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SOMACLONAL VARIATION - A GATEWAY FOR VARIETAL IMPROVEMENT OF NON-FLOWERING SUGARCANE GERMPLASMS

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ABSTRACT

The research was carried out at the Bangladesh Sugarcrop Research Institute (BSRI) to evaluate the feasibility of incorporating five exotic non-flowering sugarcane genotypes into a traditional breeding programme through somaclonal variations. To generate genetic diversity, explants from the leaf sheath of donor plant were grown on modified MS media supplemented with 3.5 mgl^{-1} 2,4-D. Callus formation occurred within six to twelve days of cultivation. For shoot regeneration, MS medium supplemented with 1.0 mgl^{-1} BA + 0.5 mgl^{-1} NAA was utilized, while 5.0 mgl^{-1} NAA was used for roots from micro shoots. 834 somaclones were acclimatised and planted in the field for evaluation, where 520 somaclones survived in the G_0 generation. On the basis of three years of field study under three generations, three somaclones were chosen for their profuse flowering and better agronomic traits. The somaclones were found to be significantly different from the mother plants.

Key words: Sugarcane, non-flowering, somaclonal variation.

Introduction

Clonal propagation via tissue culture (often referred to as micropropagation) can be accomplished quite quickly and in a tiny space (Krishna *et al.,* 2008; Eftekhari *et al.,* 2012). The uniformity of individual plants within a clone population is a major advantage of clonal cultivars in commercial production (Krishna and Singh, 2013). However, genetic variability occurs in undifferentiated cells, isolated protoplasts, calli, tissues, and morphological characteristics of plants grown in vitro (Bairu *et al.,* 2011; Currais *et al.,* 2013). Larkin and Scowkraft (1981) developed the term "somaclonal variation" in 1981 to refer to plant variants obtained from cell or tissue culture. The process of somaclonal variation enables the development of variations with increased cane yield potential, disease resistance, and flowering behavior from susceptible and non-flowering sugarcane parents. The development of enhanced sugarcane varieties may be aided by the production of somaclonal variations rather than conventional breeding (Alam *et al.,* 2015). Somaclonal variations require less space and time for in vitro screening of desirable features than cross seedlings of perennial crops, which require a substantial amount of land and time. It may have several applications in plant breeding, and by applying appropriate in vitro selection pressure, the recovery of such novel variations can be accelerated (Jain, 2001; Lestari, 2006). In case of sugarcane, somaclonal diversity enables the development of variants with increased cane yield potential, disease resistance, and flowering behavior derived from susceptible and non-flowering parents. The development of somaclonal variations may be used in lieu of conventional breeding to improve varieties (Alam *et al.,* 2015). Bangladesh Sugarcrop Research Institute (BSRI) maintains 1159 clones, 331 of which are flowering. There are some non-flowering clones that exhibit desirable agronomic characteristics (e.g. plant height, number of millable canes, girth, yield, etc.) and are resistant to biotic stresses, i.e. disease and insect pests, as well as abiotic stresses such as salinity, drought, flood, and water logging, among others. However, despite the presence of the target gene pool in the germplasm, we are unable to reintroduce them into desired clones for varietal development using conventional breeding methods. As a result, we are falling behind in terms of fully exploiting the existing germplasm. However, somaclonal variation induced by plant tissue culture may provide an alternative avenue for incorporating those non-flowering clones into breeding programmes by increasing genetic variability, which will undoubtedly increase the likelihood of obtaining desirable variations. BSRI has already created a somaclonal variety (BSRI Akh 43) from Isd 18 that was sensitive to

red rot. Additionally, BSRI has a reputation for developing somaclones; however, non-flowering clones received little attention. So this study was hence carried out to generate genetic variability in non-flowering clones via plant tissue culture and to develop flowering somaclones for use in conventional breeding.

Materials and Methods

The study was done between 2016 and 2020 at the Bangladesh Sugarcrop Research Institute's (BSRI) laboratory and field sites. Five valuable exotic sugarcane germplasm lines, namely Co 62-101, Co 65-02, Cp 44-101, Cp 48-103, and Q 69, were used as pamonthrent materials in this study. Table 1 summarizes the salient characteristics of them. Leaf sheaths from 6-8old field grown plants were collected aseptically and in vitro cultured using the plant tissue culture technique. To generate somaclonal variations, MS and Modified MS (MMS) medium supplemented with auxin and cytokine were employed. MMS medium mixed with 2,4-D (3.5 mgl^{-1}) was utilised to dedifferentiate tissues in order to produce callus from the explants. However, callus redifferentiation to generate organs (shoots and roots) was performed on MS medium treated with auxin and cytokine alone or in combination (Fig. 1).

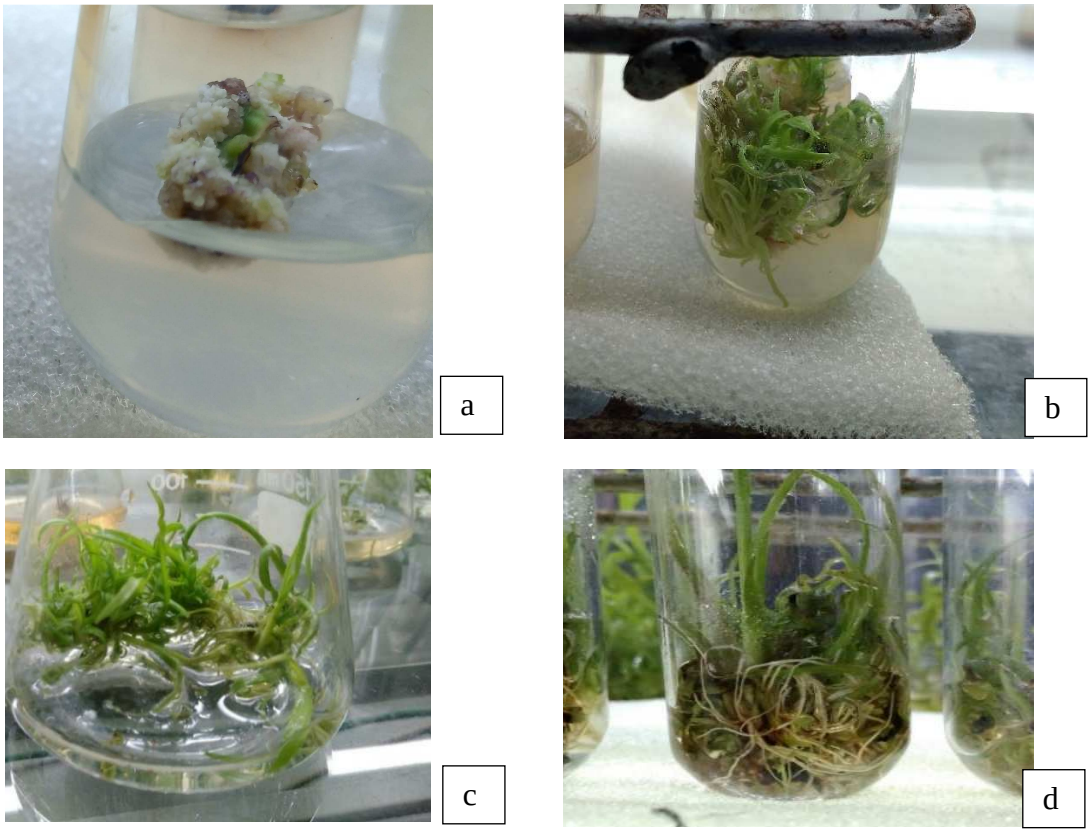

Fig. 1. Steps of developing flowering somaclones from the non-flowering sugarcane. a. Callus induction, b. shoot initiation from callus, c. multiplication of shoot and d. root initiation in micro shoots.

The pH of the medium was adjusted to 5.7 and it was autoclaved for 20 minutes at 121° C. The cultures were incubated at 25±1°C in an air-conditioned culture chamber using white fluorescent bulbs with an intensity of around 3000 lux. Following in vitro plantlet development, the culture containers were switched from control to normal condition and left unplugged for three days. The plantlets were removed from the medium, properly washed with running tap water, and set up in garden soil containing cell-u-pack in order

to acclimate to the natural environment under plastic cover. Acclimatized somaclones (seedlings) were planted in the field under G_0 generation in 2017-18 cropping season at a distance of 1.0 m row to row and 0.5 m plant to plant. Following field evaluations for millable canes/clump and growth vigour, selected variations were planted in a 1m × 4m plot at the G_1 generation (in 2018) for further evaluation. At the G_1 generation, selection was made on the basis of germination percentage, number of tillers per clump, flowering behavior, field brix percent and overall appearance. Selected somaclones were then planted in a 4m × 4m plot at the G_2 generation for further study.

Table 1. Salient feature of the selected sugarcane genotypes

Sl.	Name of the clones	Origin	Importance	Reason of tissue culture
1.	Co 62-101	Coimbatore, India	High yielding Self detrashing	Due to non-flowering nature unable to use in hybridization program
2.	Co 65-02	Coimbatore, India	High sucrose content Profuse tillering ability	
3.	CP 44-101	Canal point, USA	High sucrose content Self detrashing	
4.	Cp 48-103	Canal point, USA	High sucrose content	
5.	Q 69	Queensland, Australia	High yielding Suitable for juice	

Results and Discussion

Callusing of explants: For callus induction, explants from all genotypes were grown in MMS medium supplemented with 3.5 mgl^{-1} 2,4-D. Callus formation began within 6-12 days of culture in this medium, and by 20-25 days, 70 to 90% of the total volume of explants had formed into callus. Differences in the callus percentage were observed. CP 44-101 produced highest number of 76% callus from the explants (Table 2). The majority of cultivated explants developed loose compact creamy white embryogenic callus (Fig.1 a) capable of regeneration. Alam *et al.* (2003) previously used 3.0 mgl^{-1} 2,4-D in MMS medium to induce callus in sugarcane. It was discovered that both the number and quality of callus were critical for successful regeneration.

Regeneration of shoots from the callus: To regenerate shoots, two to three weeks old calli were grown on MS medium supplemented with 1.0 mgl^{-1} BA and 0.5 mgl^{-1} NAA. Maximum cultivated calli developed green plantlets on this medium (Fig.1 b). Within 7-12 days of culture, the callus of different genotypes responded differentially to begin shoots. In this study highest number of shoots/culture 36.13 were regenerated from the callus of CP 48-103 and lowest 9.35 by the callus of Q 69 after four weeks of culture f Q 69. The variants of Co 62-101 demonstrated the longest shoot length of 3.84 cm, while Cp 48-103 exhibited the shortest shoot length of 2.98 cm (Table 2). The regenerated shoots were multiplied using the same hormonal mixture in liquid MS media (Fig. 1c). According to Alam *et al.* (2002) this hormone combination was effective in regenerating shoots from sugarcane callus. Seema *et al.* (2011) observed that when different growth regulators were used, regeneration began with the formation of green dots on callus within a week on regeneration media.

Root induction: Micro shoots (2.5-4 cm height) were planted on MS media supplemented with 5.0 mgl^{-1} NAA. Within 9-15 days of cultivation, plantlets of all genotypes generated an average of 3–5 roots (Fig.1 d). Alam *et al.* (2003) showed efficient root development of sugarcane micoshoots on MS supplemented with 5.0 mgl^{-1} NAA. Seema *et al.* (2011) also reported on the use of IBA at various concentrations in MS medium containing 4.0 gl^{-1} sucrose for rooting sugarcane micro-shoots. The entire micro-shoot was acclimatised to the natural environment.

Field evaluation: At the G_0 generation, a total of 834 acclimatized somatic variants from five genotypes were planted at the BSRI breeding field, of which 520 survived in 2017-18 (Table 2). Intercultural

operations such as weeding, mulching, fertilizer and insecticide treatment were carried out on a need-to-know basis. Following cane maturity, 83 somaclones were selected from the G_0 generation based on their millable canes/clump and growth vigour, and were planted in a 1m x 4m plot at the G_1 generation for further assessment in 2018-19. The following year's selection criteria included germination percentage, quantity of millable canes per plot, field brix percentage (Brix%), and blooming behavior. 18 variations out of 83 shown superior performance in this G_1 generation; these were selected and planted in a 4m × 4m plot at the G_2 generation in the year 2019-20 for further evaluation. Fig. 2 depicts the field evaluation and somaclonal scenario. Between the G_0 and G_2 generations, it was noticed that some somaclones of Co 65-02, Cp 44-101, Cp 48-103, and Q 69 exhibited profuse flowering despite the donor plants being non-flowering. Following field evaluation, three flowering somaclones were chosen, each one from Cp 44-101, Cp 48-103 and Q 69.

Table 2. Effect of hormones on somaclones development

Sl.	Name of the Clones	% Callus induction	Number of shoots/culture	Average shoot length at 15th Day (cm)	Number of survived somaclones in field
1.	Co 62-101	48	22.25	3.84	46
2.	Co 65-02	68	32.67	3.25	220
3.	CP 44-101	76	9.35	3.22	38
4.	Cp 48-103	56	36.13	2.98	140
5.	Q 69	54	18.82	3.15	76

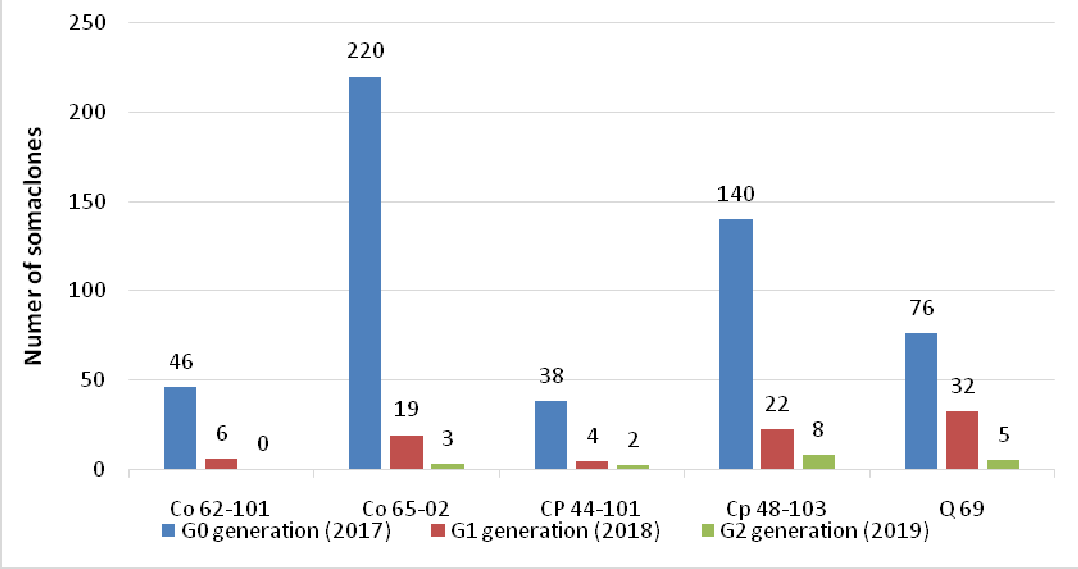

Fig. 2. Evaluated somaclones in the field condition at different stages

Conclusion

Somaclonal variation is an efficient way of creating variability in the existing plant genetic resources. Desirable change in terms of flowering behavior of three germplasm was observed in this study. We also observed genotypes perform differently in case of callus induction, shooting initiation and root development. The developed three flowering somaclones will be used for conventional breeding approach for improvement of sugarcane germplasm. Therefore, it can be concluded that somaclonal variation is an efficient way of utilizing non-flowering germplasm of sugarcane in varietal improvement.

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