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SUGARCANE HYBRIDISATION AND DETECTION OF TRUE HYBRIDS USING MOLECULAR APPROACHES: FIELD STUDY AND STATISTICAL ANALYSIS

D Angeline Kiruba, S Sangeetha, S Sanjay, M Yogeshwaran

Government College of Technology, Department of Industrial Biotechnology, Coimbatore, Tamil Nadu, India

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Corresponding Author:

E-Mail:

angelinekiruba@gct.ac.in

ABSTRACT

Sugarcane production serves a wide range of industries by providing industrial raw materials, bioenergy, sugar, and support for economies worldwide. Its various contributions highlight the crop's importance in agriculture and other industries, making it a significant crop in the world's agricultural landscape. The development of novel plant cultivars possessing desired qualities such as resistance to disease, increased yield, improved flavour, or climate tolerance using hybridization can selectively assist to produce cultivars that possess favourable characteristics like heightened nutritional value, resilience against pests and diseases, and enhanced yield. The vast majority of sugarcane cultivars are interspecific hybrids between S.officinarum, a wild species with a high sucrose content, and S. spontantaneum, a wild species with tolerance to red rot disease and drought. A major obstacle to successful crossbreeding is genetic incompatibility across species. The complexity of the genetic systems in determining features makes hybridization efforts even more challenging. The analytical techniques are essential for comprehending, evaluating, and improving hybrid plants. These methods cover a wide range of scientific approaches used at different phases of hybridization. The review articulates the general process and the various analytical techniques available to study the viability of the hybrid generations. As breeders intend to use various germplasm resources to produce hybrids with high sucrose content and resistance to biotic and abiotic stressors, the characteristic study of the inter and intrageneric sugarcane hybrid varieties along with the field study on backcross hybrids enables selection of suitable clones with the desired agronomical traits and genotypic expressions, ensuring better clone selection.

Keywords: Yield, Nutritional value, Genetic incompatibility, Pest resilience, Sucrose content, Hybrids

1. INTRODUCTION

The basic process for the hybrid plant production begins with a selection of suitable parents based on the health and observed agronomical traits. The parent plants must be contemporized such that they flower at the same time. Later the emasculation of anthers bearing the pollen are

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collected from the flower using scissors method, excising by hand, alcohol treatment and suction method. The collected pollen from the male flower is transferred to the stigma of the female flower using a hair brush by crossing. A tag must be placed on each flower with the information about the date of the emasculation, date of crossing, names of the crossed parent plants. The crossed flowers are covered with bags made of paper, butter paper to prevent apparition by insects. Seeds from the parent plant are grown in pertinent conditions. The progenies from the parent seeds are subjected to selection of hybrids among them [40]

2. SUGARCANE HYBRIDISATION

The vast, perennial grass sugarcane (Saccharum spp., Poaceae family) is planted widely within tropical regions all over the world. Usually, axillary buds on stem (or stalk) cuttings are used for vegetatively propagating it. After planting, the first crop, known as the "plant," is typically harvested 12 to 24 months later. Following that, "ratoon" crops may be harvested at shorter or equivalent intervals of time. According to the International Sugar Organization (ISO), nearly eighty percent of the sugar produced worldwide, comes from sugarcane. With 87 member countries and its headquarters located in London, ISO is the international organization tasked with enhancing the global sugar market conditions. As an alternative energy source, sugarcane is also utilized to produce biomass and ethanol. Sugarcane is grown in more than 80 countries, and in 2002–2003, 111.8 million metric tons (t) of sugar were produced. In 2002/2003, the three nations of Brazil, India, and China collectively produced about 10 million tons of cane sugar. Australia, Mexico, Thailand, Pakistan, South Africa, USA, Colombia, Cuba, and the Philippines are other big cane sugar producing nations (each generating > 2 million t). The greatest sugar yields ever measured were 17.4 t/ha/year in Australia's Central District and 23.8 t/ha-year in Hawaii's Leeward Oahu. Three species— Saccharum officinarum in New Guinea, Saccharum barberi in India, and Saccharum sinense in China—were previously employed to produce sugar. Saccharum spp. hybrids make up most modern sugarcane cultivars, which are 90% S.officinarum and 10% S.spontaneum. It is speculated that S.robustum in New Guinea is the ancestor of the species S. officinarum, which has a high sucrose content. Most sugarcane breeders agree that S.officinarum originated from a domesticated, thick-stalked, high-sugar, low-fiber variety of S.robustum in New Guinea. During the prehistoric era, S.officinarum spread from New Guinea to Indonesia, Malay, China, India [40].

3. ADVANTAGES AND CHALLENGES OF PLANT HYBRIDISATION

The offsprings of crossing two distinct species or kinds is a hybrid plant. Compared to their parents, hybrid plants have more genetic variation. Hybridisation enhances genetic variety since each parent contributes a portion of the offsprings genotype to its genetic makeup. Conciliating the various genetic and evolutionary processes that may interact in hybrids to shape variance in ancestry along the genome represents one of the main challenges for researchers studying genome evolution after hybridisation. Many of the current models focus on the sources of selection in isolation, yet in nature, numerous demographic and selective processes occur concurrently possibly interacting on another. The benefits of plant hybridisation include fast and robust growth with resistance to many diseases and stress conditions. Modern cultivars contain about 15-20% S.spontaneum, according to research by D'Hont et al., using genomic in situ hybridisation [18]. One of the major disadvantages in producing sugarcane hybrids is that less than 5% of chromosomes are recombinant or translocated from parent clones to offsprings. Their ploidy level and genomic complexity for the expression of transgene and creating molecular markers. One of the most

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important tasks to produce fertile hybrids is selection of hybrids. The possible adversities that can occur in the genomic level include,

1) Functionally important regions of genome experience reduced introgression.
2) Dobzhansky Muller hybrid incompatibilities.
3) Linkage disequilibrium.
4) Lack of Heterosis: Though heterosis produce high fertility of offsprings than the parents the heritability is low with a difficulty to achieve in a large population.
5) Transgressive segregation: A condition in which the offsprings exhibit phenotypes that exceed the parental phenotype

Reinforcement: Production of Maladaptive individuals with decrease in mating between incipient species expressing lower fitness than parents.

The advantages of plant hybridisation include the development of hybrid varieties, short stature early maturity, insect pest management, disease resistance, drought tolerance and increase in efficiency of production. The adverse effects can cause repression of flowering, premature death, and sterility [26].

4. POSSIBLE INTERGENERIC AND INTRAGENERIC HYBRIDS OF SUGARCANE SPECIES

Most of the sugarcane cultivars are interspecific hybrids of S.officinarum which have high sucrose content and S.spontaneum which is a wild species that possess resistance to drought and red rot disease. However, the fundamental issue is that only a small percentage of hybrids possess parental traits [20]. As a result, breeders are very interested in using various germplasm resources to create hybrids that are resistant to ex-vitro stress and with a high sucrose content. [38]. The available germplasm has undergone screening for agronomic traits and resistance to biotic and abiotic stress and listed in a table 1.

Table: 1 Potential sources for different agronomic traits

TRAITS	SPECIES	OBJECTIVES	REFEREN CES
Yield	S.officinarum	Thick juicy cane with high sucrose and low fiber content. It is widely used for production of sugar, ethanol and other industrial applications.	[38]
Tolerance to water logging	S.barberi S.spontaneum S.robustum	Under prolonged flooding the tolerant clones were able to maintain high energetic growth, physiological efficiency	[13]
Drought tolerance	E.arundinaceus S.officinarum S.barberi	Under drought conditions, E.arundinaceus displayed high levels of BRK1 (BRK1CK1) genes that promote leaf epidermal cell morphogenesis and division in plants. Drought tolerant E.arundanaceus exhibits over expression	[37]
Cold tolerance	S.spontaneum S.barberi S.sinense	During low temperature conditions, cold responsive mechanisms will be activated. The transcription factors associated with cold response mechanisms are Bhl4,CAHTA,MAOS,WRKY,NAC,TRAF,C3H,AP2 .genes like LHCB-3 and RUBISCO activase in chloroplast plays an important role in cold tolerance.	[37]

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Red resistance	S.officinarum S.spontaneum E.arundunaceus	Red rot is a fungal disease caused by Colletotrichum fallatum damages sugarcane by rotting planted stalks and standing cones. Erianthus showed more complete resistance to red rot than any other species of sugarcane	[17]
Resistance to multiple pests	S.barberi S.sinense S.robustum E.arundanaceus	Erianthus exhibits higher resistance to root knot nematode (Meloidogone jauanica) and root lesion nematode	[17]

4.1 Assessment of trait performance

Following planting, the field was periodically checked to see how the plants were doing in terms of emergence. Throughout the germination process the total number of sprouting branches was counted, the overall health of the freshly sprouted young plants was examined. The total number of plants and tillers were counted during tillering phase. The state of plant's health was thoroughly observed during the whole growing season. Under typical field circumstances, the major focus of observation was on the signs of the illnesses mosaic, smut, red rot ,and pokkah boeng, as well as stem borers, ants and other pests [21].

4.2 Observation of morphological traits

The significant morphological traits to be observed are leaf shape, leaf color, number of green leaves, waxiness of the leaf sheath, leaf sheath spines and color, ligule shape, auricle shape, bud shape and position [21]. Comparing the grown hybrids, there was a substantial difference in the qualitative traits of the leaf sheath hairiness (absent, dense, sparse), ligule shape (strap, deltoid, crescent, and arch), internode shape (cylindrical, conoidal, obconical,curved), leaf blade width and prominence of the growth ring on the node, and leaf sheath spines [20].

4.3 Analysis of physiological traits

A network of enzymes such as sucrose phosphate synthase (SPS), sucrose synthase (SuSy) and invertases manage the sucrose metabolism and storage in the internodes of sugarcane plants.

Many other factors, such as cellulose, sugar transporters, transcription factors, protein kinases, and hormones signaling, also play roles in sucrose synthesis, transportation and accumulation in sugarcane stalks [21]. The photosynthetic parameters, such as photosynthesis rate and instantaneous water use efficiency (WUEi), are calculated using two factors, A and E where A is the overall photosynthetic rate and E is the overall transpiration rate.

$$WUEi = A/E$$

Sugar yield can be calculated by measuring commercial cane sugar (CCS) content, which is given by the following formula:

Sugar yield = (Cane yield * CCS)/100 [22].

4.3 SPAD (Soil Plant Analysis Development)

SPAD readings relative to leaf chlorophyll content were taken during tillering and grand growth phase. SPAD measurements were recorded on the adaxial leaf surface, that was entirely exposed to sunlight between 10:00 and 14:00 with a temperature range of 25°C [21]

5. ANALYTICAL TECHNIQUES

5.1 Genomic in situ hybridisation

GISH is a highly effective molecular cytogenetic technique that uses chromosomal DNA in-situ hybridisation tests with genomic DNA from a single species as the labelled

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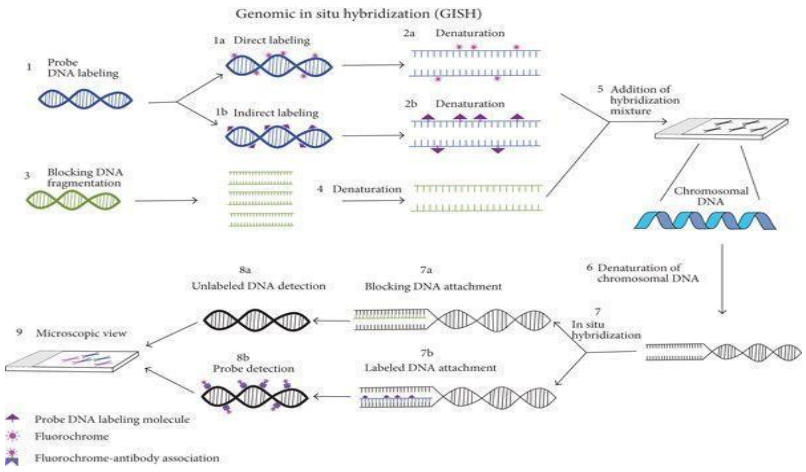
probe [35]. The GISH has made a significant contribution to the investigation of plant's karyotypic stability and selection throughout many stages of crop development [39]. The parental genomes of an intergeneric hybrid between Saccharum officinarum and Erianthus arundinaceus, a similar wild species, were also identified using GISH. The investigation verified that a n+n chromosomal transmission produced the F1 hybrids, whereas 2n+n transmission produced the BC1 cross.

The plant tissues that are most frequently utilized in cytogenetic techniques to prepare mitotic chromosomes are root-tip meristems because they contain cells that are actively dividing. Utilizing roottip meristems, most mitotic chromosomal preparations are produced. 20-liter pots filled with a 50/50 vermiculate (coarse grade) and perlite (grade 3) combination are used to grow plants in a glass house. Water and fertilizers are applied on a regular and sufficient basis. Once the roots have been collected, they can be preserved at 4°C in a 70% ethanol solution after a pretreatment that aims to stop as many metaphase cells as feasible [33].

Genomic DNA must be either directly fluorochrome-labeled or have a hapten that can indirectly associate with fluorochromes for GISH to function.Complementarity pairing between the nucleotides of the target DNA and the nucleic acid of the fluoroprobe(s) assay can be done. With the aid of a fluorescent microscope, fluorochromes enable the visualization of in situ homologous regions to the probe within the cellular structure. By connecting a digital camera to a microscope, it is possible to take long-term pictures of the fluorescent patterns on chromosomes. Figure 2.5.1.1 shows the mechanism of GISH analysis. GISH characterization for interspecific hybrids to resolve the taxonomic reclassification and intergeneric hybrids Saccharum and Erianthus were performed. The Alexa Fluor 594-5- dUTP or Rhodamine 5-d-UTP was used to label the genomic DNA of S. officinarum as "red," and the Alexa Fluor 488-5-dUTP or Fluorescein 12-d-UTP was used to label the genomic DNA of S.spontaneum as "green". Given the relative closeness of both species, the chromosomes of S.officinarum appear "orange," whilst those of S.spontaneum seem "yellow-green." There exists little cross hybridisation in the genome, the fluorochrome colours do not overlap in the F1 and BC1 of the intergeneric hybrids are not closely related than the interspecific hybrids is seen in figure 2.5.1.2 [32]

Fig 1 Mechanism of GISH



Source: Ramzan et al., 2017

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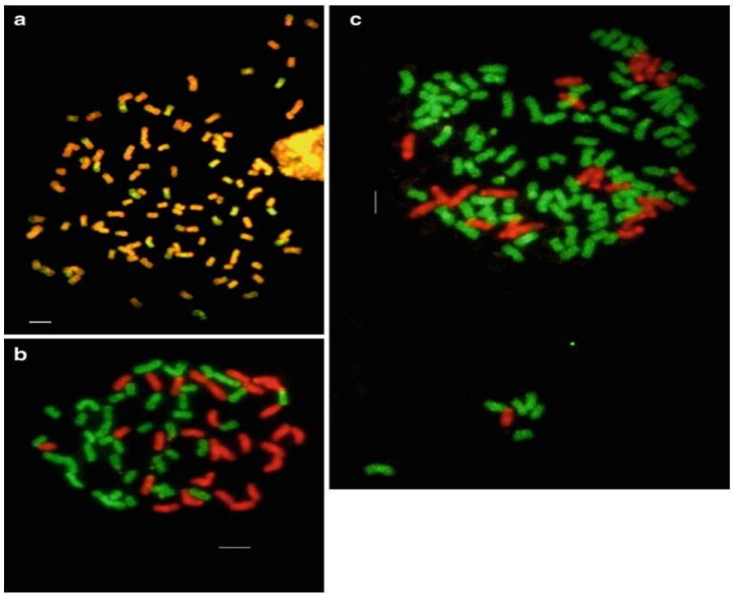
Fig 2 Characterization for interspecific hybrids to resolve the taxonomic reclassification and intergeneric hybrids Saccharum and Erianthus.



Source: Piperidis et al., 2021 GISH analysis of whole genomic DNA from S.spontaneum (green) and S.officinarum (orange) revealed the interspecific chromosome makeup of an unusual S.officinarum; recombined chromosomes appeared in both colours. GISH analysis of total genomic DNA from S.officinarum (green) and E.arundinaceus (red) revealed the intergeneric chromosomal makeup of an F1 (b) and a BC1 (c).

5.2 Restriction fragment length polymorphism

The polymorphism of restriction fragments is a tool to identify close linkages between marker and the gene of interest. The technique serves as a tool to address the drawbacks of backcross breeding as the RFLP markers bind the genes to be transmitted tightly, allowing for plant segregation at the seedling stage before the phenotype expressed [23]. Studies on genetic variability indicates that nearly all commercial clones are backcross descendants of S.officinarum and S.spontaneum. For profitably using the advantageous features, a thorough characterization and assessment of the genetic resources at hand is required. RFLP analysis to study the genetic diversity was done with maize probes to distinguish clones of different species Saccharum and genera Erianthus, Miscanthus. The three fundamental species Spontaneum, Officinarum, and Robustum are all obviously different from one another. S.officinarum has the lowest genetic variability, S.spontaneum has the most, and S.robustum has an intermediate level. Together, the nuclear DNA profiles of S.officinarum and S.spontaneum identify the secondary species S.barberi and S.sinense, confirming their hybrid origins between these two species [23].

TRAIT EXPRESSED IN VARIOUS CARBOHYDRATE METABOLISM STUDIES

The following results listed in table 2 were obtained with the RFLP tests utilized to map QTLs for fibre content, cane yield, Pol, and

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tonnes of sugar per hectare (TSH) using sugarcane genomic probes and expressed sequence tags (ESTs) that resembled genes primarily engaged in carbohydrate metabolism.[31] Pol-Polarimeter readings, TSH-Top sugar High

Table 2 Number of marker trait associations

	PLANT CANE	RATOON CANE	PLANT & RATOON CANE
Fiber yield	15	5	7
Cane yield	4	8	7
Pol	11	8	8
TSH	8	12	5

5.3 Polymerase chain reaction

PCR markers have shown to be effective and easy-to-use diagnostic instruments for tracking genuine hybrids. The 5s rRNA spacer was amplified via polymerase chain reaction. Cycling conditions were: 94ºC for 3 min; followed by 35 cycles of 1 min at 94ºC, 2 min at 55ºC, 1 min at 72ºC and a final extension step at 72ºC for 5 min [7]. Of the seven E.arundinaceus samples that were analysed, six revealed a main band of roughly 370 bp, and one showed a fragment that was marginally smaller at 360 bp. The DNA of the two parents and all eight clones that resulted from the intergeneric cross were amplified using the identical primers. Three clones are recognised as intergeneric hybrids since they have both parental bands. The five further clones of the progeny had the S.officinarum band exclusively, indicating that they were likely the product of S.officinarum selfing. As a result, three of the eight clones that were examined were found to be intergeneric hybrids, while the remaining five were most likely S.officinarum selfs [10].

Screening two putative intergeneric backcross populations of Saccharum and Erianthus using 500bp 5S rDNA primers, the parents, grandparents, and prospective backcross progeny showed distinct bands, indicating that the clones were the hybrids of the F1 generation of the two parents. However, the Saccharum 5SrDNA band being monomorphic, it is impossible to distinguish if the BC1 progeny are actual BC1s or selfs of the F1 generation [7].

5.4 SSR (Simple Sequence Repeats) markers

The breeding performance of any crop can be increased once the effect of the target traits DNA markers in the reference population has been calculated.Clones with high genetic values can be identified as a seedling using these markers, early in the breeding cycle. The SSR (Simple sequence Repeats are microsatellites made up of repeats of dinucleotides, trinucleotides that are useful to study the genetic diversity and several regulations of transcription factors and mRNA stability.

A foundation on the SSR assessment of sugarcane genetic variabilit was established by evaluating 221 SSR primer pairs created by the International Sugarcane Microsatellite Consortium. Of these, five US sugarcane clones and 67 primer pairs (30%) are highly polymorphic with PIC values ranging from 56 to 80% [30].

The genus Erianthus offers numerous unique, superior qualities that are useful for breeding sugar cane hybrids, including high sugar content, resistance to abiotic stress, and survive at barren environments. Owing to the extremely limited genome data for Erianthus, cross-transferability from Saccharum was confirmed using 20 EST-SSRs that produced obvious bands and polymorphic markers across 48 accessions from 39 Saccharum and 9 Erianthus in this study. The

results demonstrated that Chinese sugarcane cultivars from group IV (25/39) had a lower genetic divergence than cultivars from other countries [42].

5.5 SNP (Single Nucleotide Polymorphism) markers

Single base pair positions (SNPs) in genomic DNA represent the points at which distinct sequence alternatives become less common in a population of normal individuals, while the least common allele has an abundance that is at least 1% higher. It is estimated that One SNP is found for every 70 bps in the maize genome and one for every 20 bps in the wheat genome[5].

The SNP markers capture the majority of genetic variation for the traits day to flowering, gender, pollen viability, fibre, TCH, CCS. Variation in FAT clone performance explains 96% of variation in fibre between the total cane harvested and commercial cane sugar by SNP markers [18]. This valuable data explains the higher heritability of the genomic estimated breeding values that can be achieved.

Studies to evaluate genomic selection of multiple sugarcane populations reveal if alleles are

present in the commercial clones but does the data is not sufficient to interpret the dosage levels.Thus, the author concludes that the methodology to calculate the allelic dosage and incorporation in genomic prediction models is a necessity to develop practical breeding applications in future [2].

6. FIELD STUDY AND STATISTICAL ANALYSIS

The molecular cytogenetic techniques are usually performed for 150 to 200 plants of a single clone are analysed. The agronomical traits of around 1200 to 1500 plants of a single clone are analysed for the statistical study purposes [38]. Table 2.6.1 consists of the analysis of agronomical traits expressed by the different clones of sugarcanes.

Table 3: Analysis of agronomical traits expressed by the different clones of sugarcanes [38].

Clone	No of Millable stocks	Stalk height (cm)	Stalk diameter (cm)	Sucrose %	Purity %	Red rating
Parent	70.45	190.10	2.45	16.28	85.24	S
F1	151.25	235.33	1.26	7.88	62.99	R
BC1	121.14	230.50	2.10	14.18	80.75	R
BC2	127.60	245.60	2.34	14.01	81.64	MR

From the table 3 it is inferred that the various improvements in the agronomical characters between the parental and successive generations. The millability, height and diameter of the stalk of the F1 generation and the backcrosses are better than the parental clones. The BC2 generation has the best value for millability, stalk height and stalk diameter among the all generations studied.

The sucrose content was found lesser in hybrids than the parental clones. The margin of difference in the sucrose content between the parental clone and F1 generation is way higher than the back crosses. BC1 clones seems to have better sucrose content than BC2 clones by a small margin.

The purity of the sugarcane is higher in the back crosses than F1 generation, when compared with the parental clone. The red rot resistance can be observed in the F1 and BC1 clones which are highly resistant (R). The BC2 clone is moderately resistant (MR) to red rot disease and the parental clones are susceptible (S) to red rot disease.

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From the observed agronomical traits, it is inferred that the BC1 clone shows better phenotypic traits than other clones since it has a desirable level of purity, sucrose content as well as red rot resistance. The BC1 clones would be better choice for the sugarcane breeders to get a profitable yield without the impact of the red rot disease, which is a major concern for sugarcane cultivation in the Indian subcontinent. [38].

Table- 4: GISH Analysis Results Of Different Clones Of Sugarcanes

Clone	Chromosome	Chromosome from E.procerus	Chromosome from commercial clone	% of foreign chromosome expression
S.officinarum	40	0	40	0
E.procerus	104	104	0	100
F1	80	40	40	50
BC1	94	20	74	21
BC2	103	10	93	10

The GISH analysis listed in table 4 of the sugarcane clones reveal that there is a 50% expression of the foreign chromosome in the F1 generation but the agronomical traits of the F1 clone is not in the desirable range as expected by the sugarcane breeders. The expression of the Erianthus chromosome in the backcrosses is 21% and 10% in BC1 and BC2 clones respectively. The expression of better agronomical traits can be seen in BC clones than the F1 clones[33]. Among BC1 and BC2 clones, BC1 clone is best suited for sugarcane breeders since its yield is better when compared with BC2 clone in terms of agronomical characters expressed.

The range, mean, phenotypic and genotypic variance could be used to analyse the variability among sugarcane hybrids [40]. The Burton and Vane method can be used to calculate genotypic (σ^2_g) variance and phenotypic (σ^2_p) variance [15]. The equations are,

$$\sigma^2_g = (\sigma^2_t - \sigma^2_e)/r; \qquad \sigma^2_p = \sigma^2_g + \sigma^2_e;$$

where, σ^2_g is genotypic variance

σ^2_t is mean square of the particular trait

σ^2_e is mean square of error (environmental variance)

r is the number of replications

σ^2_p is the phenotypic variance [40].

The formula derived by Singh et al., can be used to calculate the phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV)

$$GCV = (\sigma^2_g)^{0.5}/x$$

$$PCV = (\sigma^2_p)^{0.5}/x$$

Where x stands for grand mean of the trait.

Allard et Al., derived a formula for heritability (H^2),

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$H^2 = (\sigma^2_p / \sigma^2_g)*100$

The ANOVA tool is used to obtain the statistical values described by Gomez et al., [15].

$P_{ij} = \mu + G_i + B_j + eij$

Where P_{ij} stands for Phenotypic observation,

G_i stands for genotype

B_j, eij are empirical constants [4].

The BC1 clones of the sugarcane hybrids are best suited for sugarcane breeders in terms of agronomical traits as well as genotypic expressions [38]. Further hybridisation of commercial clones with Erianthus would give us better choices of clones which need to be intervened in the near future according to the needs of the sugarcane breeders [33].

CONCLUSION

As a tropical and subtropical vegetative-propagated crop, sugarcane (Saccharum spp.) covers 80% of the world's sugar production. S.officinarum and S.spontaneum are the two species that were artificially crossed to create the huge genomes of modern sugarcane cultivars, which are highly polyploid and aneuploid hybrids. Due to the low fertility and complicated genetic makeup of sugarcane under natural growth conditions the conventional breeding techniques are time-consuming, costly and labour-intensive. Sugarcane with its highly polymorphic genome imposes the need for alternate approaches that can help breeders to cultivate sustainable crops. Several studies indicate the possible genomic causes of the difficulties in breeding of fertile hybrids. However, the development of several molecular techniques and genomic studies aids the breeders to produce crops with enhanced resistance towards herbicides, diseases and tolerance to stressful abiotic conditions. Every methodology designed acts as a powerful tool to develop the intended hybrid varieties with the results they provide. With a high sucrose content, S.officinarum, and S.spontaneum, a wild species resistant to drought and red rot disease, are interspecific hybrids that produce most sugarcane cultivars.

Through the field study of the backcross hybrids the suitable clones with the desired agronomical traits and genotypic expressions are generated. It is crucial to emphasize that to ensure the effectiveness of this strategy in complex polyploid genomes, a reliable genetic transformation technique that permits the GE system to be introduced into the various genetic backgrounds of elite sugarcane varieties acquired through traditional breeding programs is required. In terms of genotypic expressions and agronomical features, sugarcane breeders would benefit most from using the BC1 clones of the hybrids. We would have better clones of sugarcane cultivars based on the demands of sugarcane breeders if commercial clones were further hybridized with Erianthus species.

STATEMENTS AND DECLARATIONS

Ethics approval and Consent to participate (include appropriate approvals or waivers):

Not applicable. Ethical approval and consent to participate was not required for this review article as it does not involve primary research on human or animal subjects.

Consent for publication (include appropriate statements):

Permissions were obtained from the copyright holders for the use of figures,data, tables from previously published works. Details of these

permissions are provided in the references section.

Availability of data and materials (data transparency):

The data that support the findings of this review are available in the referenced. All sources cited in this review are publicly available through their respective journals or databases.

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Author contributions:

Dr. D. Angeline Kiruba:

Conceived and designed the entire study.

S. Sangeetha:

Reviewed and prepared the manuscript data for advantages and limitations of sugarcane hybridization, analytical procedures.

S. Sanjay:

Reviewed and prepared the manuscript data for sugarcane hybridization, field study and statistical analysis.

M. Yogeshwaran:

Reviewed and prepared the manuscript data for intergeneric and intrageneric hybridization.

All authors read and approved the final manuscript.

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