Bangladesh J. Sugarcane, 36 : 37-47 *June, 2015*

Improvement of Sugarcane Germplasm through Somaclonal Variation

R. Alam*, M.A. Rahman, K.M.R. Karim, H.M. Tarique, R.K. Ganapati, A.M.S. Rahman and Sk.A. Mannan
Breeding Division,
Bangladesh Sugarcrop Research Institute, Ishurdi-6620, Pabna, Bangladesh

ABSTRACT

The research was conducted at Bangladesh Sugarcrop Research Institute (BSRI) for the improvement of ten sugarcane germplasm through somaclonal variation. Among them 7 indigenous and 3 exotic germplasm were selected for the study. To create genetic variability the leaf sheath explants of donor plants were placed on modified MS medium supplemented with 4.0 mgl^{-1} 2,4-D, callus was initiated within 7-10 days of culture. MS medium supplemented with 1.0 mgl^{-1} BA + 0.5 mgl^{-1} NAA was used for shoot regeneration and 5.0 mgl^{-1} NAA was used for rooting from micro shoots. In total, 2064 somatic variants were acclimatized and planted in the field for evaluation. On the basis of field evaluation for four years under three generations 18 variants were selected for the characters of profuse flowering, resistant (R)/ moderately resistant (MR) to red rot disease and as the source of foreign gene pool from degenerating parent. The selected variants were significantly differed from each other for the characters studied.

Key words : Sugarcane, somaclonal variation, callus, regeneration, acclimatization

INTRODUCTION

The growth of plant cells *in vitro* and their regeneration into whole plants is an asexual process that involves only mitotic division of the cells. 'Somaclonal variation' was coined to refer to the genetic variation among such plants. This variation is important for its control and possible suppression with the aim of producing genetically identical plants, and for its use as a tool to produce genetic variability, which will enable breeders the genetic improvement. Somaclonal variation provides a valuable source of genetic variation for the improvement of crops through the selection of novel variants (Unai *et al.* 2004). Breeder can obtain advantage from cross incompatible plants by this process to develop new genotypes. Variety development programme of sugarcane mainly depends on hybridization with a limited number of parent materials followed by selection; it required several years to complete selection cycle. Besides, cross incompatibility among different species and requirements of flowering plants is also the main constraint for successful sugarcane hybridization. Somaclonal variant plant production technique offers to circumvent sexual barriers in plant breeding. The *in vitro* culture conditions of somatic tissues can be mutagenic and regenerated plants derived from organ cultures, calli, protoplasts and somatic embryos sometimes can show phenotypic and genotypic

* Corresponding author: R. Alam, Senior Scientific Officer,
e-mail: alamr69@yahoo.com

variation (Orbovic, 2008). Skirvin (1994) reported that some, or all, of the somaclones may be physically different from the stock donor plants. Usually, variability occurs spontaneously and can be a result of temporary changes or permanent genetic changes in cells or tissue during *in vitro* culture. Temporary changes result from epigenetic or physiological effects and are nonheritable and reversible (Kaeppler, 2000). In contrast, permanent changes are heritable and often represent expression of pre-existing variation in the source plant (Larkin, 1981). This investigation was attempted to take advantage from some valuable but endangered indigenous and exotic sugarcane germplasm through *in vitro* culture following the objectives:

1. Development of somaclonal variants from degenerating exotic sugarcane germplasm *in vitro* for getting superior one
2. Selection of flowering variants to broaden the hybridization scope

MATERIALS AND METHODS

The study was conducted at the laboratory and field of Bangladesh Sugarcrop Research Institute (BSRI) in the year of 2005-11. Ten valuable and endangered sugarcane germplasm viz., Isd 2-54, BS 96, LJ-C, Isd 16, Isd 17, Isd 18, NSM, CP 50-70, L 5 and Ni 8 were subjected in this study as parent materials. Salient feature of those are shown in Table 1.

Leaf sheath as explant of 6-8 months old field grown plants was collected aseptically following plant tissue culture technique for *in vitro* culture. MS and Modified MS (MMS) medium supplemented with auxin and cytokine were used to develop somaclonal variants. 2,4-D (4.0 mgl^{-1}) supplemented MMS medium was used for de-differentiation of tissues to develop callus from the explant. But re-differentiation of callus to form organ (shoots and roots) was done on MS medium supplemented with auxin and cytokine alone or in combinations. pH of the medium was adjusted to 5.7 and autoclaved at 121 ^{0}C for 20 minutes. The cultures were incubated in an air conditioned culture room at 25±1^{0}C and illuminated by white fluorescent tubes of intensity about 3000 lux. After development of *in vitro* plantlets the culture vessels were transferred from control to normal condition and makes them unplugged for three days. The plantlets were pull-out from medium, thoroughly washed the roots under running tap water and pricked up at garden soil containing cel-u-pack to acclimatize at natural environment under plastic covered condition. Acclimatized somatic variants (seedlings) were planted in the field under G_0 generation maintaining 1.0 m row to row and 0.5m plant to plant distance in 2005-06 cropping season. After field evaluation in respect of millable canes/clump and vigour of growth selected variants were planted in 1m x 4m plot at G_1 generation for further evaluation. On the basis of germination percentage (G%), number of tillers per hector (NT 10^3 ha^{-1}), number of millable canes per hector (NMC10^3 ha^{-1}), field brix per cent (Brix %) and cane yield (tha^{-1}) selection was done at G_1 generation and selected variants were planted in 4m x 4m plot at G_2 generation for further evaluation. The replicated trials were done at G_3 generation with the selected variants of G_2 generation. Pathological test of the selected variants was done at G_3 generation by pathology division of BSRI. Data on G% and NT 10^3 ha^{-1} were collected after 90 and 150 DAP and, NMC10^3 ha, Brix % and cane yield (tha^{-1}) characters at the time of harvest. Collected data were analyzed following the methods of Singh and Singh (1979).

Table 1. Salient feature of parent materials

Germplasm	Source of collection	Importance	Reason of culture
Isd 2-54	Institute bred	1. High yielding and well adopted 2. Resistant to red rot disease	1. Non-flowering 2. Unable to use in hybridization programme
BS 96	Institute bred	1. High yielding and well adopted 2. Resistant to red rot disease	1. Non-flowering 2. Unable to use in hybridization programme
LJ-C	Local collection	1. Well adopted 2. Resistant to red rot disease	1. Non-flowering 2. Unable to use in hybridization programme
Isd 16	Institute bred	1. High cane and sugar yielding 2. Flowering and early maturing	1. Susceptible to red rot disease
Isd 17	Institute bred	1. High cane and sugar yielding 2. Self detrashing	1. Non-flowering 2. Unable to use in hybridization programme 3. Highly susceptible to red rot disease
Isd 18	Institute bred	1. High cane and sugar yielding 2. Good ratooner	1. Non-flowering 2. Unable to use in hybridization programme 3. Highly susceptible to red rot disease
NSM	Local collection	1. High sucrose contain 2. Huge tillering behaviour	1. Non-flowering 2. Unable to use in hybridization programme 3. Highly susceptible to red rot disease
CP 50-70	Exotic source	1. Source of foreign gene 2. Detrashing nature	1. Non-flowering 2. Unable to use in hybridization programme
L 5	Exotic Source	1. Source of foreign gene 2. Thick and high sucrose contain	1. Non-flowering 2. Unable to use in hybridization programme 3. Highly susceptible to red rot disease
Ni 8	Exotic source	1. Source of foreign gene 2. High sucrose contain 3. Soft fiber	1. Non-flowering 2. Unable to use in hybridization programme 3. Highly susceptible to red rot and wilt disease

RESULTS AND DISCUSSION

Callusing of explants

The explants of all varieties/genotypes were cultured in MMS medium supplemented with 4.0 mgl^{-1} 2,4-D for induction of callus. In this medium callus formation was started within 7-10 days of culture and after 3 weeks 70 to 90% of total volume of explant formed into callus. Maximum of the cultured explants formed loose compact, creamy white and embryogenic callus which were capable of regenerating plants (Figure 3.A). Similar results were also reported by Khan *et al.* (1998). Previously Alam *et al.* (2003) work with 3.0 mgl^{-1} 2,4-D in MMS medium for callusing of sugarcane. It was observed that quantity and quality of callus were equally important for obtaining good regeneration. Distinction between ebryogenic and non-embryogenic callus was performed on the basis of callus external aspect as reported by Gandonou *et al.* (2005).

Regeneration of shoots from the callus

Two to three weeks aged calli were cultured on MS medium supplemented with 1.0 mgl^{-1} BA and 0.5 mgl^{-1} NAA to regenerate shoots. On this medium maximum cultured calli produced green plantlets while some of them exhibited both albino and green plantlets (Figure 3.B). The callus of different genotypes was responded differently to initiate shoots within 7-12 days of culture. In this study highest number of shoots/culture 26.0 ± 1.37 were regenerated from the callus of CP 50-70 and lowest 7.82 ± 0.09 by the callus of Isd 17 after four weeks of culture. The highest shoot length 2.07 ± 0.08 was exhibited by the variants of Isd 17 whereas, NSM the lowest 0.87 ±0.24 (Table 2). The regenerated shoots were multiplied in liquid MS medium using same hormonal combination (Figure 3.C). This hormonal combination was suitable for regeneration of shoots from the callus of sugarcane reported by Alam *et al.* (2002). Karim *et al.* (2002) found better shooting response from the callus of sugarcane on MS supplemented with 1.0 mgl^{-1} BA and 0.5 mgl^{-1} IBA. Seema *et al.* (2011) reported that regeneration started with the appearance of green dots on callus within a week on regeneration medium when works with different growth regulators. Callus derived albino plantlets confirmed the induction of genetic variability was reported by Shepard *et al.* (1980).

Root induction

The well developed microshoots (2.5-4 cm height) were rooted on 5.0 mgl^{-1} NAA supplemented MS medium. In this medium the plantlets of all the varieties/genotypes produced average 3-5 numbers of roots within 9-15 days of culture (Figure 3.D). Efficient root formation of sugarcane micoshoots on 5.0 mgl^{-1} NAA supplemented MS were reported by Kuasha *et al.* (2012), Karim *et al.* (2003) and Alam *et al.* (2003). Using IBA at different concentration in MS medium with 4.0 gl^{-1} sucrose also reported by Seema *et al.* (2011) and Khan *et al.* (2009) for rooting of sugarcane microshoots. Only well developed plantlets showed root growth from the nodal primordia reported by Khan *et al.* (1998). The complete microshoots were acclimatized to the natural environments under plastic covered moist condition.

In total 2329 acclimatized somatic variant from ten varieties/genotypes were planted at BSRI breeding field at G_0 generation among them 2064 were survived (Figure 1). Intercultural operations like irrigation, weeding, mulching, fertilizer and insecticide

application etc were performed as and when required. After maturity of canes 78 somaclones were selected from G_0 generation in respect of millable canes/clump and vigourity of growths, and planted in 1m x 4m plot at G_1 generation for further evaluation. Next year selection was made on the basis of germination percentage (G%), number of tillers per hector (NT 10^3 ha), number of millable canes per hector (NMC10^3 ha), field brix percentage (Brix %) and cane yield (tha^{-1}) characters. From this generation 22 variants out of 78 showed better performance than others; those were selected and planted in 4m x 4m plot at G_2 generation in the year of 2008-2009 for further evaluation. After evaluation of G_2 generation 18 variants were selected and planted at G_3 generation, in 2009-10. Field evaluation and selection of variants have been shown in Figure 2.

It was observed from G_0 to G_3 generation that the somaclones of Isd 17 T_7, Isd 18 T_1 and Isd 18 T_2, CP 50-70 T_5, L 5 T_1 and NSM T_1 showed profuse flower though the donor plants were non-flowering (Figure 3.E and 3.F). A permanent genetic change was received by these somaclones in cells or tissue during *in vitro* culture. According to Sengar *et al.* (2011) flowering variability of these six somaclones was obtained by passing the genotypes through tissue culture cycle. These six flowering variants were included in breeding nursery of BSRI and utilizing in variety development programme.

Analysis of variance for five characters of the selected variants and respective parent was done according to Singh and Singh (1979) and shown in Table 3. It is clear that genotypic item was highly significant against all the characters included in this study, indicated that the tested materials were significantly different from each other in respect of these characters. Which illustrate sugarcane genotypes callus derived via somaclones showed variation from their parents. Similar result was reported by Skirvin *et al.* (1994) and Orbovic *et al.* (2008) when working with sugarcane somaclones.

Red rot is a severe disease for sugarcane caused by *Colletotrichum falcatum* Went; it may damage the total cane production. So, the breeders have to work for red rot disease resistant genotypes. Hence, the selected somaclones were tested against red rot disease reaction by pathology division and results are shown in Table 4. It is evident from the table 4 that six variants *viz.* Ni 8 T_2, Isd 18 T_1 and Isd 18 T_2, LJC T_1, LJC T_2 and LJC T_3 are resistant (R)/moderately resistant (MR) to red rot disease. Izquierdo *et al.* (1988) found regenerated plantlets having high to moderate resistance to rust disease using leaf explants of susceptible plant. The exotic donor genotype of Ni 8 did not survived in our environment though bearing some importance. Its four somaclones viz., Ni 8 T_1, Ni T_2, Ni T_3 and Ni T_4 were found suitable and growing frequently in the field obviously this is the improvement of the genotype. The improvement of Ni 8 through somaclonal variation is agreed with the findings of Karp (1995); Mehta and Angra (2000); Predieri (2001) and Unai *et al.*(2004).

Regarding tested characters most of the variants showed better mean performance compare to their respective parent, mean data were presented in the Table 4 with C.D. (critical difference) values. This table showed that among the four variants of Ni 8, Ni 8 T_2 revealed highest cane yield 101.81 (tha^{-1}) followed by Ni 8T_3 (96.69 tha^{-1}) and Ni 8 T_1 (94.49 tha^{-1}) which were significantly different against parent yield of 67.38 t ha^{-1}. The variants of Isd 16 T_1 (93.90 tha^{-1}) and Isd 16 T_4 (104.16 tha^{-1}) showed 18%

and 26% higher yield than parent (82.40 tha^{-1}). In the midst of three variants of Isd 18, the yield of Isd 18 T_2 (122.00 tha^{-1}) and Isd 18 T_1 (102.81 tha^{-1}) was highly significant when tested againt it's parent yield of 84.24 tha^{-1}. Out of seven variants of LJ-C, LJ-C T_3 revealed significantly highest cane yield of 122.18 tha^{-1} followed by LJ-C T_2 and LJ-C T_1 with 113.53 and 102.95 tha^{-1}, respectively where as its parent showed 52.28 tha^{-1}. Mean differences in percentage (%) of the somaclones comparing to their parent for five characters were summarized in the Table 5. From this table it is apparent that the somatic variants of Ni 8 T_1, Ni T_2, Isd 18 T_3 and LJ-C T_{1-7} showed significantly different compared to their parent in respect of G%. In case of NT and NMC the variants of Ni 8 T_1, Ni T_2, Ni T_3, Isd 18 T_3 and LJ-C T_1, T_2 and T_3 showed significant difference to the respective parent as they showed more than 20% mean difference against parent. But in respect of brix% it was less than 20%, so for this character somaclones are analogous to their parent.

From the present study it can be concluded that somaclonal variation technique is supportive to develop variants having higher cane yield potential, disease resistant and flowering behavior from susceptible and non-flowering parent. Development of somaclonal variants may act as alternative to conventional breeding for developing improved sugarcane varieties.

Table 2. Effect of MS supplemented with BA (1.0 mgl^{-1}) in combination with NAA (0.5 mgl^{-1}) on growth and differentiation of shoots from callus after 3 weeks of culture

Variety/ Genotype	No. of shoots/ culture (Mean ± SE)	Shoots Length (Mean ± SE)	Variety/ Genotype	No. of shoots /culture (Mean ± SE)	Shoots Length (Mean ± SE)
Isd 2-54	17.3± 0.71	1.20 ± 0.05	Isd 18	23.75 ± 1.08	1.52 ±0.18
Isd 16	22.8 ± 0.29	1.42 ± 0.13	NSM	19.66 ± 0.44	0.87 ±0.24
BS 96	9.04 ± 1.11	1.73 ± 0.36	CP 50-70	26.0 ± 1.37	1.48 ±0.44
LJ-C	15.65 ± 0.32	1.55 ± 0.29	L 5	21.59 ± 3.26	1.72 ±0.06
Isd 17	7.82 ± 0.09	2.07 ± 0.08	Ni 8	22.04 ± 0.81	1.15 ±0.21

Table 3. Analysis of variance of sugarcane genotypes (somaclones and donor plants) for different characters

Items	df	G %		NT (10^3 ha^{-1})		NMC (10^3 ha^{-1})	
		MS	F	MS	F	MS	F
Geotypes	21	137.07	5.79**	735.26	4.35**	566.42	3.76**
Repli	2	276.77	11.68**	2967.21	17.56**	2599.40	17.26**
Error	42	23.69		168.98		150.60	
Total	65						

Items	df	Brix %		Yield (tha^{-1})	
		MS	F	MS	F
Geotypes	21	7.39	11.48**	1129.65	7.56**
Repli	2	1.72	2.67**	2435.02	16.30**
Error	42	0.64		149.37	
Total	65				

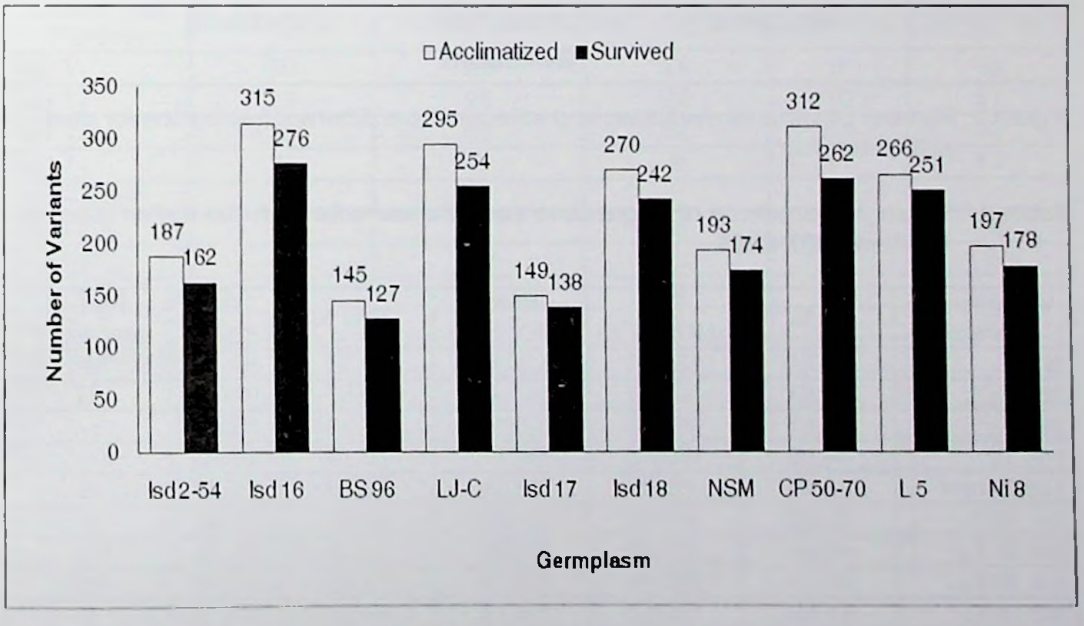

Figure 1. Number of acclimatized variants and their survivability in the field

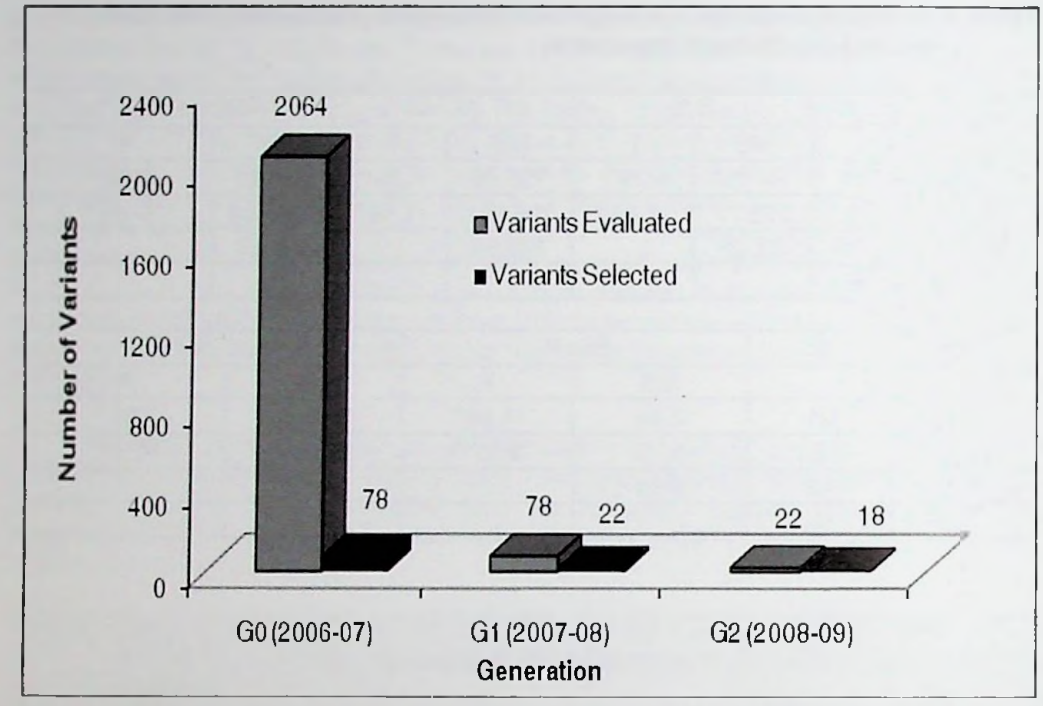

Figure 2. Number of variants evaluated and selected from different generation for next year plantation

Table 4. Mean performance of sugarcane somatic variants with the donor plant at BSRI in 2007-2009

Variants with Parents	G %	NT (10^3 ha^{-1})	NMC (10^3 ha^{-1})	Brix %	Yield (tha^{-1})	Red rot reaction **
Ni8 T_1	50.00	130.42	120.63	24.33	94.49	MS
Ni8 T_2	53.67	121.04	111.46	23.67	101.81	R
Ni8 T_3	54.67	119.38	113.75	23.53	96.69	-
Ni8 T_4	44.00	105.63	99.17	23.78	76.03	-
Ni8 Parent	42.00	96.88	89.54	21.50	67.38	S
Isd 16 T_1	51.62	124.38	91.46	22.67	93.90	S
Isd 16 T_2	44.44	125.63	86.25	21.20	87.23	S
Isd 16 T_3	45.14	121.46	84.79	23.33	97.62	MS
Isd 16 T_4	41.67	113.13	89.79	23.20	104.16	S
Isd 16 Parent	41.20	114.67	89.21	20.67	82.40	S
Isd18 T_1	38.00	90.42	87.50	21.00	102.81	R
Isd18 T_2	38.00	120.00	114.38	22.20	122.00	MR
Isd18 T_3	42.67	87.50	90.21	19.53	82.15	-
Isd18 Parent	34.67	90.42	90.21	19.33	84.24	HS

Variants with Parents	G %	NT (10^3 ha^{-1})	NMC (10^3 ha^{-1})	Brix %	Yield (tha^{-1})	Red rot reaction **
LJC T_1	39.00	114.38	104.17	20.85	102.95	R
LJC T_2	49.00	107.92	101.67	21.34	113.53	R
LJC T_3	39.00	118.54	108.13	21.33	122.18	R
LJC T_4	35.67	81.25	75.42	20.67	57.82	-
LJC T_5	42.67	90.42	82.92	20.80	60.81	-
LJC T_6	37.67	90.42	84.38	21.00	75.94	-
LJC T_7	36.67	101.67	94.17	18.25	78.47	-
LJC Parent	26.33	81.25	63.75	20.50	52.28	MR
Grand mean	42.17	106.67	94.22	21.58	88.95	
C.D (0.5)	6.87	18.36	17.33	1.13	17.26	

** R = Resistant to red rot disease and MR = Moderately resistant to red rot disease
S = Susceptible to red rot disease, MS = Moderately susceptible to red rot disease

Table 5. Mean differences of the somaclones (in percentage) comparing to their respective parent

Variants with Parents	G %	NT (10^3 ha^{-1})	NMC (10^3 ha^{-1})	Brix %	Yield (tha^{-1})
Ni8 T_1	30	23	24	9	43
Ni8 T_2	28	25	22	10	51
Ni8 T_3	19	35	32	13	40
Ni8 T_4	5	9	8	11	13
Ni8 Parent					
Isd 16 T_1	-6	6	-5	13	18
Isd 16 T_2	-8	10	-3	3	6
Isd 16 T_3	10	8	3	10	14
Isd 16 T_4	-15	-1	1	12	26
Isd 16 Parent					
Isd18 T_1	10	0	4	9	22
Isd18 T_2	10	33	34	15	45
Isd18 T_3	23	-3	7	1	-2
Isd18 Parent					
LJC T_1	48	41	63	2	97
LJC T_2	86	33	59	4	117
LJC T_3	48	46	70	4	134
LJC T_4	35	0	18	1	11
LJC T_5	62	11	30	1	16
LJC T_6	43	11	32	2	45
LJC T_7	39	25	48	-11	50
LJC Parent					

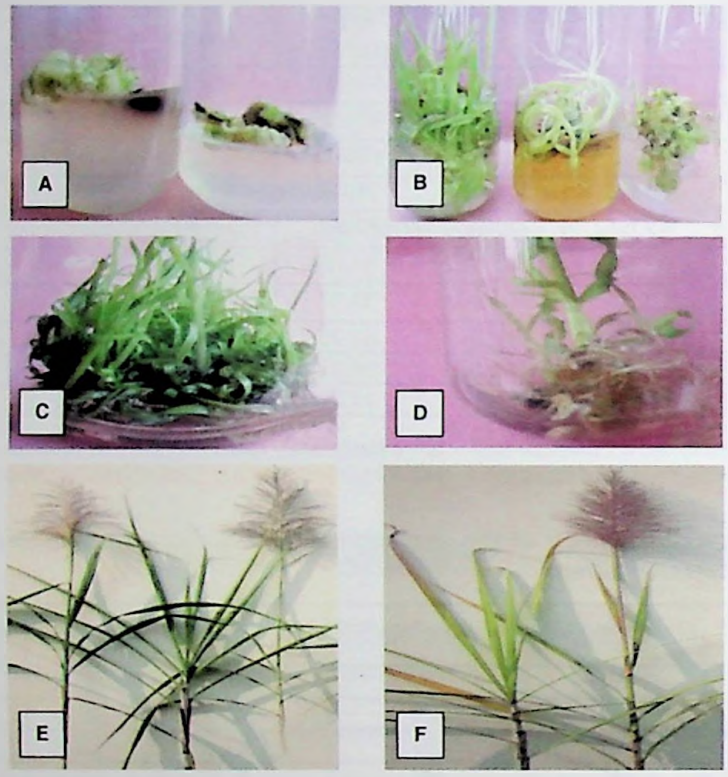

Figure 3. Development of somaclonal variant plant (A-D) and selection upon flowering behaviour (E-F)

A: Explant formed callus

B: Shoot initiation from callus, albino plantlets and shoot multiplication in solid MS

C: Multiplication of shoots in liquid MS

D: Rooting of microshoots

E: Flowering variants and non-flowering donor plant of Isd 18

F: Flowering variant and non-flowering donor plant of Isd 17

REFERENCES

Alam, R.; Rahman, A.B.M.M. and Gupta, S. 2002. *In vitro* plant regeneration from leaf sheath cultures of sugarcane via organogenesis. *Pl. Cell Biotech. and Mole. Biol.*, **3** (3&4): 131-136.

Alam, R.; Mannan, Sk.A.; Karim, Z. and Amin, M.N. 2003. Regeneration of sugarcane (*Saccharum officinarum*) plantlet from callus. *Pak. Sugar Journal,* **58**(1): 15-19.

Baksha, A.K.M.R.; Alam, R.; Karim, Z.; Mannan, S.K.; Rahman, A.B.M.M. and Gupta, S. 2003. Anther culture and plant regeneration in sugarcane *(Saccharum officinarum*). *Pl. Cell Biotech. and Mole. Biol.*, **4**(3&4): 179-184.

Larkin, P. and Scowcroft, W. 1981. Somaclonal variation a novel source of variability from cell cultures for plant improvement. *Theoretical and Applied Genetics*, **60**: 197-214.

Orbovic, V.; Calovic, M.; Viloria, Z.; Nielsen, B.; Gmitter, F.; Castle, W. and Grosser, J. 2008. Analysis of genetic variability in various tissue culture-derived lemon plant populations using RAPD and flow cytometry. *Euphytica*, **161**: 329-335.

Skirvin, R.M.; McPheeters, K.D. and Norton, M. 1994. Sources and frequency of somaclonal variation. *Hort. Science*, **29**:1232-1237.

Kaeppler, S.M.; Kaeppler, H.F. and Rhee, Y. 2000. Epigenetic aspects of somaclonal variation in plants. *Plant Molecular Biology*, **43**:179-188.

Karim, M.Z.; Alam, R.; Baksha, R.; Paul, S.K.; Hossain, M.A. and Rahman, A.B.M.M. 2000. *In vitro* clonal propagation of sugarcane (*Saccharum officinarum*) variety Isd 31. *Pak. J. Biol. Sci.*, **5**(6):659-661.

Mahmud, K.; Nasiruddin, K.M.; Hossain, M.A.; Hassan, L. and Basar, M.K. 2012. Development of somaclones of sugarcane through biotechnological technique in Bangladesh. *Proc. ISNPSR*, 15-18 Oct. 2012, SBI, Coimbator, India. pp. 9-10.

Mehta, Y.R. and Angra, D.C. 2000. Somaclonal variation for disease resistance in wheat and production of dihaploids through wheat x maize hybrids. Genetics and *Molecular Biology*, **23**:617-622.

Predieri, S. 2001. Mutation induction and tissue culture in improving fruits. *Plant Cell Tissue Organ Culture*, **64**:185-210.

Karp, A. 1995. Somaclonal variation as a tool for crop improvement. *Euphytica*, **85**: 295-302.

Unai, E.; Iselen, T. and Garcia, E. 2004. Comparison of characteristics of bananas (*Musa sp.*) from the somaclone CIEN BTA-03 and its parental clone Williams. *Fruit*, **59**: 257-263.

Gandonou, C.; Abrini, J.; Idaomar, M. and Skali, S.N. 2005. Response of sugarcane (*Saccharum* sp.) varieties to embryogenic callus induction and *In vitro* salt stress. *Afr. J. Biotech.*, **4** (4): *350*-354.

Shepard, J.F.; Bidiney, D. and Shahin, E. 1980. Potato protoplast in crop improvement. *Science*, **208**: 17-24.

Khan, I.A.; Khatri, A.; Ahmad, S.M.; Siddiqui, S,H; Nizamani, G.S.; Khanzada, M.H.; Dahar, N.A. and Khan, R. 1998. *In vitro* mutagenesis in sugarcane. *Pak. J. Bot.*, **30** (2): 253-261.

Khan, I.A.; Gaj, M.D. and Maluszynski, M. 1999. *In Vitro* mutagenesis in sugarcane callus culture. *Mutation Breeding Newsletter*, **44**: 19-20.

Khan, I.A.; Dahot, M.U.; Seema, N.; Yasmine, S.; Bibi. S. and Khatri, A. 2009. Genetic variability in sugarcane plantlets developed through *In vitro* mutagenesis. *Pak. J. Bot.*, **41 (**1): 153- 166.

Seema, N.; Oad, F.C.; Khan, I.A.; Tunio, S.; Siddiqui, M.A.; Yasmin, S.; Khatri, A. and Bibi, S. 2011. Influence of phytohormone on the organogenesis of sugarcane. *Pak. J. Bot.*, **43** (3): 1531-1534.

Singh, R.K. and Chaudhary, B.D. 1979. Biometrical methods in quantitative genetic analysis. Rev. Edi., Alyani Publishers, India. pp. 39-57.