Research Article

Assessment of Soma-Clonal Variation CP67-1026 Mutant of Sugarcane through Morphological and Molecular Procedures

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ABSTRACT

Present studies were conducted on to assess the soma clonal variation in CP67-1026 mutant of sugarcane through methodological and molecular procedures. It was concluded that the Soma-clonal variation does occur in the procedure of reproduction of soma-clonal variants under invitro circumstances. Significant alterations among the sugarcane soma-clones for various parameters were studied. Out of which soma-clone-3 showed significantly best performance among all other soma-clones of NIA-2010 genotype of sugarcane. As this soma-clone gives desirable features like high cane yield and maximum sucrose content that could be used directly as commercial variety as other commercial varieties used at industrial level. This soma-clone will be designated with such combinations of features which to give maximum development in their sugar yield No. 3 was diverse soma-clone among all soma-clones. It was due to hereditary distinction among all the characters of soma-clones. These desired somatic clones might be used as parents in hybridization programs with other disease-resistant varieties to confer primary disease resistance, ultimately producing agronomically superior varieties in sugarcane crops.

Keywords: Vegetables, Fruits, Multan, Pesticide residue, Gas Chromatography**.**

INTRODUCTION

Sugarcane crop is a tropical, giant-growing, light-heat sensitive, monocotyledonous perennial herb belonging to the genus Saccharum (Obeid, 2010; P., 2012). This crop is highly polyploid and allopolyploid, with a highly heterozygous complex genome, and the number of chromosomes in diploid cells in the cultivar is 80-12 (Perera et al., 2023). Sugarcane improvement in various countries relies on various technologies such as conventional breeding, mutation breeding, somatic clonal variation and genetic engineering (Shahid, 2012). Further, used to assess genetic diversity, this is an important tool for enhancing sugarcane genotypes since parents with different inherent materials can be crossed by breeders to obtain viable and superior offspring. Genetic divergence is studied through one or more multivariate techniques, such as principal component analysis and cluster analysis that have been used over the past four decades for a variety of crops and different breeding objectives. These techniques help quantify the amount of variation in genetic material and designate crop populations with similar or dissimilar genetic makeup (Singh et al., 2017). Though, although conventional breeding has promoted the agronomic improvement of developed varieties, it also has limitations such as narrow gene

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pool, poor fertility, and long breeding cycle, making further development difficult. In addition, recent varieties have variable chromosome numbers and rarely flower. Sustaining sugarcane yields, increasing productivity, tolerance to biotic and abiotic stresses, managing nutrients and improving sugar recovery are some of the factors for crop improvement programs (Esechie, 1994; Shahid et al., 2011). For obtaining viable varieties in sugarcane the soma clonal variation performs better to overcome many difficulties in cane breeding technology (Sally et al., 2021). Additionally, regenerated soma-clones from this technique show variation and has viable superior qualities as well as quantities as comparison to other cultivars that obtained through conventional methods. Assessment of genetic variability in tissue cultured plants will assist plant breeders in selecting appropriate materials for their breeding programs (Rea et al., 2017). The DNA molecular marker has high stability, large quantity, and high polymorphism, and is more suitable for evaluating the collection of sugarcane germplasm resources. (RAPD) Randomly amplified polymorphic DNA markers offer several advantages over other polymorphism detection technologies, including RFLP and other markers. These include cheap, fast, relatively easy assays, requiring small amounts of template DNA to produce genome-specific fingerprints of multiple amplification products, not requiring DNA sequence information, and the advantages of fluorescence. Because of these advantages, RAPD is often used to characterize variability (R. B. Doule, 2008; R.N. Pandey, 2012). In sugarcane, RAPD is used to assess levels and patterns of variation among cultivars, species, and members of the sugarcane complex and to identify putative markers associated with phenotypic traits (Sharma, 2008). In contrast, sugarcane contains large amounts of polysaccharides, polyphenols, RNA, and proteins, which are sources of contamination in obtaining pure DNA that can be used in subsequent enzymatic reactions (R. B. Doule, 2008). Specific protocols for the isolation of genomic DNA from dry sugarcane leaf tissue without the use of liquid nitrogen (N2) were recently developed. This DNA has been successfully used as a RAPD marker for genetic diversity studies. DNA is commonly extracted from plants, including sugarcane, using commercial kits (Sally et al., 2021) . This study aimed to report the induction of somatic clonal variants in the sugarcane cultivar CP67-1026 and to further test these variants using the RAPD molecular marker.

MATERIALS AND METHODS

Experimental Design

Present studies were carried out in the field as well as under laboratory for molecular based analysis of morphological and molecular characteristics of the sugarcane soma-clones. The experiments were being done at the experimental farm of Nuclear Institute of Agriculture (NIA) Tandojam. Where the double budded seeds of sugarcane clone NIA-2010 were implanted at the trial Farm of NIA. The experiment was being conducted through Randomized Completely Block Design proposed with three different replications. The field size was 25 x 5m2 row to row distance 1.5 meter. The sowing in month of October in the year (2020) and ordinary agronomical and plant protection observes (weeds management and pest control, fertilizer utilization, (earthling, and irrigation) were subsequent during the growing period. Different stools were arbitrarily obtained from separately plot to regulate sugar substances conferring to sugarcane.

Molecular Analysis

The molecular analysis of samples was taken out at maturity period from the experimental farm (NIA), for this 14 randomly selected soma-clones of the genotype NIA-2010 were tagged for the analysis of variations under laboratory observations through DNA extraction as well as PCR amplification by using (RAPD) molecular markers.

Qualitative and Quantitative Parameters

The morphological (external) characters were studied and analyzed after 12 months through qualitatively and qualitatively parameters related to sugarcane plants in the field.

Stalk Height

The total height of stalk was calculated in centi-meters from the exterior of soil to the topmost noticeable crosswise mark of the cane at 12 months of the growing period of cane of sugar.

Measurement of Cane Grith

The grith of cane of each plant was measured by using specific scale the vernier-callipers from which the diameter of stalks was measured in centi-meters at the central of internode part of individually stalk's top, mid and bottom to get the average stalk grith at 12 months.

Number of Tillers

Number of stalks per stool were calculated in separately plot of entirely replications at 120 days afterward establishing. stalks population in separately plot was attained by enchanting the cumulative overall for completely the 10 rows.

Internode Length

The length of internode was distinguished by calculating length in (cm) amongst 2 nodes from 3 parts viz lowest, central, and topmost part of the cane and be an average to acquire the mean length of internode at one year.

Germination Percentage

This approximates the capability of a population of seeds. The formula for germination percentage is:

Germination% = Germinated seeds / Total seeds x 100

Cane Weight

The remaining plot was collected discretely. The stalks were detached and then mass of millable canes for individually trial treatment were noted in kilogram and changed into tons per hectare.

Brix Percentage

The percentage of brix of the sugarcane juice were measured by using the standard instrument brix hydrometer in the measuring cylinder.

Sucrose Percentage

The percentage of pure sucrose was determined by using polarimeter machine, after purification of sugarcane juice by using 2 g of lead acetate.

Extraction of raw juice

The volume of juice was measured through 2 L volumetric cylinder. Observations were taken in triplicate and averaged subsequently.

Purity Percentage

The percentage of purity of the sugarcane juice were calculated according to the formula: sucrose %, purity % = Brix % x 100

Sugar Recovery

The recovery percentage was recorded and being as reported previously: Sugar recovery% X purity %
Factor = 100 (Fiber % + physical impurities % + water free sugar %).

Sugar Yield

This is the total amount of sugar present in the cane of sugarcane plant. It is measured through the formula: Sugar yield = CCS % x cane yield / 100

RESULTS

Plant Height (cm)

The results of fourteen soma-clones and NIA-2010 genotype showed that the plant height varies among the variety of NIA-2010 and their soma-clones that was measured at the maturating period of sugarcane plants. Out of which the best performance observed in soma-clone SC-3 was about 302.33cm, followed by SC-12 had 296cm as comparison to NIA-2010 that had 233cm of plant height were recorded. The results of soma-clones and their plant height characteristic indicated that the lowest performance showed in SC-2 that was approximately 154.33cm followed by SC-5 had 166.33cm in height observed. However, remains other soma-clones of sugarcane possessed optimal responses.

Number of Internodes

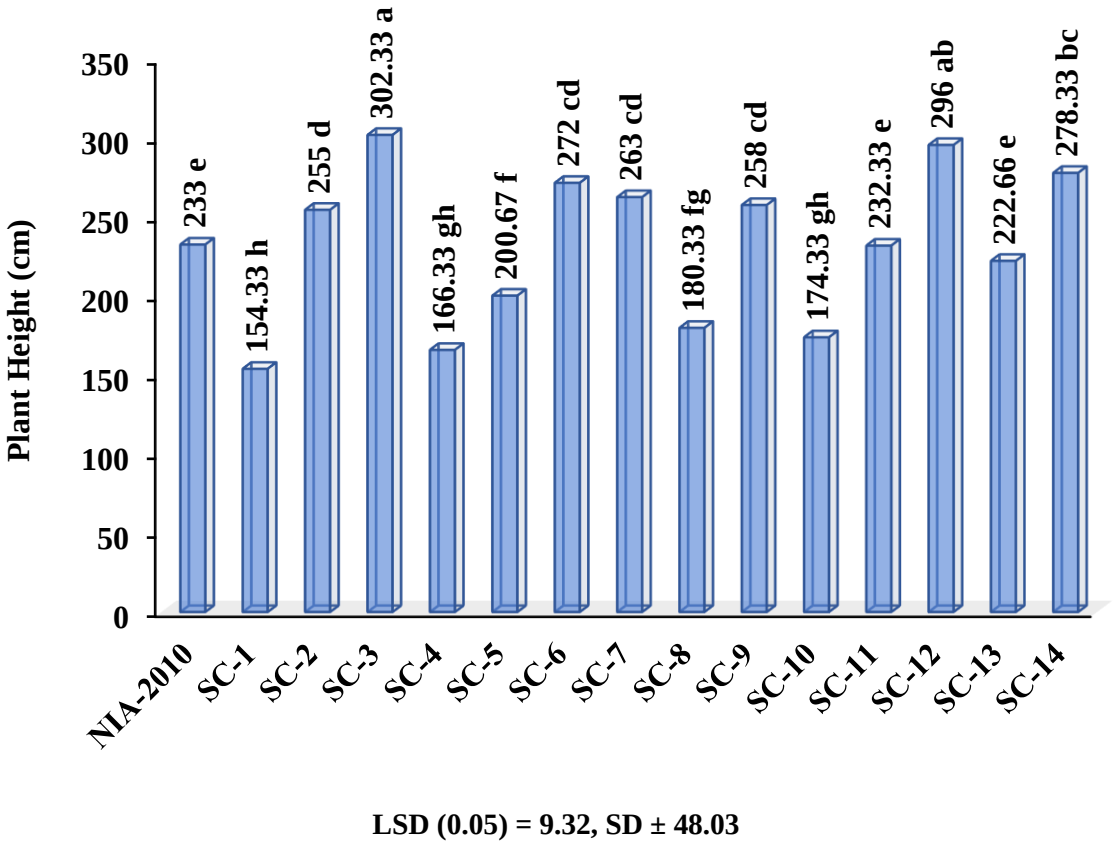
The results of 14 soma-clones and NIA-2010 variety showed that the number of internodes varies among the variety of NIA-2010 as well as their soma-clones that measured at the maturating period of sugarcane plants. Out of which the best performance observed in SC-3 was about 38 followed by soma-clones SC-6, SC-2, SC-11, SC-9, SC-14 that was 34.33, 32.33, 30, 29.33, 29, respectively. Whilst controlled variety NIA-2010 and SC-13 had approximately equal value i.e., 28.67. On the other side the lowest number of internodes were observed in SC-2 followed by SC-8, SC-4, SC-13, SC-5 SC-7 and SC-10 (25.33, 25.67, 26, 26.33, 27, 27.67 respectively).

Length of Internodes (cm)

The results of NIA-2010 variety and their 14 soma-clones presented that the length of internodes that was measured at the harvesting time of sugarcane plants. Based on the comparison of length of internodes parameter in soma-clones and their controlled variety the highest length of internodes was observed in SC-3 that possessed 17.83cm followed by SC-3 had 14.36cm. The results of NIA-2010 variety and their soma-clones indicated that the lowest length of internodes was observed in SC-8 and SC-10 which was near to 10.66cm in measurement followed by SC-11 had 11.33cm of length of internodes. Besides these soma-clones the other remains soma-clones showed optimal responses for the length of internodes in cane of sugarcane.

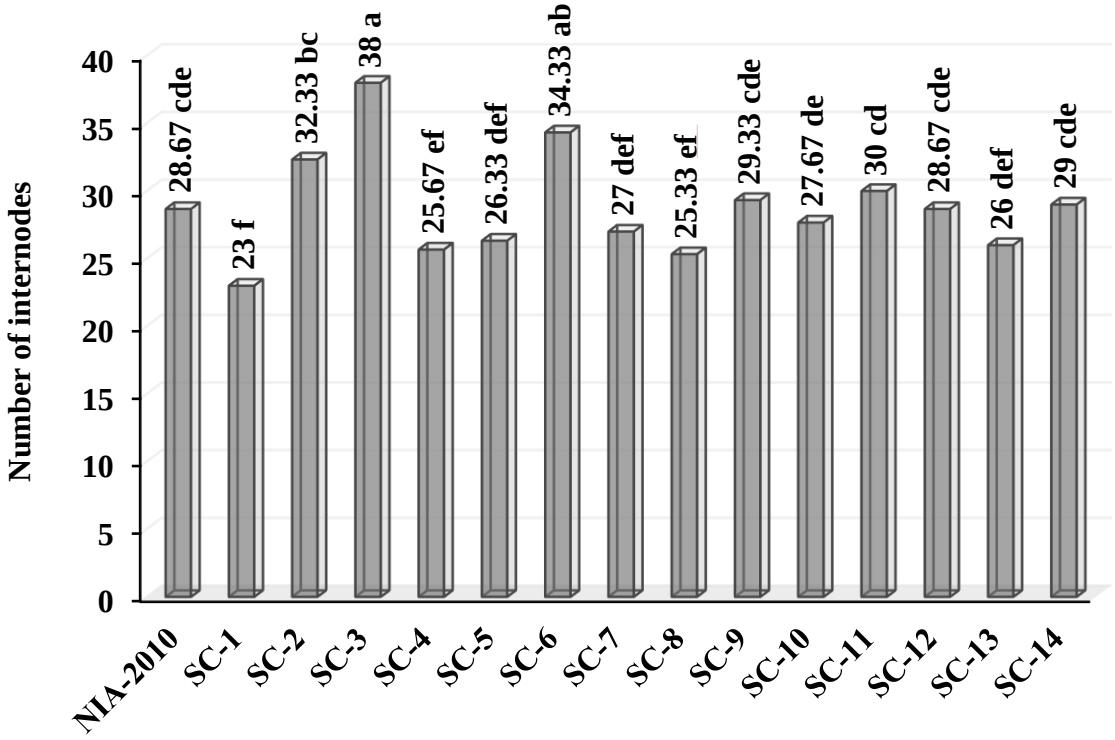
Grith (cm)

The analysis of variance proposed that the cane girth of sugarcane diameter was significant ($P<0.05$). It is apparent from the results that the greatest grith of cane was appeared in SC-2 that was about to 2.21cm, followed by NIA-2010 and SC-10 had equal values nearly 2.16cm. While the lowest cane of grith was analysed in SC-13 which was almost to 1.81cm followed by SC-12 (1.87) cm in grith of sugarcane soma-clones. The analysis of variance indicated that the remains soma-clones had moderate responses observed in sugarcane soma-clones.



LSD (0.05) = 9.32, SD ± 48.03

Figure 1. Determining the height of plant (cm) of different soma-clones of sugarcane.



LSD (0.05) = 1.89, SD ± 4.25

Figure 2. Examining the number of internodes per cane of different soma-clones of sugarcane.

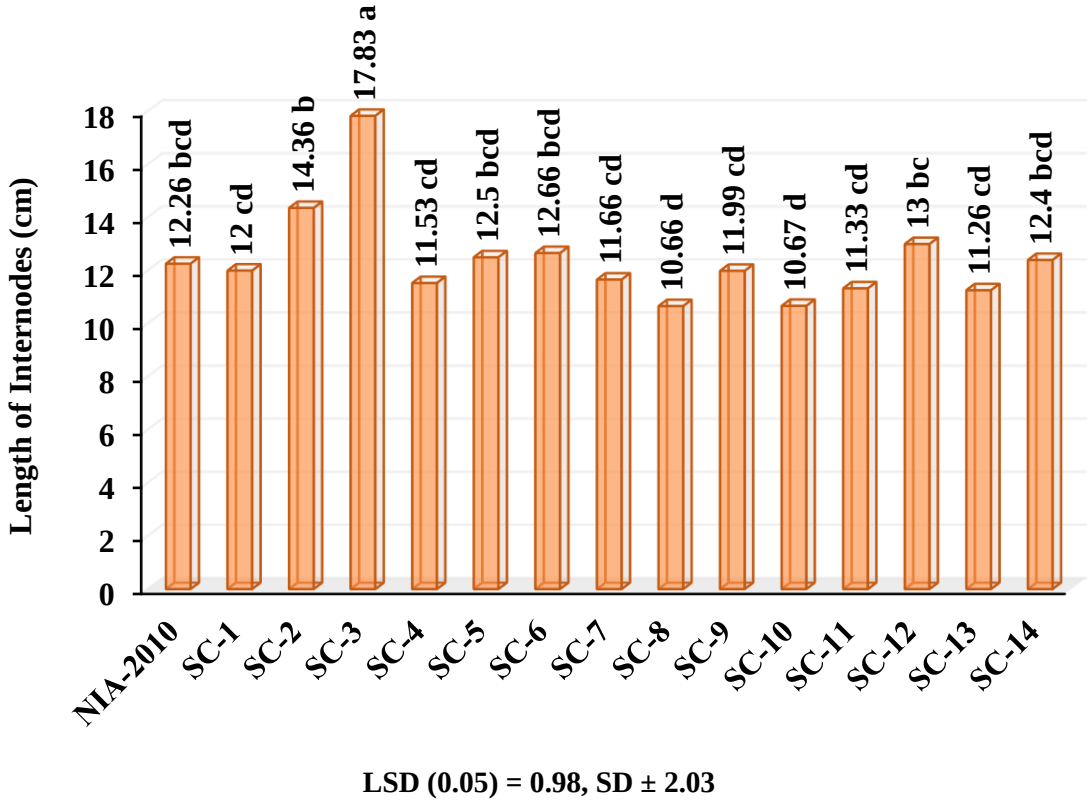


Figure 3. Evaluating the length of internodes per cane of different soma-clones of sugarcane.

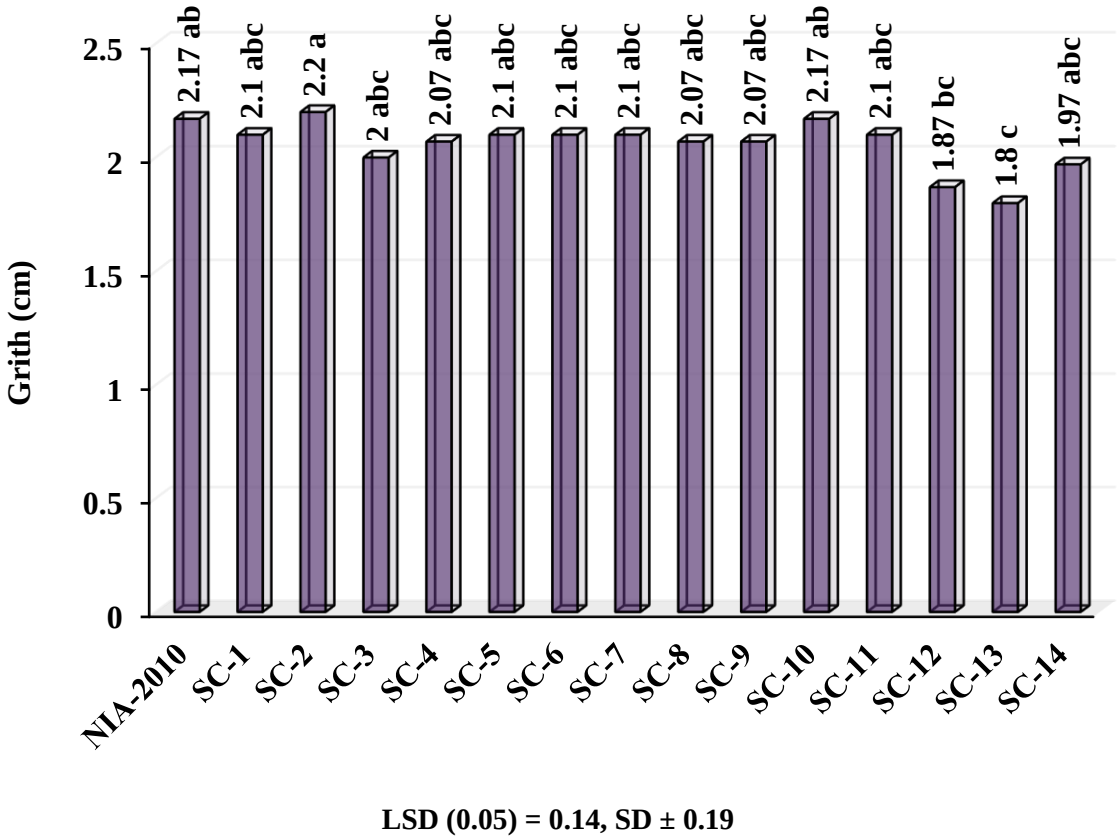


Figure 4. Determining the grith (cm) per stalk of different soma-clones of sugarcane.

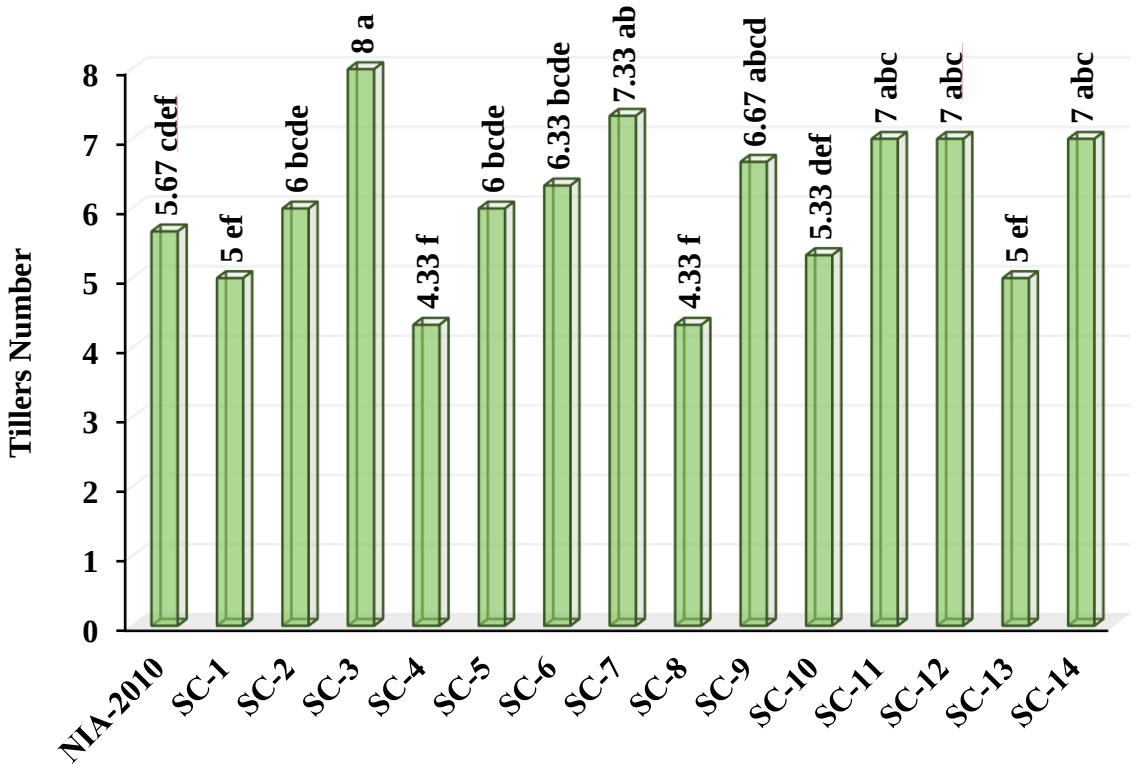
Number of Tillers

The statistical scrutiny of variations proved that the clones and their physiological parameters were extremely noteworthy, although their interaction were non-significant at 5% possibility level. The results compared the 14 soma-clones presented that the

number of tillers differs with the variety of NIA-2010. The tiller numbers were measured at the growing time of sugarcane plants. the analysed data indicated that the maximum number of tillers appeared in SC-3 which was around to 8.02, followed by SC-7 had 7.33 in numbers. On the other side the minimum number of tillers observed in SC-4 and SC-8 was approximately 4.33 followed by SC-14 had 5.01 in numbers. Nevertheless, the genotypes of the other residual soma-clones had middle values among these extreme values for the number of tillers in cane of sugarcane plants.

Cane Weight (kg)

The analysis of variance proposed that the effect of different cane weight in soma-clones of NIA-2010 were highly significant (P<0.05). The analysed data showed that the highest cane weight was observed in SC-4 that was about to 9.66kg in weight followed by SC-13 that 9.01kg in weight of cane. However, the lowest cane weight of sugarcane plant is SC-2 that was about to 5.83kg in weight of cane followed by SC-5 had 6.33kg in weight of cane. though, the remaining soma-clones of sugarcane had mid values among these highest as well as lowest values for the cane weight of sugarcane plants.

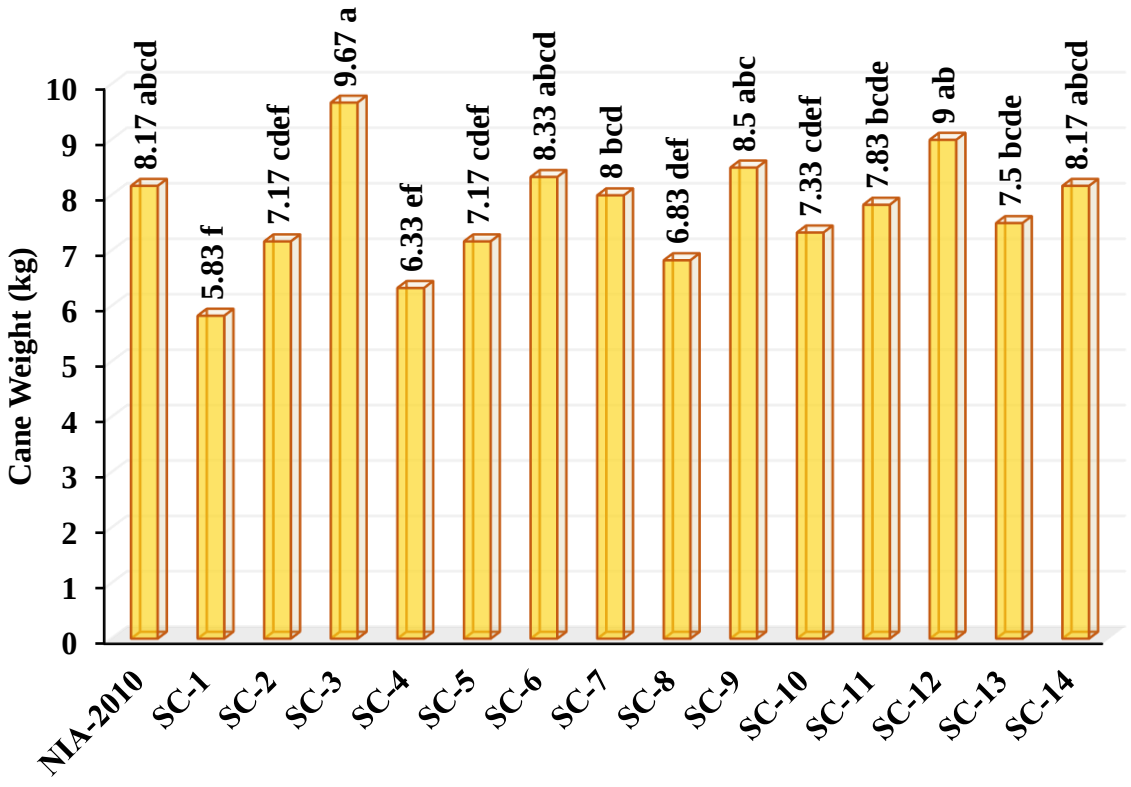


LSD (0.05) = 0.61, SD ± 1.26

Figure 5. Determining the number of tillers per plant of different soma-clones of sugarcane.

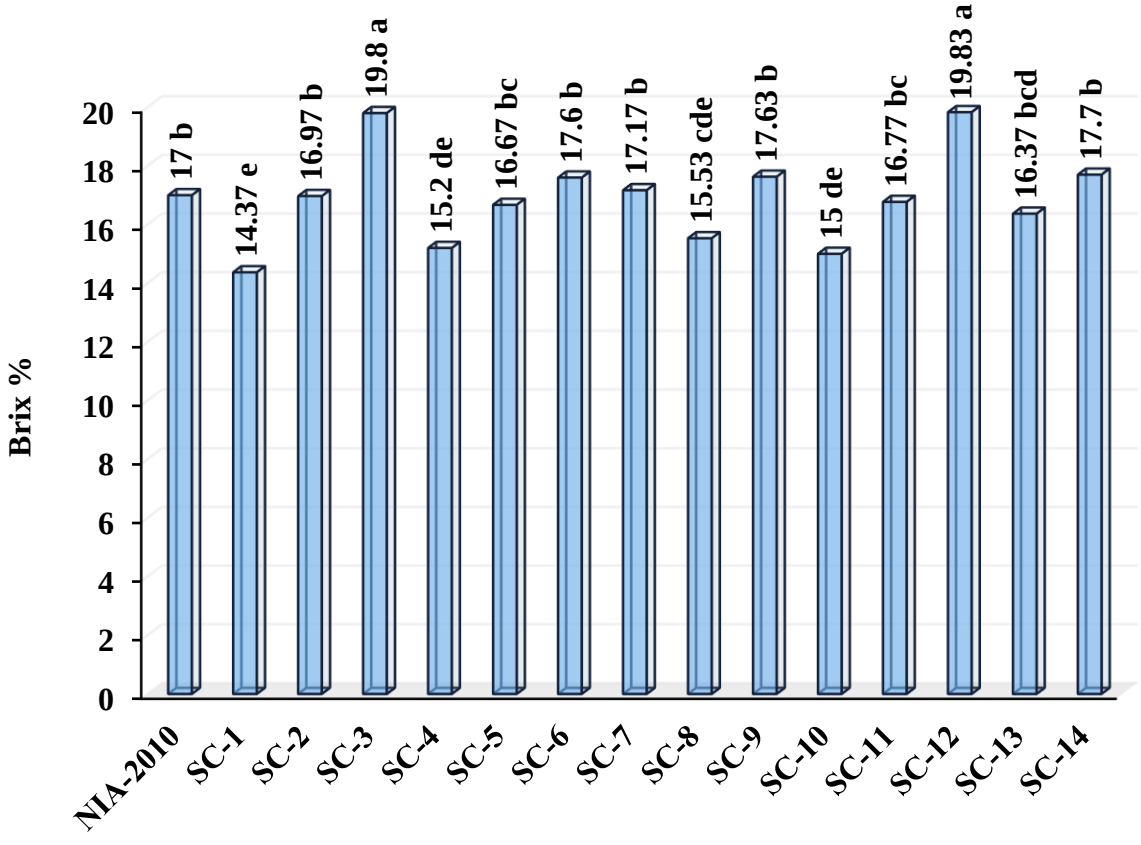
Brix Percentage

Most of the sugar relating characters of plants are guided by the brix % and this trait has prime importance in agronomic research of sugarcane plants. The results of 15 soma-clones presented that the brix % varies among the variety of NIA-2010 soma-clones that was measured at the post harvesting time of sugarcane plants. from these soma-clones the highest percentage observed in SC-13 that was about to 19.83% followed by SC-4 that had 19.81%. the analysis of variance indicated that the lowest percentage of brix was observed in SC-2 that was about to 14.37% followed by SC-11 had 15.01% of brix of soma-clones. Regardless, the middle values are to the remaining soma-clones of sugarcane plants.



LSD (0.05) = 0.73, SD ± 1.25

Figure 6. Determining the weight of stools per plant of different soma-clones of sugarcane.



LSD (0.05) = 0.63, SD ± 1.66

Figure 7. Determining the brix percentage of different soma-clones of sugarcane.

Sucrose (%)

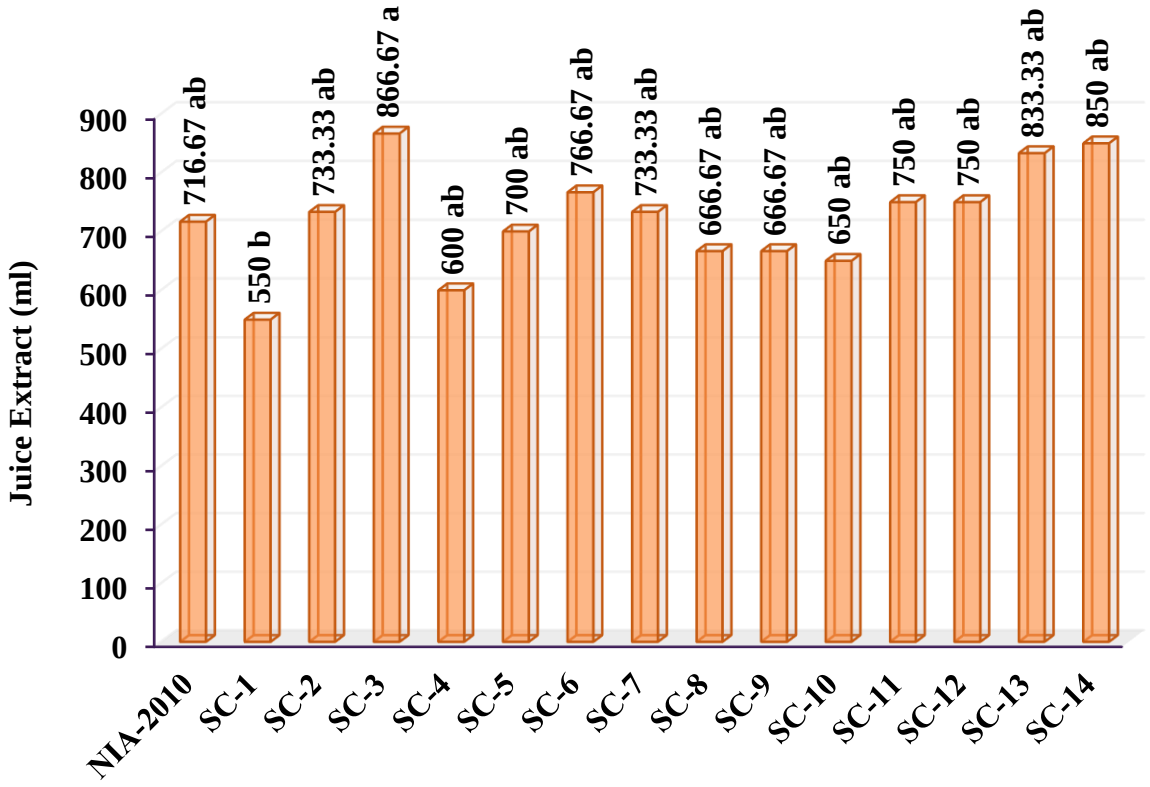
The statistical analysis of variance specified that varieties, structural parameters were vastly significant. The results of fourteen soma-clones along with NIA-2010 genotype showed that the sucrose% varies among the was measured at the post harvesting period of sugarcane plants. out of which the maximum percentage observed in SC-3 that was almost to 13.4% followed by SC-14 had 13.387% of sucrose. Nonetheless, the analysis of variance indicated that minimum percent of sucrose observed in SC-4 that was close to 11.3% followed by SC-7 had 11.62% of sucrose in soma-clones of sugarcane. Beside these percentages of sucrose, the remaining soma-clones of NIA-2010 had intermediate values of sucrose.

Juice Extract (ml)

Juice extraction is a physiological parameter and physiological stages can be influenced by various internal as well as external factors of sugarcane plants. The analysis of variance indicated that the effect of different volume of juice extraction in soma-clones of NIA-2010 were highly significant (P<0.05). the analysed data indicated that the highest volume observed in SC-4 that was about to 866.6ml followed by SC-14 had 833.3ml. However, results further showed that the the lowest volume of juice extraction observed in SC-2 that was about to 550.1ml followed by SC-5 that was almost to 600.1 ml in volume of juice extraction of sugarcane soma-clones. Furthermore, the other soma-clones had mid values among that highest as well as lowest values of sugarcane soma-clones.

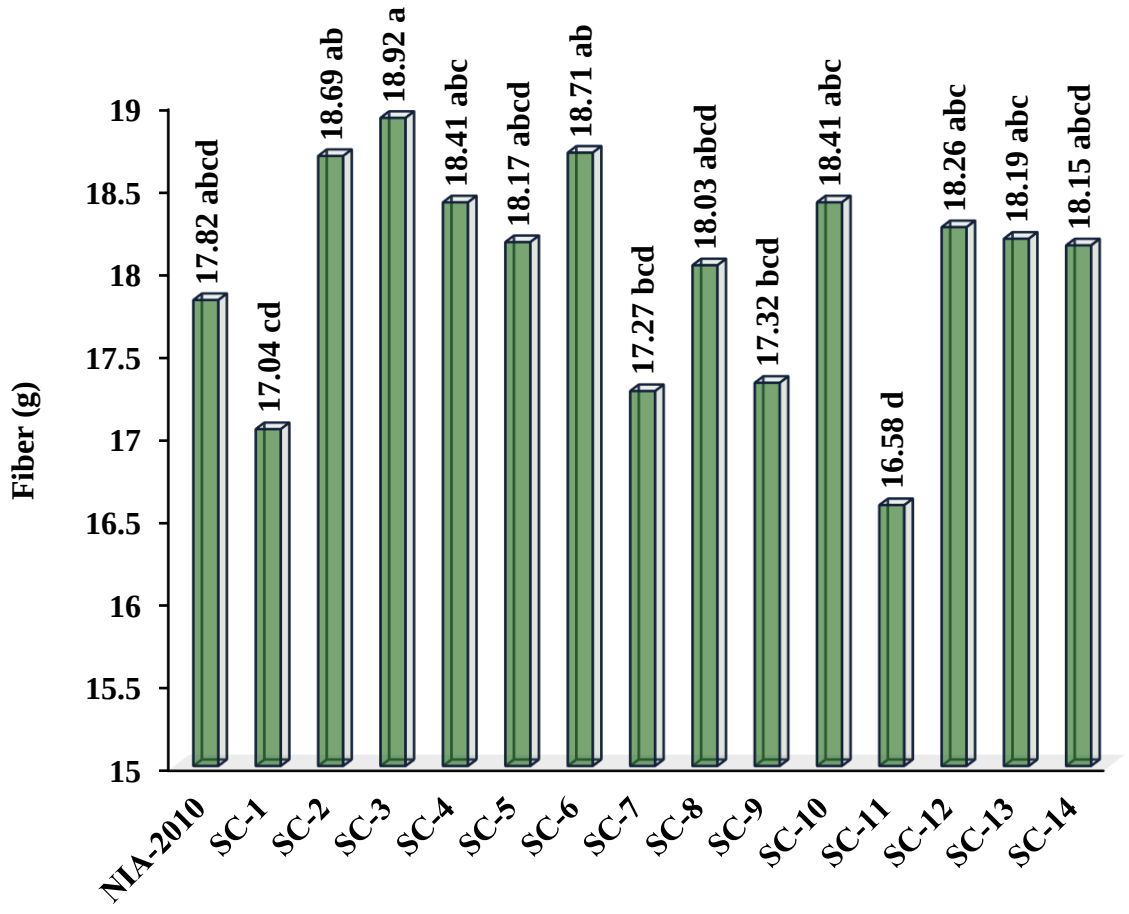
Fiber (g)

The analysis of variance proposed that the fiber of soma-clones was significant (P<0.05). It is apparent from the results in Table-9.1 that 18.92g observed maximum value of SC-3, closely followed by SC-6 was 18.71g recorded, respectively. There were adverse effects of fiber analysis that the SC-11 was about to 16.58g recorded followed by SC-1 had 17.04g. However, the remaining soma-clones had moderate response with respect to fiber analysis in grams of sugarcane soma-clones.



LSD (0.05) = 138.56, SD ± 171.74

Figure 8. Determining the juice extraction (ml) of different soma-clones of sugarcane.



LSD (0.05) = 0.71, SD ± 1.01

Figure 9. Determining the fibre (gm) of different soma-clones of sugarcane.

Discussions

The goal of present study was established for the assessment of various molecular parameter and their effects on the performance for soma-clonal variation in sugarcane variety NIA-2010 and their soma-clones were utilized in this research work. In vitro plant soma-clonal variation is regularly the most imperative advance for fruitful usage of different biotechnological procedures for product enhancement. To carry on sugarcane crop development database, it is essential to recognise the soma-clones, as well as their parent NIA-2010 which contribute to have invisible capability to be utilized. High yielding soma-clones with fit environmental constancy and phenotypically mandatory traits could be precisely used for normal growing. Additionally, use of enhanced high yielding genotypes to enable creation efforts is approximately prerequisite for sugarcane crop. It is important to represent soma-clones because its obtaining data will support in the collection of superior genotypes (Khan*, 2019). Hereditary composition and distinction in their basis propose that alterations in genotype (Mazza, 2000).

Muhammad et al., (2001) (Muhammad Mujahid, 2001) assessed that soma-clonal alteration as a significant tool for induced distinction during in vitro cultures. Numerous bases for soma-clonal distinction have been projected, which contained variations in number of chromosomes in sugarcane clones (Obeid, 2010). Nevertheless, hereditary expression extremely reliant on the atmosphere that is genotype and environmental interaction effects which may unstable transversely the environments. This recommends that the experimental constituents possess valuable hereditary resources for cultivar of traits, thus can widely be utilized for succeeding breeding programs (Oliveira et al., 2002).

Information related to hereditary diversity and relationships of advanced sugarcane genotypes is still limited, obstructive the effective use and maintenance of its soma-clones. Compared with the agronomic parameters, the (RAPD) amplification assessment are more powerful approach to inform the characterization

and relationships as well as variability among sugarcane soma-clones and the determination is much sophisticated to identify distinct soma-clones (P., 2012). The (RAPD) amplification information was efficiently used to acquire a similarity matrix and for the generation dendrogram. Resemblance matrix imitates the hereditary connection amongst the sugarcane soma-clones and parent (Nighat seema, 2014) presented that the fourteen soma-clones as well as variety NIA-2010 of sugarcane crop formed six distinct clusters on the dendrogram. Out of which the maximum similarity was achieved between parents and its soma-clones are NIA-2010 and SC-3. While minimum similarity noted between NIA-2010 and SC-10. Shahid et al., (2012) also publicised that the great hereditary resemblance observed among sugarcane varieties by using (RAPD) molecular marker and outcomes of dissimilar sequences or meditations can transposons with additional magnification products.

It is proposed that the (RAPD) marker bands probably characterize mostly repetitive DNA sequences. Polymorphism in repetitive DNA sequences has commonly been detected during plant proliferation by soma-clonal variations (Sharma, 2008) and undergoes more changes than the coding sequences. Maximum 8 bands were enlarged with primer OPN-09 and minimum 1 band was augmented with primer OC-19 for the assessment of hereditary constitutions of NIA-2010 genotype and their soma-clones. Shahid et al., (2012) (Shahid et al., 2011) who established a technique to acquire intact somatic metaphase cells of sugarcane crop. Additionally, these experimental analyses suggested either that few genotypes are more vulnerable to soma-clonal variation, or that the in vitro unpredictability is appropriately significance of a genotypes contrasted with culture medium interaction.

CONCLUSION

It was concluded that the Soma-clonal variation does occur in the procedure of reproduction of soma-clonal variants under invitro conditions. Significant alterations among the sugarcane soma-clones for various parameters were studied. Out of which soma-clone-3 showed the best performance among all other soma-clones of NIA-2010 genotype of sugarcane crop. As this soma-clone gives desirable features like high cane yield and maximum sucrose content that could be used directly as commercial variety as other commercial varieties used at industrial level. This soma-clone will be designated with such combinations of features which to give maximum development in their sugar yield among all soma-clones. It was due to hereditary distinction among all the characters of soma-clones. This soma-clone must be utilized as parent in hybridization programmes, where it crossed with other diseases resistant varieties to impart major diseases resistance ultimately yielding agronomically superior varieties in sugarcane crop. As for the functionality of DNA templates sharp and well-marked bands were obtained by using the (RAPD) molecular marker approach. This soma-clone assessed through (RAPD) approach. For obtaining this alteration for DNA extraction method of soma-clone of sugarcane that allowed the optimizing a protocol for getting high DNA quality and high quantity.

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