcane cultivars NCo 310 and GT 54-9 on single bud cuttings yielded reduction in germination percentage compared to controls was reported [104]. They observed the significant increase in internode numbers in NCo 310 cultivars at all the doses wherein GT 54-9 cultivar it was increased only at dose of 3 kr. Mutagenic treatment were carried in six commercial sugarcane varieties cultivated by Indian

sugarcane farmers in order to induce mutations [105]. Fifty sugarcane mutants were obtained which exhibited a number of morphological characters, disease resistance and higher sugar content compared to other varieties.

In vitro mutagenesis technology was used to develop sugarcane mutants using sugarcane calli. Calli treated with a treatment dose of 6kr showed no response and shoots/plants failed to regenerate but other calli treated with doses below 6kr responded and developed well. Calli treated with 0.5Kr had a good effect on some agronomic characters like plant height, number of tillers per plant, cane thickness and number of green leaves per plant. The use of gamma radiations studies on sugarcane and observed that 4-8 Kr was the sensitivity range dose for optimum generation of cane buds was reported [106].

Three sugarcane varieties Isd-2/54, *Nagarbari* and *Latarijaba* were treated with different doses of gamma rays viz., 20, 30 and 40 Gy [107]. The mutants were challenged with red rot spore suspension which yielded 37 resistant plants and 151 moderately resistant plants. From the gamma rays (20, 40 and 60 Gy) treatment of four sugarcane varieties Isd-2/54, Isd-16, *Nagarbari* and *Latarijaba*, the mutants SCM-12, SCM-14 and SCM- 15 variants selected showed tolerance to waterlogged conditions in MV6 [74].

Sugarcane calli regenerated on medium containing partially purified *Colletotrichum falcatum* toxin at a range of 0.05% to 0.5% were induced mutation with sodium azide gamma-rays. In which minimum plants regenerated with maximum callus death at 0.5 % toxin concentration. Regenerated plants were resistant to red rot disease from callus which was insensitive to red rot toxin [108].

Somatic mutations were induced in vegetative cuttings of three sugarcane clones namely NI-98, NIA- 2004 and BLA which were treated with different doses of gamma rays ranging from 0, 10, 20, 30, and 40 Gy which showed negative impact on different agronomical at 30 Gy and 40 Gy whereas 20 Gy showed an enhancing effect on plant height and cane yield [75].

The callus cultures were induced using spindle explants grown on MS media with 2,4-D and from three commercial sugarcane varieties *viz*., CoJ 64, CoJ 83 and CoJ 86. The callus and callus derived shoots were exposed different doses of gamma radiations and resulted in varying percentage of shoot regeneration. Highest number of shoot regeneration and shoot proliferation was observed in 20 Gy and lowest in 60 Gy whereas 80 Gy dose was recorded 100% lethality. The optimum gamma radiation dosage for both mutagenesis of callus and shoots derived from callus was found to be 60 Gy which showed a very big variability with respect to number of canes, cane girth, cane height and sucrose content of different irradiated clones of the same variety under field conditions [109].

In vitro mutagenesis technique was used to select salt tolerant lines in popular sugarcane (*Saccharum officinarum L*) cv CoC 671, Co 86032 and Co 94012. The embryogenic cultures were treated to different gamma radiation doses (10-50Gy) and challenged with different levels of NaCl (42.8 - 256.7 mM) which showed plant regeneration dose treatments of 10 and 20Gy irradiated calli up to 171.1 mM NaCl selection. The regenerated plantlets were evaluated at Field level to study the various agronomical parameters like cane yield and sucrose [83].

In vitro mutagenesis technique is used for different sugarcane clones viz., NIA-98, NIA-0819 and BL4 in which genetic variability was induced mainly using apical meristematic region as an explant for callus induction medium. Callus was exposed different doses of gamma rays which resulted in maximum callus proliferation, plant regeneration and growth in control treatment and minimum was observed at the treatment of 40 Gy. The treatment 30 and 40Gy doses showed maximum chlorophyll mutation frequency and maximum number of roots and root length in control followed by 10 Gy. Negative responses on tillering potential of the regenerated plants were recorded at 30Gy and 40Gy treatments [110].

Mutation breeding was used to select a sugarcane variety Guifu 98-296 and field evaluation results showed that Guifu 98-296 cane yields was estimated at 118.95t/hm2, sugar yield at 16.65t/hm2, with a significant increase of 32.98% and 29.07%, respectively, compared to ROC 22 which is the main sugarcane variety in Guangxi [84].

2.2.2 Chemical mutagenesis

The importance of mutagenesis to introduce variability, random genetic mutations in plants by using physical mutagens to include: gamma rays

and UV light or chemical mutagens namely: ethyl methane sulfonate (EMS), sodium azide (NaN_3) and 5-azacitidine agents were described [111]. The most commonly used chemical mutagen is Ethyl methane sulfonate because of its ability to induce valuable traits in many species, including sugarcane [77].

Sodium azide (NaN_3) is effectively used chemical mutagen in agricultural, medical, and organic synthesis research which induces random mutations [112-114]. The mechanism of action of sodium azide (NaN_3) depends on its ability to produce an organic metabolite (β-azidoalanine moiety [N_3–CH_2–CH (NH_2)–COOH]) which induces chromosomal changes at relatively lower rates than other mutagens [115, 116].

The chemical mutagenic treatment sugarcane callus from meristematic leaf whorl explant of cv Co 86032 with EMS (0.5%) at different time intervals from 1 to 3 hrs. The treatment results showed no regeneration of calli treated with EMS for 2 hours and 15 healthy plants were regenerated from calli treated for 2.5 hours and calli treated with EMS for 3 hours induced regeneration at lower rate and only three healthy plants [76].

Chemical mutagen ethyl-methane sulfonate (EMS) with different concentration doses were used to induce mutations on callus derived from two commercial varieties namely, CP48-103 and CP57-614 in Iran [117]. The findings established the significance of regression coefficient at a = 0.01 whereas the regression equation for the relationship between utilized dose of EMS and mortality of calli obtained was Y= -8.18 +5.28X.

Sugarcane varieties viz., Co 419, Co 527, Co 1287, Co 740 and Co 775 were treated with physical and chemical mutagens for Induction and isolation of mutants obtain desirable traits as depicted in Table 5 [118].

The development of new sugarcanes genotypes with a high tolerance for water stress was reported using ethyl methanesulfonate (EMS) mutagenesis in the sugarcane cultivar Khon Kaen 3. They have used 16 mM of EMS for 4 h induced callus mutagenesis (survival rate, 57.5%) followed by treating the survival rates of calli with 10 mM of EMS for 2 and 4 h in selective media with 15% PEG were higher than that of non-EMS-treated calli. The EMS treated calli survived and grew on selective media with 20% PEG and no growth of non-EMS-treated calli. Based on their research results it was concluded that EMS mutagenesis and evaluation using *in vitro* and greenhouse methods were successful in developing new sugarcane clones with high water-stress tolerance [119].

The chemical EMS induced mutation was reported in sugarcane for smut disease resistance was reported. Eleven Ethyl Methyl Sulfonate (EMS)-induced mutants of the Indian sugarcane cultivar CoC 671 were evaluated for smut disease resistance along with agronomic and quality traits viz. early maturity, high sucrose, and high cane yield and for quality. The smut resistant mutants were found to be superior w.r.t juice and sugar quality parameters. Mutants TC 2819 and TC 2826 exhibited superior for sucrose content (20–24%) than parent CoC 671 (18–21%), respectively, at 10^{th} and 12^{th} month of maturity. Their study concluded that EMS-induced genetic variability for the mutants with smut resistance and agronomic traits in sugarcane [120].

Single bud setts of sugarcane variety Co 1148 were exposed to both physical mutagens

Table 5. Different sugarcane varieties treated through mutagenesis

No.	Sugarcane variety	Mutagen used	Mutation desired
1.	Co 419	Gamma-rays 3, 5 and 7 kr	Study of radiosensitivity
2.	Co 527	Gamma-rays 3 and 5 kr	Glabrous leaf sheath. Non-flowering, early maturity
3.	Co 1287	Gamma-rays 3, 5 and 7 kr; EMS 0.10 - 0.80%	Smut resistance; increase in sucrose content
4.	Co 740	EMS 0.10 - 0.80%	Smut resistance
5.	Co 775	Gamma-rays 3, 5 and 7 kr	G1abrous leaf sheath, red rot resistance.

Source: [118]

(gamma rays) and chemical mutagens (EMS and SA) followed by inoculation of clones with red-rot isolate. Different parameters like mean, range and C.V % were used to estimate brix number, sucrose percent, purity coefficient, commercial cane sugar, juice extraction % and fibre content in regenerated mutagenic population of sugarcane. Observations showing significant increase in mean values, range and C.V % were recorded for those identified quality traits in comparison with control [121].

Four replications of seven multiple-bud mutants including one gall-forming mutant (US 94-12) were tested with normal sugarcane cultivars for 2 years using randomized complete block design. Whereas normal cultivars produced one shoot per node when grown in the greenhouse while two shoots per node were obtained from multiple-bud genotypes, but US 94-12 that produced 4.5 shoots per node compared to others [122].

Different molecular markers are being used to study genetic diversity and selection of parents for planning crossing between parents from divergent backgrounds which will be applicable in genetic improvement programmes. Several researchers have shown that microsatellite repeats have the ability to be applied for studies on genetic diversity [123,124]. One of the important tools used to study complex genomes such as sugarcane was the use of molecular genetic markers [125]. Molecular markers help to reduce time needed for the development of new varieties in breeding program as they are used to select economic traits during the early stages of growth as well as in the choice of the best parents in a cross.

RAPD a molecular marker technique was used to study the variability obtained from mutation breeding (gamma rays) in sugarcane. Brown rust susceptible sugarcane genotype B4362 were exposed to both physical and chemical mutagenic treatment using tissue culture in which only five brown rust resistant mutants with hypersensitive response to *Puccinia melanocephala* were selected [126]. Occurrence of variations in molecular, morphological, and agronomic traits was recorded in different brown rust resistant mutants. There was significant increase in sugar yield for two mutants as well as stalk length, stalk number, and stalk diameter also increased.

3. SUMMARY AND CONCLUSION

All commercial Agri. biotech companies should take a lead and heavily invest in developing commercial transgenic sugarcane, targeting developing non-flowering genotypes and sucrose accumulation as priority traits. Due to technological innovations in molecular biology and biotechnology, has led to discovery of flowering genes and their functions, expanding knowledge about effect of flowering on sugarcane production and productivity are expected to accelerate research in sugarcane. Availability of improved germplasm due to combination of transgenic and conventional breeding, followed by appropriate crop management practices will make a significant impact in productivity improvement and yield stability for commercial crop production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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