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Research Article

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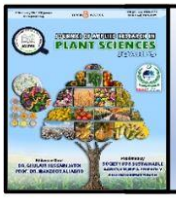

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Evaluation of Soma-clonal Variation in NIA-2010 Variety of Sugarcane (*Saccharum officinrum* L) Through Morphological Techniques

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Abstract

Present studies were conducted on evaluation of soma-clonal variation of NIA-2010 variety (Pedigree number CP67-1026) through morphological procedures with the aims at discussing how soma-clonal variations can be analyzed through morphological approaches in gaining knowledge about the underlined regulatory controlled mechanisms in plants. *In vitro* experiments were conducted in the Laboratory of Nuclear Institute Atomic Energy (NIA), Tandojam during the year of 2021. The results so far achieved opened further window for deep analysis with reference to functional analysis of soma-clonal variated plants with different approaches. Results on the performance for soma-clonal variation in sugarcane variety NIA-2010 and their soma-clones revealed the best performance of varieties, which showed that the plant height (302.33cm) was observed in SC-3, whereas; the consequences of varieties and diverse parameters indicated that the highest number of internodes plant^{-1} were recorded (38) in SC-3 and lowest number of internodes plant^{-1} were accomplished (23) in SC-1. The consequences of soma-clones indicated that maximum weight stool^{-1} (kg) was observed (9.67kg) in SC-3. The output of different physiological features indicated that highest values were remarkably noticed of brix (%) (19.83%) in SC-13, sucrose (%) (13.4%) in SC-3, fiber (%) (18.92g) in SC-3. The results of various agronomic parameters such as germination % observed (82.01%) in SC-6, sugar recovery (%) (8.56%) in SC-8, purity (%) (85.27%) in SC-8, cane yield (tonnes per hectare) (96.67 tonnes per hectare) in SC-3 for the assessment of morphological constitutions of NIA-2010 genotype and their soma-clone

Keywords: CP-671026 mutant, Morphological techniques, Soma-clonal variation, Sugarcane

Introduction

Sugarcane (*Saccharum spp*.) is considered as the most valuable plants, which is cultivated in approximately ninety (90) countries of the globe. It is widely used in each day of Live's individuals and its industrial use proposed for dietary and economic sustenance (SASA 2012). It is creating 80% of the world's raw sugar which is mostly used for biofuel in developed countries (Setta et al. 2014).

In sugarcane plants, the production of cane and percentage of sucrose are relative variables specified that four parameters viz., germination percentage, tillers numbers, plant length and grith diameter (Khan et al. 2012). Percentage of sucrose differs from 8-16% reliant on genotype of cane, its development, and properties of soil, environment, and agricultural practices. Sugarcane gave many by-products such as press mud and bagasse that used as fertilizer which helps to improve physio-chemical, and biological status of soil, which eventually enhance yield and improve the quality of agricultural products (Pandey and Devkota, 2020).

Obstacles of sugarcane low yield: There are severe obstacles of low yield in sugarcane that hampers varietal development are flowering as well as sets of seed under ordinary environments of Pakistan. The fundamental facilities for cultivation of hybrid seeds and plantation of unapproved and low yielding varieties and improvement at the genomic level in varieties of sugarcane are deficient in Pakistan (Tiawari et al. 2009). While various other factors persuades distinctive responses at the metabolic as well as physiological level in sugarcane crop (Sanghera et.al 2019).

Recent advancement and resolving constraints through Biotechnology: The ancient and recent

approaches of biotechnology, significantly provided to resolving the some of these constraints. By obtaining the maximum cane output is only feasible through enhancement in sugarcane by accepting biotechnology as well as latest hybridization techniques. The procedures of biotechnology including the tissues culture and genomic engineering are being applied for the inherited development of sugarcane (Sobhakumari, 2012). Sugarcane plant is the individual of Gramineae family that belongs to genus Saccharum where *invitro* multiplications are utilized and economically viable. Because of which plant rejuvenation by tissue cultures procedures have potential for alternative improving the qualitative and productive approaches in sugarcane crop (Behera and sahoo, 2009).

The utilization of tissue cultures for production of soma-clonal variation that used for enhancing velocity and efficacy of breeding procedure to progress the availability of current germplasms of crop and generate new distinction for the crop enhancement (Wang et al. 2005). Soma-clones may itself have abundant applications in plant breeding and hereditary improvements and recovery of such novel cultivars can be increased by applying suitable in vitro selection procedure (Lestari, 2006). Additionally, Soma-clonal variations perform as a significant approach to defeat the limitations of conventional breeding in the crop. Additionally, *invitro* soma-clonal distinction that enhance the regularity as well as applications of physio-chemical mutagens for callus cultures. Such artificial mutagenesis has the capability to produce beneficial modifications in the genotypes (Patade and Suprasanna 2008). Soma-clones reproduced through this method express distinction for diverse constraints such as yield, maturity, drought tolerance, sugar recovery and disease resistance. Inherited variability in tissue cultures-derived plants is supportive for breeder or scientist to choose the suitable substance for breeding programs (Smiullah et al. 2011). Thus, keep in view the genome complex aneupolyploid and difficult vegetative mode of propagation in selection and identity issues in breeding, the planned study has been hypothesized to observe the analysis of Soma-Clonal variation of CP67-1026 through morphological procedures.

Materials and Methods

Experimental Design: The present study was carried out in the field as well as in the sugarcane molecular based laboratory (Plant genomic and Tissue culture laboratory of Nuclear Institute of Agriculture, Tandojam for the analysis of morphological characteristics of the sugarcane soma-clones. A total of 14 soma-clones were used and analyzed and further proceed to grow in the field. The experiments were being done at the investigational farm of Nuclear Institute of Agriculture (NIA) Tando Jam. Where the double budded seeds of sugarcane clone NIA-2010 were implanted at the trial Farm of NIA. The experiment was laid out through Randomized Complete Block Design with 3 different consequent replications. The field size was approximately 25 x $5m^2$ row to row distance 1.5 meter. The sowing was done in month of October 2020 and ordinary agronomical and plant protection observes (weeds management and pest control, fertilizer utilization, (earthling, and irrigation) were subsequent applied during the growing period. 3 stools were arbitrarily obtained from separately plot to regulate sugar substances conferring to sugarcane Lab Manual of Queensland Sugarcane Mills, while 3 rows from individually plot were collected to note the yield data.

Qualitative and Quantitative Parameter: 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 Girth: The girth 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 girth 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

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

Commercial Cane Sugar (CCS) Percentage: The

CCS percentage defined as the total recoverable sugar percent in sugarcane plant.
The CCS percentage was calculated using the formula as below.
CCS % = +(1.5 x sucrose x (1- (fibre + 5) / 100)) – (0.5 x brix x (1- fibre+ 3) / 100))
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 of the sugar was calculated according to Sugar recovery % x purity %
Factor = 100 (Fiber % + physical impurities % + water free sugar %).
Cane Yield: Cane yield was determined by harvesting plots of each variety. Cane weight was measured for each plot and then converted into cane yield on per hectare basis.
Sugar Yield: 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
Statistical Analysis: The obtained data was noted and exposed to analysis of variance (ANOVA) under linear models of statistics to perceive statistical modifications amongst diverse morphological and molecular parameters of sugarcane plant by utilizing computer programs, Student Edition of Statistics (SWX), Version 8.1 (Analytical Software, 2005).

Results

Variation in quantitative characteristics of somaclones of sugarcane: The results of fourteen soma-clones of NIA-2010 variety showed overall the finest execution; while, plant height (302.33cm) was observed in SC-3 and the lowest performance was seen in SC-2 that was approximately (154.33cm) Figure 1. The consequences of varieties and diverse parameters indicated that the highest number of internodes plant^{-1} were recorded (38) in SC-3 and lowest number of internodes plant^{-1} were accomplished (23) in SC-1. The output of different features indicated that highest number of tillers plant^{-1} was observed (8.02) in SC-3, the lowest number of tillers plant^{-1} (4.33) were noted in SC-4 and 8 as well. The results were seen that the maximum internodes length (cm) enlistment was observed (17.83cm) in SC-3 and minimum internodes length (cm) was indicated (10.66cm) in SC-8 and 10 as well. The best response regarding cane grith (cm), enlistment was seen (2.21cm) in SC-2 and the lowest cane of grith was analysed in SC-13 which was almost to (1.81cm). The consequences of soma-clones indicated that maximum weight stool^{-1} (kg) was observed (9.67kg) in SC-3 and minimum weight stool^{-1} (kg) was showed (5.83) in SC-1. The output of soma-clones and their parent NIA-2010 indicated that maximum juice extraction volume was observed (866.6ml) in SC-4 and minimum juice extraction volume was achieved (550.1ml) in SC-2. The results of various agronomic parameters showed that greatest germination % observed (82.01%) in SC-6 and lowest germination % indicated (62.66%) in SC-10. The results of soma-clones presented that highest cane yield (tonnes per hectare) were observed (96.67 tonnes per hectare) in SC-3 and lowest cane yield (tonnes per hectare) were achieved (58.33 tonnes per hectare) in SC-1.
Variation in qualitative characteristics of soma clones of sugarcane: The physiological features indicates that among the fourteen different soma-clones, the output of different the highest brix (%) was achieved under the percentage of brix that was (19.83%) in SC-13 and the lowest percentage was observed (14.37%) in SC-2 Figure 2. The products of soma-clones and different parameters indicated that maximum sucrose (%) was recorded (13.4%) in SC-3 and minimum sucrose (%) was enlistment (11.3%) in SC-4. The output of soma-clonal variants presented that the highest fiber (%) were recorded (18.92g) in SC-3 and the lowest fiber (%) were seen under the grams of (16.58g) in SC-11. The outcomes of soma-clones and diverse physiological characteristics indicated that maximum commercial cane sugar percentage (CCS%) were accomplished that highest percentage showed (9.1%) in SC-9 and lowest percentage observed (6.15%) in SC-4. The results of somaclones and numerous agronomic constraints showed that maximum sugar recovery (%) was recorded (8.56%) in SC-8 and minimum sugar recovery (%) was noted (5.78%) in SC-3. The best response of purity (%) was obtained (85.27%) in SC-8 and lowest response of purity (%) was achieved (61.06%) in SC-3. The outcomes of soma-clones indicated that the best response regarding sugar yield (tonnes per hectare) were seen on (7.95 tonnes per hectare) in SC-3 and lowest response regarding sugar yield (tonnes per hectare) were noted (4.47 tonnes per hectare) in SC-1.

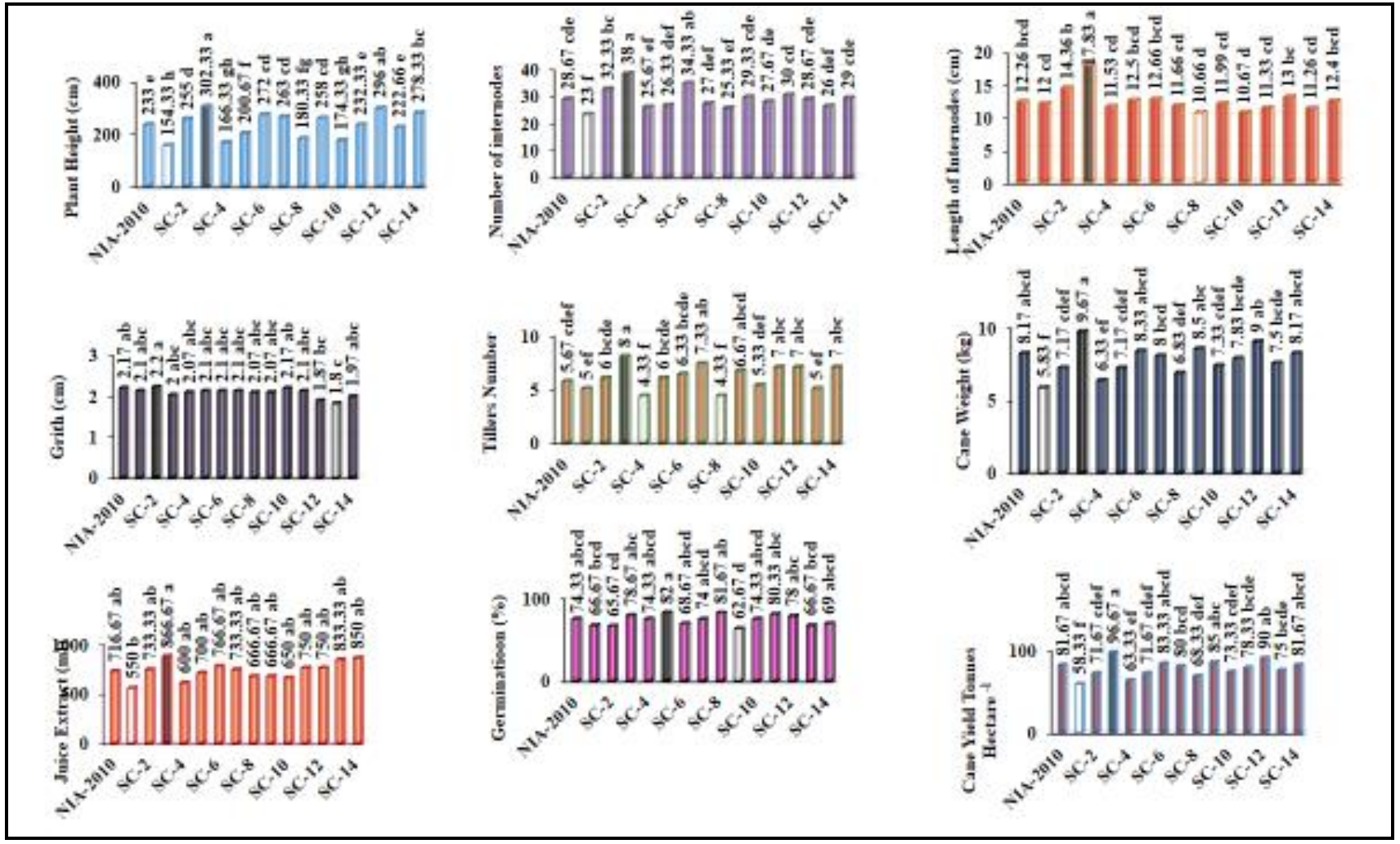

Figure 1 Determining the height of plant, number of internodes, length of internodes, girth, tillers number, cane weight, juice extract, germination percentage and cane yield tons per hectare of different soma-clones of sugarcane.

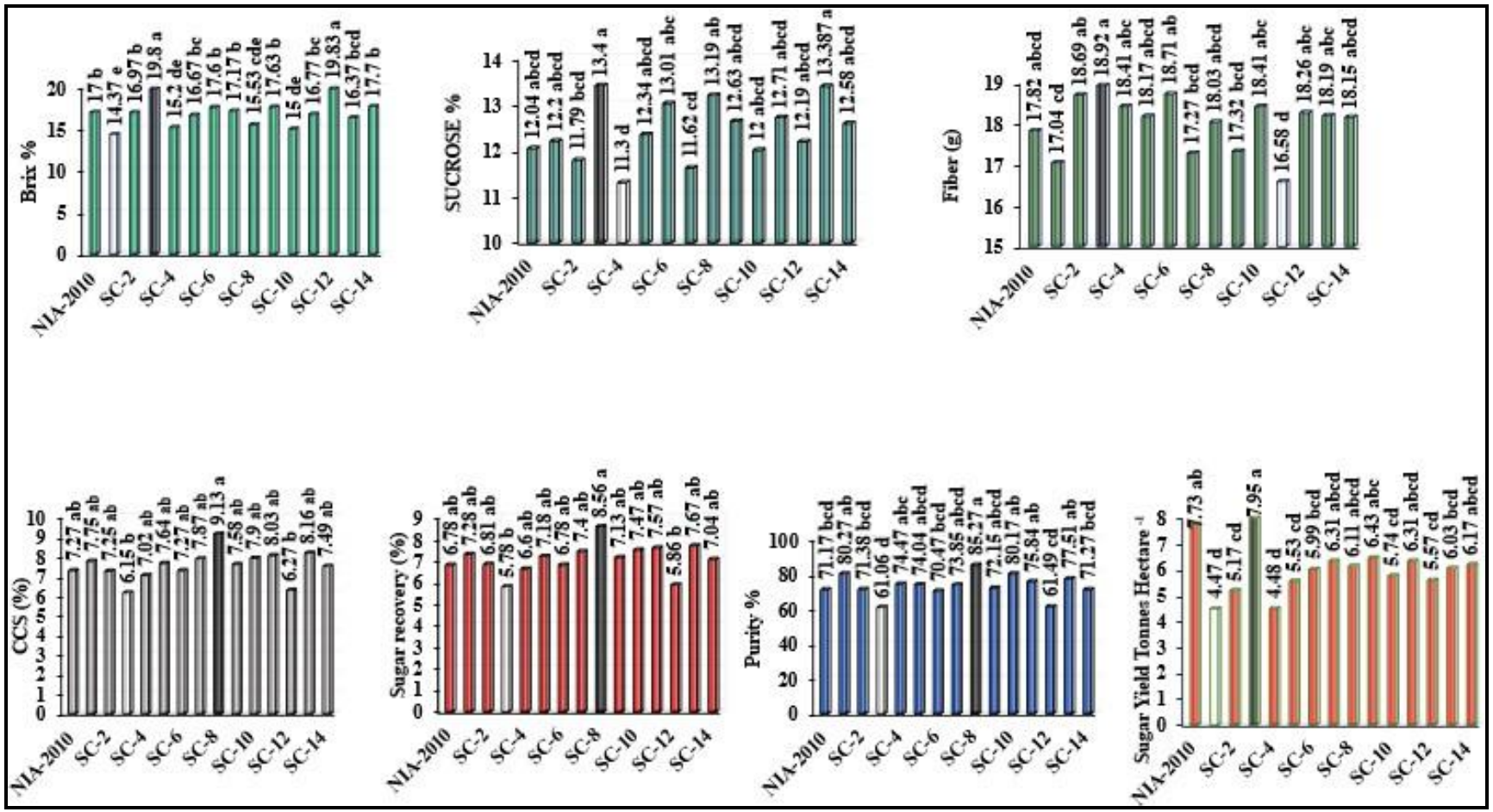

Figure 2 Determined the brix %, sucrose %, fiber, CCS%, sugar recovery %, purity% and sugar yield tons per hectare of different soma-clones of sugarcane

Discussions

The goal of present study was established for the assessment of various physiological and 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 recognize 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 (Arrey and Mih, 2016). Hereditary composition and distinction in their basis propose that alterations in genotype (Thippeswamy et al., 2003).

Seema et al., (2014) 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 (Leva et al. 2012). Chaudhary and Joshi (2005) also informed a significant variation amongst the verified sugarcane soma-clones for diverse parameters and ascribed to the circumstance that utilised clones were established from numerous parentages with various hereditary and environmental backgrounds. The similar results obtained from Khan et al., (2017) that showed that as plant height rises the number of internodes also increases because these parameters are proportional to each other. Javed et al., (2000) also publicised that the number of tillers plant^{-1} was the main causative factor for the cane yield in sugar cane soma-clones. This enlargement rate difference may be because of soma-clonal variation, which influences agronomical characteristics. Hoy et al., (2003) also detected that smaller cane grith diameter in (cm) and increased number of tillers in the plants reproduced from soma-clonal variations of immature leaves. Information related to hereditary diversity and relationships of advanced sugarcane genotypes is still limited, obstructive the effective use and maintenance of its soma-clones. Similarly, Zucchi et al. (2002) and Doule et al. (2008) utilised this technique by (RAPD) approach to analyse tissue culture induced soma-clonal variations in sugarcane varieties. 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 (Lal et al., 2008). The (RAPD) amplification information were 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. Mujahid et al (2001) 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. Nair et al., (2002) also published 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.

Conclusion

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 somaclones for all the sixteen parameters studied. Out of which soma-clone-3 showed the best performance among all other soma-clones of NIA-2010 genotype of sugarcane crop. Consequently, NIA-2010 is a viable variety, and its average yield around 1800mds/acre. Also it has best tolerance power against biotic and abiotic stresses. 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 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.

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