

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 (Online) • Volume 3 • Number 7 (July-2016)

Journal homepage: www.ijcrbp.com



Original Research Article

doi: http://dx.doi.org/10.20546/ijcrbp.2016.307.008

Somaclonal Variation an Aid for Sugarcane Improvement

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Abstract

Variation in natural population occurs as a result of crossing, the evolution of ecotypes, mutations and natural selection. These processes have been accelerated by plant breeders with the refinement in the techniques of selection aided by modern scientific methods. It was now find that sugarcane plants obtained through tissue culture produce a range of variation as wide as that obtained through other means. Populations of subclones were studied for economic characters and the data analyzed show a significant variation from the donor parent CoC 671. The morphological variations *viz.*, stem colour, intermodal alignment, leaf characters, waxiness, etc. were distinct and stable in the somaclones. The somaclonal variant TC 434 was moderately resistant to red rot disease by nodal method of inoculation as well as found drought tolerance with earliness. The present study suggests that somaclonal variation can be exploited to develop and improve agronomical traits, increase resistance to biotic and abiotic stress in crops like sugarcane.

Article Info

Accepted: 10 June 2016 Available Online: 06 July 2016

Keywords

Drought tolerance Red rot Somaclonal variation Sugarcane Tissue culture

Introduction

Sugarcane is a major crop and plays important role in socio-economic conditions of the state. The sugarcane yield levels remained stagnant or decreased in some years due to varietal degeneration and susceptibility to biotic and abiotic stresses. To ensure the genetic diversity among the cultivated varieties, it is essential to identify a few more early maturing, high yielding and high sugar varieties with resistant to biotic and abiotic stresses. The introduction of useful mutation as a means of crop improvement has a special appeal in breeding vegetatively propagated plants. The major advantages of this procedure is that, once an economically important trait is detected, it can be kept by asexually means without having to go through purification and fixation process such as have to be carried out in a

sexually propagated species (Krishnamurthi, 1977). Tissue culture studies were initiated in late 1970's and protocols were standardized for tissue and meristem culture. Several clones were developed through tissue culture with improved productivity and eliminating certain defects like spines, leaf drying, disease susceptibility etc. Somaclones with smut resistance were developed from susceptible clones. Tissue culture work in sugarcane was initiated by the Hawaiian Sugarcane Planters Association Experiment Station, Hawaii, USA (HSPA) in 1961 (Nickell, 1964 and 1967). They have demonstrated that variability occurs in plants regenerated from any form of cell culture and somaclonal variation for the variation displayed among such plants. The Hawaiian work clearly demonstrated that the variability noticed in sugarcane somaclones have a clear genetic basis.

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Sugarcane varieties released so far are evolved through hybridization involving different varieties used as female and male parents. Since sugarcane varieties flowers and seed sets at sugarcane Breeding Institute, Coimbatore (Tamil Nadu, India) every year crosses are made, seedlings are raised and brought through different selections stages after careful evaluation by different research institutes to evolve new sugarcane varieties. In the present study different approach was made, without sexual cycle, somaclones were developed through tissue culture technique. CoC 671 a variety with high sucrose and good yield was under cultivation in many states like Maharashtra, northern Karnataka, Gujarat, but the variety was susceptible to two major diseases, sugarcane smut and red rot.

An abiotic stress includes drought, salinity, extreme temperature; chemical toxicity and oxidative stress are serious threats to agriculture, and natural states of environment (Wang and Altman, 2003). Drought is one of the principal environmental stresses limiting crop productivity around the world (Watanabe, 2002). It is known that somaclonal variation can bring about desirable agronomic changes in the progeny and increased sugar yield in sugarcane. Earlier studies showed variants having differentiation ability in Saccharum spp. (Liu et al., 1972; Banshali and Kishan, 1982). For imparting disease resistance CoC 671 was used in many crosses with resistant varieties but none of the seedling population obtained with high sucrose content. Hence to obtain the disease resistance, drought tolerance with high sucrose content like CoC 671, asexual improvement through tissue culture were utilized at Vasantdada Sugar Institute's, Pune Tissue Culture laboratories. TC 434, TC 435, TC 436, TC 1190, TC 1191, TC 1193, TC 1195, TC 1196, TC 1197 and TC 1200 are some of the somaclones derived from CoC 671 for the present study.

Materials and methods

Callus culture was established from immature inflorescence of CoC 671 sugarcane variety. Callus inductions and plant differentiation were achieved as per mentioned below.

Protocol standardized for tissue culture / somaclonal variation

Sugarcane variety CoC671 plants were grown and immature inflorescence explants were collected for raising callus. The explants material was washed several

times with tap water with few drops of liquid soap. The outer old leaf base coverings were removed carefully without damaging the internal young tissue, followed by surface sterilization with 0.1 % Mercury chloride for 40 to 50 min and followed by three washes of sterile distilled water in the laminar flow and in absolute alcohol for 2 to 3 min. After removing outer sheaths, innermost inflorescence segments cut in to 3-6 mm pieces and inoculated on callus induction medium (CIM).

Murashige and Skoog (MS) (1962), basal medium, with supplemented with 2mg, 3mg and 4mg/l 2, 4-D and 5, 10 and 15% PEG (6000) containing 10% coconut milk and 2% sucrose were used for callus induction. The pH of the medium adjusted to 5.8 and the cultures kept in the dark at $25\pm1^{\circ}$ C.

The well-developed calli were kept on shoot regeneration MS medium consisting $0.001\,\text{mg/l}$ NAA + $0.0009\,\text{mg/l}$ Kinetin and 2% sucrose and 0.8% agar. The regenerated shoots were separated and grow further for root development on White's medium consisting 5 mg/l NAA + 3% sucrose.

The well developed 293 rooted plantlets were separated and transferred to soil mixture containing small polybags and maintained 25±1°C temperature and 70% relative humidity in the greenhouse for primary hardening. Out of which the well-hardened 199 plantlets transferred for secondary hardening in open environment. Later 167 plants were planted in ground nursery for field performance evaluation. Flowchart for the development of somaclonal variants is given in Fig. 1.

Field trial

The somaclones along with standards were planted in replicated trials in Randomized Block Design (RBD) and yield contributing characters *viz.*, cane diameter (cm), single cane weight (kg), number of millable canes ('000/ha), millable height (cm), tillering at 120 days ('000/ha) and sugar yield contributing characters *viz.*, brix %, sucrose %, commercial cane sugar %, juice purity % were recorded at 10 and 12 months of the crop age.

Drought tolerance

Along with the yield, quality and disease resistance the selected somaclones also screened for drought tolerance. A field experiment was conducted to select for drought tolerant somaclones derived from sugarcane variety CoC

671. A split plot design was used, with contrasting irrigation levels imposed as whole plot treatments and genotypes as subplots. The unit plot size was 7 rows \times 7 meter. To screen for drought tolerant sugarcane somaclones, moisture stress was imposed by withholding irrigation from 60th day after planting up to 150th day after planting (I2) and compared with the regular irrigated crop as control (I1). Somaclones were studied for drought tolerance along with parent variety CoC 671. Tolerance level was measured based on cane yield data. The productivity of sugarcane under moisture/drought stress conditions could be used as one of the key selection criteria for drought tolerance. In the present study, number of millable canes ('000/ha), cane yield (t/ha) and sugar yield (t/ha) of the somaclones were evaluated as field trial screening criteria.

Red rot disease resistance

Early maturing sugarcane variety CoC 671 is highly susceptible to red rot. Maharashtra State is free from red rot disease hence for testing of red rot disease the selected somaclones derived from CoC 671 were sent to Regional Sugarcane Research Station, Navsari, Gujarat State, India for nodal as well as plug method of inoculation. After resistance found in TC 434 this entry again sent to Sugarcane Breeding Institute, Coimbatore and Thiruvella, India for confirmation of the results at different States of India.

Results and discussion

Sucrose and cane yield improvement in the somaclones over the donor parent

Somaclones of CoC 671 were obtained from ground nursery seedlings on the basis of brix% at 10th and 12th month age of crop as well as single cane weight at the age of 12th month. Such only 10 promising somaclones were selected and advanced in rod –row trial as compared to the donor parent CoC 671 and another standard Co 86032. The cane yield and yield contributing characters were recorded in selected somaclones along with juice quality parameters (Table 1). None of the clone was found to be significantly superior over CoC 671 (129.78 t/ha) while TC 436 (141.28 t/ha), TC 434 (138.80 t/ha), TC 1191 (134.09 t/ha) and TC 435 (132.30) were found numerically superior over to donor parent CoC 671.

The sucrose % at 12^{th} month's age of crop of somaclones viz., No. TC 1197 (21.89%), TC 1191 (21.70%), TC 434

(21.12%) and TC 435 (21.04%) were superior as compared to CoC 671 (20.52%) where as Commercial Cane Sugar % recorded highest in somaclone No. TC 435 (15.95%) followed by TC 1191 (15.81%) and TC 1197 (15.74%) respectively.

On the basis of yield and juice quality parameters six somaclones *viz.*, TC 434, TC 435, TC 436, TC 1191, TC 1196 and TC 1197 were advanced for further study under field trial for evaluating for yield and quality parameters as compared to the donor parent CoC 671 (Table 2). Out of six somaclones, maximum sucrose % recorded in somaclones No.TC 434 (22.53), TC 436 (22.01) and TC 1196 (21.95) at the age of 12th month of crop. The cane yield of somaclone TC 434 (154.06 t/ha) and TC 1191 (150.40 t/ha) recorded numerically higher over donor parent CoC 671 (149.44 t/ha). Considering the superiority of three somaclones forwarded for further studies and evaluated with other promising somaclones developed by VSI.

The juice quality parameters along with cane yield and its contributing characters data recorded in two plants and one ration crops are depicted in Table 3. Among the selected somaclones derived from donor parent CoC 671, TC 434 found to be highest sucrose percentage at 10 months (21.51%) as compared to CoC 671 (21.33%). The improvement in the sucrose percentage also recorded in other somaclones viz., TC 2776 (21.41%). The earliness for the maturity of canes in terms of sugar was improved through the use of somaclonal variation technology. The cane yield tones/ha for the somaclone TC 906 (140.15 t/ha) recorded significantly higher as compared to the donor parent CoC 671 (126.19 t/ha) followed with numerically higher in TC 922 (133.91 t/ha) and TC 434 (131.13 t/ha) respectively. The other parameters like CCS % (Commercial Cane Sugar), Brix % at 10 and 12 Months recorded in the somaclones as compared to the donor CoC 671. The maximum CCS % was recorded in the TC 434 with 16.79% and Brix % (24.07%) at 12 months of age crop against donor CoC 671 (CCS-16.70% and Brix- 23.85%) respectively. The similar improvement in somaclones over donor parent for cane and sugar yield reported by Jalaja et al. (2006) using utilization of somaclonal variation technology and mentioned about this techniques for rectifying specific defects. The data recorded in the somaclone derivatives showed that TC 434 has early maturing characters (at 10 months) with better yield. The morphological features of the donor parent CoC 671 and the somaclonal variants given in Table 4 and Fig. 2. The same somaclone TC 434 also tested for the drought trial.

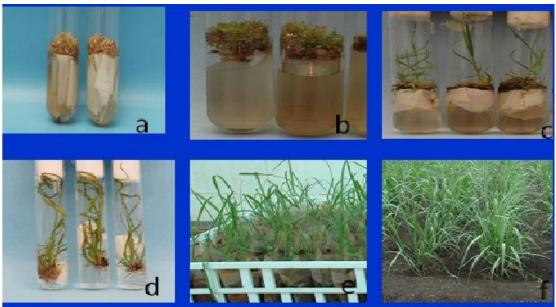


Fig. 1: Flowchart for the development of somaclonal variants.

- a) Callus induction in liquid medium
- c) Shoot elongation in liquid medium
- e) Hardening in green house
- b) Shoot initiation in solid medium
- d) Root formation in liquid rooting media
- f) Planting in field



Fig. 2: Morphological variations in the somaclones derived from donor parent CoC 671. a. Stem colour of CoC 671 b. Leaf canopy of CoC 671

- d. Leaf canopy of TC 434
- c. Stem colour of TC 434
- e. Stem colour of TC 435
- f. Leaf canopy of TC 435

- g. Stem colour of TC 1191
- h. Leaf canopy of TC 1191

Table 1. Field and quality performance of somaclones derived from CoC 671 sugarcane variety in preliminary trials.

| Sr. No | Somaclones | CCS (t/ha) | Cane yield (t/ha) | Cane diameter (cm) | Single cane weight (kg) | No. of millable canes/ha ('000) | Millable height of cane (cm) | Sucrose % 12 months | CCS % 12 months | Brix % 12 months | Sucrose % 10 months | CCS % 10 months | Brix % 10 months |
|-----------|-------------|------------|-------------------|--------------------------|----------------------------|------------------------------------|------------------------------------|---------------------|--------------------|---------------------|---------------------|--------------------|---------------------|
| 1 | TC 434 | 21.24 | 138.80 | 2.84 | 1.46 | 94.73 | 247.00 | 21.12 | 15.28 | 22.29 | 21.40 | 15.41 | 22.85 |
| 2 | TC 435 | 21.08 | 132.30 | 2.95 | 1.52 | 87.27 | 247.44 | 21.04 | 15.95 | 22.00 | 20.84 | 15.06 | 22.05 |
| 3 | TC 436 | 20.79 | 141.28 | 2.94 | 1.58 | 89.60 | 253.34 | 20.34 | 14.70 | 21.51 | 19.18 | 13.70 | 20.86 |
| 4 | TC 1190 | 18.16 | 123.30 | 2.92 | 1.46 | 84.47 | 261.00 | 20.14 | 14.74 | 20.70 | 17.84 | 12.85 | 19.01 |
| 5 | TC 1191 | 21.18 | 134.09 | 2.85 | 1.37 | 98.00 | 266.78 | 21.70 | 15.81 | 22.85 | 18.35 | 13.44 | 20.82 |
| 6 | TC 1193 | 20.35 | 133.18 | 2.86 | 1.51 | 88.20 | 276.67 | 21.05 | 15.30 | 22.00 | 19.45 | 13.89 | 21.14 |
| 7 | TC 1195 | 19.37 | 133.30 | 2.78 | 1.43 | 93.80 | 263.44 | 20.21 | 14.55 | 21.58 | 19.65 | 13.97 | 21.57 |
| 8 | TC 1196 | 20.24 | 141.10 | 2.96 | 1.53 | 92.60 | 276.44 | 19.84 | 14.36 | 20.99 | 19.40 | 13.83 | 21.17 |
| 9 | TC 1197 | 20.80 | 123.77 | 2.87 | 1.25 | 98.93 | 265.78 | 21.89 | 15.74 | 22.62 | 19.00 | 13.49 | 20.90 |
| 10 | TC 1200 | 18.42 | 118.76 | 3.11 | 1.32 | 90.53 | 274.22 | 21.49 | 15.54 | 22.73 | 20.08 | 14.35 | 21.81 |
| | Standards | | | | | | | | | | | | |
| | CoC 671 | 19.27 | 129.78 | 2.78 | 1.44 | 89.83 | 248.63 | 20.52 | 14.95 | 21.33 | 20.48 | 14.68 | 22.06 |
| | Co 86032 | 18.19 | 141.17 | 2.80 | 1.35 | 104.93 | 235.05 | 18.91 | 12.87 | 18.50 | 16.83 | 11.85 | 18.82 |
| | SE <u>+</u> | 1.15 | 5.69 | 0.13 | 0.06 | 2.33 | 4.82 | 1.31 | 0.72 | 0.83 | 0.65 | 0.48 | 0.61 |
| | CD 5% | 3.38 | 16.68 | 0.27 | 0.19 | 6.83 | 14.15 | 3.84 | 2.12 | 2.42 | 1.90 | 1.39 | 1.77 |
| | CV | 10.01 | 7.43 | 2.13 | 7.79 | 4.35 | 3.22 | 2.37 | 8.37 | 6.63 | 5.78 | 5.93 | 4.97 |

Table 2. Field and quality performance of selected somaclones derived from CoC 671 sugarcane variety.

| Sr. | Somaclones | CCS (t/ha) | Cane | Cane | Single cane | No. of millable | Millable | Sucrose % | CCS % | Brix % | Sucrose % | CCS % | Brix % |
|-----|-------------|------------|--------------|----------|-------------|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| No | | | yield (t/ha) | diameter | weight | canes/ha ('000) | height of | 12 months | 12 months | 12 months | 10 months | 10 months | 10 months |
| | | | | (cm) | (kg) | | cane (cm) | | | | | | |
| 1 | TC 434 | 25.07 | 154.06 | 2.96 | 1.59 | 96.89 | 256.67 | 22.53 | 15.29 | 23.91 | 19.94 | 14.18 | 21.87 |
| 2 | TC 435 | 22.20 | 148.35 | 2.99 | 1.47 | 101.00 | 246.67 | 21.08 | 14.67 | 22.89 | 21.04 | 15.07 | 22.73 |
| 3 | TC 436 | 21.53 | 136.92 | 2.98 | 1.46 | 94.00 | 252.33 | 22.01 | 14.27 | 23.41 | 19.83 | 14.05 | 21.94 |
| 4 | TC 1191 | 21.11 | 150.40 | 3.06 | 1.57 | 95.44 | 247.67 | 19.70 | 13.78 | 21.46 | 17.93 | 12.63 | 20.08 |
| 5 | TC 1196 | 20.83 | 132.23 | 2.99 | 1.50 | 88.11 | 238.67 | 21.95 | 14.28 | 23.54 | 20.01 | 14.33 | 21.59 |
| 6 | TC 1197 | 17.81 | 120.98 | 2.84 | 1.38 | 87.78 | 256.00 | 20.65 | 14.44 | 22.67 | 19.33 | 13.68 | 21.44 |
| | Standards | | | | | | | | | | | | |
| | CoC 671 | 22.40 | 149.44 | 2.94 | 1.52 | 97.99 | 242.33 | 21.09 | 14.32 | 23.03 | 19.68 | 13.93 | 21.82 |
| | Co 86032 | 24.54 | 164.44 | 2.80 | 1.68 | 98.55 | 292.67 | 20.74 | 14.09 | 22.43 | 20.13 | 14.44 | 21.68 |
| | SE <u>+</u> | 1.98 | 7.81 | 0.33 | 0.11 | 3.42 | 11.69 | 0.79 | 0.62 | 0.59 | 0.61 | 0.49 | 0.51 |
| | CD (5%) | 5.79 | 17.62 | 0.88 | 0.31 | 7.08 | 24.00 | 2.30 | 1.80 | 1.71 | 1.79 | 1.46 | 1.49 |
| | CV | 8.24 | 12.24 | 1.03 | 2.39 | 9.21 | 12.37 | 1.20 | 1.37 | 1.36 | 1.48 | 1.17 | 1.28 |

Table 3. Pooled of two plant and one ration data on cane yield, quality and their contributing characters in selected Tissue culture somaclones.

| Sr No | Somaciones | CCS (t/ha) | Cane yield (t/ha) | Cane diameter (cm) | Single cane weight (kg) | No. of millable canes/ha ('000) | Millable height of cane (cm) | CCS % 10M | CCS % 12M | Sucrose % 10M | Sucrose % 12M | Brix % 10M | Brix % 12M | Fibre % | No. of inter nodes/cane |
|----------|-------------|---------------|----------------------|--------------------------|----------------------------|--|------------------------------------|--------------|--------------|---------------|---------------|---------------|---------------|---------|-------------------------|
| 1 | TC 878 | 19.35 | 118.25 | 2.91 | 1.19 | 99.47 | 255.89 | 14.90 | 16.34 | 20.77 | 22.41 | 22.34 | 23.23 | 14.11 | 20.33 |
| 2 | TC 1191 | 17.00 | 106.81 | 2.85 | 1.04 | 96.68 | 220.78 | 15.27 | 15.99 | 21.21 | 22.02 | 22.63 | 23.08 | 13.21 | 22.67 |
| 3 | TC 906 | 22.96 | 140.15 | 3.12 | 1.31 | 103.37 | 235.56 | 14.65 | 16.33 | 20.36 | 22.45 | 21.76 | 23.41 | 14.29 | 22.55 |
| 4 | TC 922 | 22.04 | 133.91 | 3.07 | 1.34 | 92.25 | 250.10 | 15.36 | 16.49 | 21.11 | 22.67 | 22.01 | 23.66 | 14.37 | 22.44 |
| 5 | VSI 435 | 19.86 | 124.02 | 2.80 | 1.15 | 100.02 | 223.71 | 13.88 | 16.00 | 19.31 | 22.03 | 20.68 | 23.05 | 13.98 | 22.44 |
| 6 | TC 2776 | 16.69 | 103.48 | 2.80 | 1.13 | 83.33 | 235.78 | 15.50 | 16.18 | 21.41 | 22.29 | 22.55 | 23.36 | 14.48 | 22.11 |
| 7 | TC 434 | 22.17 | 131.31 | 2.98 | 1.33 | 96.17 | 249.48 | 15.60 | 16.79 | 21.51 | 23.09 | 22.60 | 24.07 | 14.36 | 22.28 |
| | Standards | | | | | | | | | | | | | | |
| | CoC 671 | 21.10 | 126.19 | 2.98 | 1.45 | 87.54 | 222.05 | 15.48 | 16.70 | 21.33 | 22.93 | 22.35 | 23.85 | 14.81 | 21.44 |
| | Co 94012 | 19.34 | 122.94 | 3.10 | 1.39 | 87.78 | 255.63 | 14.70 | 15.78 | 20.33 | 21.76 | 21.47 | 22.87 | 14.13 | 21.55 |
| | Co 86032 | 22.82 | 145.88 | 2.96 | 1.37 | 107.29 | 226.99 | 14.35 | 15.72 | 19.82 | 21.64 | 20.89 | 22.63 | 14.39 | 21.70 |
| | SE <u>+</u> | 1.78 | 8.36 | 0.13 | 0.06 | 8.74 | 11.76 | 0.34 | 0.20 | 0.45 | 0.27 | 0.49 | 0.28 | 0.66 | 0.95 |
| | CD at 5% | 3.65 | 12.43 | 0.27 | 0.13 | 18.11 | 20.53 | 0.69 | 0.42 | 0.92 | 0.56 | 1.02 | 0.57 | 1.68 | 1.97 |
| | CV % | 9.32 | 11.63 | 5.12 | 4.91 | 11.28 | 10.03 | 2.14 | 1.54 | 2.63 | 1.48 | 2.78 | 1.44 | 2.24 | 5.31 |

Drought tolerance

Cane yield is the product of genetic potential of a variety and environmental conditions through agronomic management. Maximum cane yield was obtained in non-stress condition i.e. in control (115.53 t/ha) where as the moisture stress reduced the cane yield up to 99.02 t/ha (Table 5). These results are supported by the findings of earlier workers (Singh et al., 2006; Inman-Bamber and Smith, 2005; Smith et al., 2005; Ghaffar et al., 2013).

The moisture stress had minimum adverse effect on TC 434. In TC 434 due to moisture stress cane yield was reduced by 4.54% over the control treatment (120.77 t/ha). The donor parent CoC 671 had noted 27.49% reduction in cane yield due to moisture stress over the cane yield obtained in control treatment (123.57 t/ha). The yield data revealed that the differences among genotypes were not significant. TC 434 has recorded higher cane yield (123.64 t/ha) over the donor parent CoC 671 (106.58 t/ha).

Table 4. Morphological differences between donor CoC 671 and selected somaclones.

| Sr. | D | Donor parent | Somaclones | | | | | | |
|-----|--------------------------|--------------------------|--------------------------|----------------------|---------------------------------|--|--|--|--|
| No | Descriptor | CoC 671 | TC 434 | TC 435 | TC 1191 | | | | |
| 1 | Parentage | Q 63 X Co 775 | Somaclone | Somaclone | Somaclone | | | | |
| | | | CoC 671 | CoC 671 | CoC 671 | | | | |
| 2 | Stem colour (Exposed) | Purple | Reddish pink | Pink | Dark purple | | | | |
| 3 | Stem Colour (Un-exposed) | Light greenish yellowish | Greenish yellow | Greenish yellow | Light greenish yellowish | | | | |
| 5 | Corky patches | Absent | Absent | Absent | Absent | | | | |
| 6 | Internode shape | Cylindrical | Abconoidal | Abconoidal | Cylindrical | | | | |
| 7 | Internode alignment | Zigzag | Slightly zigzag | Straight | Zigzag | | | | |
| 8 | Internode diameter | 2.75 cm | 2.63 cm | 2.71cm | 2.78cm | | | | |
| 9 | Pithiness | Absent | Absent | Absent | Absent | | | | |
| 10 | Internode splits | Absent | Absent | Absent | Absent | | | | |
| 11 | Internode waxiness | Absent | Medium | Medium | Absent | | | | |
| 14 | a) No. of root eye rows | Two-three | Two | Two | Two-three | | | | |
| | b) Arrangement | Irregular | Irregular | Irregular | Irregular | | | | |
| 15 | Bud size | Small | Small | Small | Small | | | | |
| 16 | Bud shape | Ovate | Round | Round | Ovate | | | | |
| 17 | Bud cushion | Absent | Absent | Absent | Absent | | | | |
| 18 | Bud groove | Absent | Absent | Absent | Absent | | | | |
| 20 | Leaf width | Broad | Medium | Medium | Medium | | | | |
| 21 | Lamina colour | Green | Dark green | Green | Green | | | | |
| 22 | Leaf carriage | Open drooping | Semi drooping | Semi drooping | Open drooping | | | | |
| 23 | Leaf sheath colour | Dark purple | Purple with slight green | Green with purple | Dark purple | | | | |
| 24 | Leaf sheath waxiness | Absent | Medium | Medium | Absent | | | | |
| 25 | Leaf sheath spines | Hard, more | Few, hard, deciduous | Few, hard, deciduous | Few, hard, deciduous | | | | |
| 26 | Leaf sheath clasping | Easily detrashing | Slightly tight | Medium | Slightly tight | | | | |
| 27 | Dewlap colour | Light green | Green with dark brown | Dark green | Light green | | | | |
| 28 | Ligular process | Present | Absent | Absent | Absent | | | | |
| 30 | Flowering | Non Flowering | Non-flowering | Non-flowering | Flowers in south Maharashtra | | | | |

The moisture stress adversely affected on number of millable canes of all genotypes. The genotypes grown under non-stress condition i.e. control treatment has recorded maximum number of millable canes (83.97 '000/ha) as compared to the crop affected due to

moisture stress (78.68 '000/ha). The decrease in number of millable canes due to moisture stress is also reported by Hemaprabha et al. (2004 and 2006) and Silva et al. (2008). Significant difference among the genotypes for number of millable canes was observed and it might be

due to the genetic potential of the genotypes to withstand moisture stress. Among the genotypes significantly higher number of millable canes was noted in TC 434 (85.55 '000/ha) over the donor parent CoC 671 (72.86

'000/ha). The significant difference in number of millable canes might be due to the inherent potential of the genotypes to withstand moisture stress. These results are in line with Vasantha et al. (2005).

Table 5. Effect of water stress on yield and yield attributes.

| Variety | Number o | Number of millable canes ('000/ha) | | | ld (t/ha) | | C.C.S. (t/ha) | | | | |
|----------------|----------------|------------------------------------|--------|--------|-----------|--------|----------------|-------|-------|--|--|
| variety | I ₁ | I_2 | Mean | I_1 | I_2 | Mean | I ₁ | I_2 | Mean | | |
| Co 94012 | 74.44 | 77.61 | 76.03 | 96.50 | 86.69 | 91.59 | 13.31 | 11.93 | 12.62 | | |
| TC 434 | 88.73 | 85.55 | 87.14* | 126.52 | 120.77 | 123.64 | 18.09 | 17.17 | 17.63 | | |
| CoC 671 | 88.73 | 72.85 | 80.79 | 123.57 | 89.60 | 106.58 | 17.20 | 11.58 | 14.39 | | |
| Mean | 83.97 | 78.68 | | 115.53 | 99.02 | | 16.20 | 13.56 | | | |
| S.E. ± Mair | Treat. | | 1.46 | | | 3.57 | | | 0.65 | | |
| C. D. at 5% | Main Treat | | NS | | NS | | | NS | | | |
| S.E. \pm Sub | Treat. | | 3.17 | 3.17 | | | | 3.01 | | | |
| C.D. at 5% | Sub Treat | | 9.77 | 9.77 | | | N.S. | | | | |
| S.E. ± Inter | action | | 4.49 | | | 4.25 | | | 0.81 | | |
| C.D. at 5% | Interaction | | NS | | | 1.31 | | | NS | | |

I₁: Non-stress, I₂: Stress.

Table 6. Reaction of TC 434 to red rot disease in All India Coordinated Research Project on Sugarcane (AICRPS).

| Entry | Coimbatore | | Thiruvella | | Navsari | | |
|----------|-------------|--------------|-------------|--------------|-------------|--------------|--|
| Elitiy | Plug method | Nodal method | Plug method | Nodal method | Plug method | Nodal method | |
| TC 434 | MR | R | MS | R | MR | R | |
| Standard | | | | | | | |
| CoC 671 | HS | HS | HS | HS | HS | HS | |

HS: Highly Susceptible, MR: Moderately Resistant, R: Resistant

The commercial cane sucrose i.e., C.C.S (t/ha) is a product of cane yield and commercial cane sucrose %. The moisture stress did not affected adversely on C.C.S (t/ha). Among the somaclones highest C.C.S. (t/ha) was recorded in TC 434 (17.63 t/ha) over its donor parent CoC 671 (14.39 t/ha). The results are in consonance with Singh et al. (2001) and Singh et al. (2006) who reported that C.C.S. (t/ha) was not affected due to different levels of irrigation.

Red rot disease resistance

One of the major reasons of low productivity in sugarcane and sugar in the country is the incidence of red rot disease. Many varieties of sugarcane have gone out of cultivation due to incidence of this disease and several other popular and high yielding varieties are struggling for their existence (Pankaj Kumar et al., 2012). New varieties with high yielding capacity, higher sugar content along with resistance for disease especially red rot are evolved through breeding methods are requiring 8-10 years (Gill et al., 2007; Singh et al., 2008). A period of further 8-10 years is required to reach the seed of new varieties to the growers in remote villages for general

cultivation. In vitro techniques can be used in competitively shorter period of time (Tiwari et al., 2010). Somaclonal variation has also been employed to develop resistant clones of sugarcane for various diseases (Sreenivasan and Jalaja, 1995; Jalaja et al., 2006). Amongst the clone, TC 434 somaclone derived from CoC 671 found to be resistant by nodal method and moderately resistant to plug method at Thiruvella and Navsari location of India (Table 6).

Conclusion

The somaclonal variation in sugarcane in the present study resulted genetic improvement in terms of sucrose, earliness, red rot resistance along with drought tolerance in somaclones derived from the donor parent CoC 671. The work resolved the controversy of induced mutations role in crop improvement equal to conventional hybridization particularly obtaining novel variants *viz.*, high sugar content, multiple stress tolerance etc. where conventional breeding has many limitations. The somaclone i.e., TC 434 was identified for earliness, red rot resistance and drought tolerance may be utilized as a parent in hybridization programme for the development

of resistant genetic stocks. In the present investigation it was found that TC 434 showed superiority in cane and sugar yield under moisture stress condition compared to their donor parent variety CoC 671. These results indicate feasibility and effectiveness of creation and selection of somaclone for drought tolerance.

Conflict of interest statement

Authors declare that they have no conflict of interest.

Acknowledgement

Authors are thankful to Director General, Vasantdada Sugar Institute, Pune for the facilities provided for carrying out the work.

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How to cite this article:

Tawar, P. N., Sushir, K. V., Meti, N. T., 2016. Somaclonal variation an aid for sugarcane improvement. Int. J. Curr. Res. Biosci. Plant Biol. 3(7), 47-55. doi: http://dx.doi.org/10.20546/ijcrbp.2016.307.008