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Full Length Article

Induction of Somaclonal Variation in Selected Drought Sensitive Genotype of Sugarcane (*Sachharum officinarum*)

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Abstract

In this research study, induction of somaclonal variation in selected sugarcane genotype, CPF-248 was investigated. For this purpose, five media for callogenesis were studied and embryogenic, fragile calli were achieved on CIM3 (7.75 mg/L 2, 4-D). So, calli of three different age groups; 35 days old calli,45 days old calli and 55 days old calli, induced on this callus induction medium (CIM3) were shifted to four regeneration media. Among these media few plants were achieved from 35 days old calli on shifting to RM1 (BAP 3.25 mg/L and casein hydrolysate1.75 mg/L). Hence, for *in vitro* selection of drought tolerant somaclonal variant plants, RM1 was supplemented with 7% PEG (IVSM1), 8% PEG (IVSM2), 9% PEG (IVSM3) and 10% PEG (IVSM4). On these media, two plants were achieved and named as *in vitro* selected putative drought tolerant somaclonal variants (IPSV). These plants were then subjected to genomic as well as physiological analyses. Genomic analysis revealed that the plant obtained from IVSM1 (IPSV1) showed different DNA banding pattern to its parent plant while plant from IVSM2 (IPSV2) gave same DNA profile as parent genotype (CPF-248). Whereas physiological analyses showed IPSV1 has more water potential, osmotic potential, turgor potential, CO_2 assimilation rate, transpiration rates water use efficiency and relative leaf water contents as compared to its parent genotype as well as IPSV2. © 2018 Friends Science Publishers

Keywords: Sugarcane; Drought stress; Somaclonal variation; *In vitro* selection; Water relation
Abbreviations: IPSV: *in vitro* selected putative drought tolerant somaclonal variants; CIM: Callus Induction Medium; RM: Regeneration Medium; IVSM: *in vitro* selection Medium; PEG: Polyethylene glycol; 2, 4-D: 2,4-Dichlorophenox acetic acid; WUE: Water use efficiency; RWC: Relative leaf water contents; BAP: Benzyleaminipurine; PCR: Polymerase Chain Reaction; RAPD: Random amplified polymorphic DNA

Introduction

Sugarcane is a tall perennial grass with a stem height ranging from 2−6 m. It belongs to the monophyletic grass family Poaceae also known as Graminaea (a family of cereals, grasses and bamboos including approximately 12000 species) (Christenhusz and Byng, 2016*)*. Commercially cultivated Sugarcane botanically known as *Saccharum officinarum* is an interspecific aneuploid hybrid of *S. spontanum* and *S. officinarum* (Wang *et al*., 2010). With high levels of heterozygosity, it is a polysomatic crop which is clonally reproduced (Bakker, 1999). Sugarcane has gained the status of the imperative cash crop since remote past. At present, it is fulfilling the 80% of the worldwide sugar need. This crop is gaining importance globally for its sugar production as well as an alternative bioenergy source because of its massive dry mass production. As a C4 plant, its efficient photosynthesis and proficient production of biomass make this crop an excellent target for industrial processing and a substantial alternative for animal feed in tropical and subtropical areas of the world. There is a need to increase its productivity and quality. In spite of the massive demand, the ideal production target, per acre harvest and quality enrichment of sugarcane have not been attained.

Scarcity of water as one of the crucial environmental factor is aggravating the situation in terms of productivity of sugarcane (Venkataramana *et al*., 1986) While effective management of irrigation water is of crucial importance. There is need to reduce the wasteful usage and to reduce the production costs and sustainability (Carr and Knox, 2011). Thus, the production and maintenance of drought tolerant sugarcane germplasm is a crucial need to combat the drought stress. The use of conventional methods for the improvement of crops to combat different stresses has its importance since remote past. But these methods for sugarcane has some limitations due to its complex genome which includes long duration requirement. Its narrow genetic base and poor fertility (Suprasanna *et al*., 2011).

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Therefore, this study has been designed for the induction of somaclonal variation in drought sensitive sugarcane genotype. For this purpose, a newly approved sugarcane genotype, CPF-248 was selected because of its good agronomic features such as high yield and excellent sugar recovery and susceptible to abiotic stresses particularly drought.

Material and Methods

Drought sensitive genotype CPF-248 was selected on the basis of data provided by Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. For *in vitro* studies, different tissue culture media supplemented with combinations of growth regulators were prepared for callus induction, shoot formation and root induction.

Callogenesis

Five callus induction media were prepared as Basal MS salts, Sucrose, Myoinositol, Nicotinic acid, Glycine, Thymine HCl and Pyridoxine HCl containing 2, 4 –D as 5.75 mg/L, 6.75 mg/L, 7.75 mg/L, 8.75 mg/L and 9.75 mg/L (Table 1). pH was adjusted to 5.7−5.8 and Gellan gum powder was used to solidify the media. The media was autoclaved for 20 min at 121°C temperature and 15Psi pressure and pour into the petri plates.

For explant preparation, six-month-old, healthy sugarcane plants were selected and washed in running tapped water for 2−3 times and surface sterilization was done with 70% ethanol. Young immature leaf pieces were used as explant such as leaf rolls were peeled and cut into cylindrical pieces of approximately 2−3 mm in diameter under sterile conditions in laminar air flow and cultured on different callus induction media (CIM) as mentioned above. These plates were placed at 26±1ºC under dark condition. Sub culturing was done on to fresh callus induction media (CIM) biweekly at least three times.

In Vitro Regeneration

For regeneration studies, MS media containing Basal MS salts, Sucrose, Myoinositol, Nicotinic acid, Glycine, Thymine HCl, Pyridoxine HCl was used supplemented with different levels of BAP such as 3.25 mg/L, 4.25 mg/L, 5.25 mg/L, 6.25 mg/L) in combination with 1.75 mg/L casein hydrolysate along with (Table 2). pH was adjusted to 5.7−5.8 and Gellan gum powder was used to solidify the media. Media were autoclaved at 121°C, 15 *psi* for 20 min and poured into the tubes. Proliferated calli of 35, 45 and 55 days old, were shifted to regeneration media (RM) and incubated at 26±1ºC in 16/8 light and dark conditions. The proliferated calli were transferred on to fresh regeneration media (RM) biweekly until the development of shoots (6 weeks).

Table 1: Callus induction media (CIM) used for callogenesis

Ingredients	Callus induction media				
	CIM1	CIM2	CIM3	CIM4	CIM5
MS Salts (g/L)	4.33	4.33	4.33	4.33	4.33
Sucrose (g/L)	30	30	30	30	30
2,4-D (mg/L)	5.75	6.75	7.75	8.75	9.75
Myo-inositol (g/L)	0.1	0.1	0.1	0.1	0.1
Thiamine HCl (g/L)	2	2		2	2
Nicotinic acid (g/L)	1	1	1	1	1
Glycine (g/L)	4	4	4	4	4
Pyridoxine HCl (g/L)	1	1	1	1	1
Gellun gum powder (g/L)	2.66	2.66	2.66	2.66	2.66

Table 2: Regeneration media (RM) with different plant growth regulators (BAP and casein hydrolysate)

Ingredients	Regeneration media			
	RM1	RM2	RM3	RM4
MS salt (g/L)	4.33	4.33	4.33	4.33
Sucrose (g/L)	30	30	30	30
Casein hydrolysate (mg/L)	1.75	1.75	1.75	1.75
BAP (mg/L)	3.25	4.25	5.25	6.25
Myo-inositol (g/L)	0.1	0.1	0.1	0.1
Thiamine HCl (g/L)	2	2	2	2
Nicotinic acid (g/L)	1	1	1	1
Glycine (g/L)	4	4	4	4
Pyridoxine HCl (g/L)	1	1	1	1
Gellun gum powder (g/L)	2.66	2.66	2.66	2.66

Table 3: Root induction media

½ MS Medium	Concentration
MS Salts (g/L)	2.165
Sucrose (g/L)	30
Myo-inositol (g/L)	0.1
Thiamine HCl (g/L)	2
Nicotinic acid (g/L)	1
Glycine (g/L)	4
Pyridoxine HCl (g/L)	1
Gellun gum powder (g/L)	2.66

Rooting and Acclimatization

For the development of profuse rooting system, ½ strength MS media was prepared in which Basal MS salts, Sucrose, Nicotinic acid, Glycine, Thymine HCl, Pyridoxine HCl were used (Table 3). Plantlets were shifted to ½ strength MS medium, for root induction. For acclimatization, plants were shifted to polythene bags containing peat moss and kept in green house.

In Vitro Selection of Drought Tolerant Plants

For *in vitro* selection of drought tolerant plants, MS media supplemented with 7%, 8%, 9% and 10% PEG (polyethylene glycol: molecular weight 6000) was used. These *in vitro* selected plants were then micropropagated on micropropagation medium reported by Ijaz *et al*. (2012).

RAPD Based Genome Analysis of *in Vitro* Selected Drought Tolerant Plants

For analyzing genetic variability of *in vitro* selected drought tolerant plants, RAPD analysis was performed. Genomic DNA of *in vitro* selected drought tolerant plants and parent genotype CPF-248 was isolated by using the protocol as described by Palotta *et al.* (2000).

RAPD (PCR) Analysis

For determining genetic variability, PCR analysis of *in vitro* selected drought tolerant plants and parent genotype CPF-248 was performed. Three highly polymorphic RAPD primers (GL Decamer K-07, AGCGAGCAAG; GL Decamer L-01, GGCATGACCT and GL Decamer L-08, AGCAGGTGGA) were used as these primers are based on diversity analysis (conducted in our lab, for PhD thesis) Each PCR was carried out in a 20 µL reaction volume, containing d3H2O (12.8 µL), 10X Taq buffer (2 µL), MgCl2 (2 µL), dNTPs 2 µL), *Taq* DNA polymerase (0.2 µL, primers (2 µL), and Template DNA (1 µL). PCR conditions were set as 94°C for 5 min, 40 cycles comprising of denaturation at 94°C for 1 min, annealing of primer at 36°C for 1 min and extension at 72°C for 2 min. The final extension was carried out at 72°C for 10 min. Resolving of PCR product was done by gel electrophoresis. Gels were visualized and photographed using Gel Documentation System (GDS) of Bio-Rad.

After these experiments, the *in vitro* selected drought tolerant plants were subjected to physiological analyses and compared with field grown parent plant (CPF-248).

Physiological Analyses

Determination of water potential attributes: Water potential parameters viz, leaf water potential, leaf osmotic potential and leaf turgor pressure were determined. (1) Water Potential of leaf (+1: diagnostic leaf) was measured according to the protocol devised by Scholander *et al.* (1965). (2) For determining of leaf osmotic potential, the same leaf was stored by keeping it for seven days (-80°C) to make the cell membrane leaky for the extraction of cell sap. Then the cell sap was collected by disposable syringe and injected into the osmometer chamber (Wescor 5500). (3) The turgor potential (Ψ_p) was calculated as the difference between osmotic potential (Ψs) and water potential (Ψ_w) values ($\Psi p = \Psi_w - \Psi s$).

Gas Exchange Characteristics

In this research work, five gas exchange parameters were studied viz. net photosynthetic rate (A_N), stomatal conductance (g_s), transpiration rate (E), water use efficiency (WUE). All these parameters except WUE were performed on diagnostic leaf (leaf+1) by using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England), while WUE was measured indirectly by using the formula (A/E). Measurements were taken at peak hours of photosynthesis (11:00 am to 1:00 pm) using infrared gas analyzer (IRGA) (molar flow of air per unit leaf area 300 mL min^1, atmospheric pressure 99.9 kPa, water vapor pressure into chamber ranged from 6.0 to 8.9 mbar) and photosynthetic active radiation (PAR) was observed up to 1700 µmol m^{-2} s^{-1} during that period.

Relative Leaf Water Content

Leaf relative water content (RWC) was determined by using the method described by Mu-Qing and Ru-Kai (1998). Leaf was excised into sections of 100 mm^2 and weighed out and kept on floating for 24 h on distilled water and again weight was noted. Subsequently, over drying was done at 80ºC to get the dry weight. Then RWC was calculated by using the formula; RWC = (FW – DW) ⁄ (TW – DW (* FW = leaf fresh weight, DW = dry weight, TW = turgid weight).

Results

In this study, five callus induction media were used and the callogenesis response of genotype CPF-248, on these media were observed. Calli induced on CIM1 (5.75 mg/L 2, 4-D) and CIM2 (6.75 mg/L 2, 4-D) were non-embryogenic and little bit waxy in their appearance (plate 1a). While callus induced on CIM3 (7.75 mg/L 2, 4-D) was embryogenic and fragile (plate 1b). But, highly pigmented calli were observed on CIM4 (8.75 mg/L 2, 4-D) and CIM5 (9.75 mg/L 2, 4-D) (plate 1c). Thus, on the basis of observations, CIM3 medium was selected for calli formation. In regeneration study, calli of three age groups viz., 35, 45 and 55 day old calli induced on CIM3 were shifted to regeneration media to regenerate the plants *in vitro*. It was observed that only few plants were regenerated from 35 day old calli which was shifted on RM1 media that contained (BAP 3.25 mg/L & casein hydrolysate 1.75 mg/L) (plate 2).

***In Vitro* Selection of Putative Drought Tolerant Plants**

For *in vitro* selection of putative drought tolerant somaclonal variant(s) (IPSV), RM1 supplemented with varying level of PEG was used. Therefore, four *in vitro* selection media (IVSM) were prepared with four concentrations of PEG (7%, 8%, 9% and 10%) (Table 4). For selection on tissue culture regime, calli were induced on CIM3 and then 35 days old calli were transferred to these four IVSM. One plant was obtained on IVSM1 (plate 3a) and one plant was observed on IVSM2 (plate 3b). While increasing concentration of PEG hindered the regeneration, so, on IVSM3 and IVSM4, no plant formation was observed. Putative drought tolerant somaclonal variant plants were acclimatized and shifted to polythene bags containing peat and moss (plate 4) and then subjected to molecular and physiological analyses to evaluate them as drought tolerant somaclonal variants.

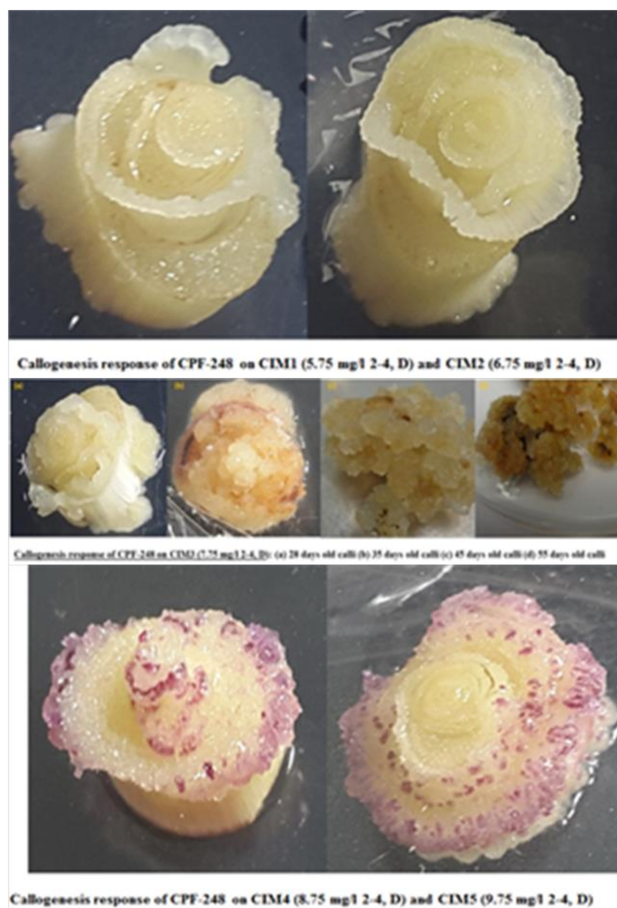

Plate 1: Callogenesis response of CPF-248 on five callogenesis media: (a) callogenesis response on CIM1 & CIM2 (b) callogenesis response on CIM3 (c) callogenesis response on CIM4 & CIM5

RAPD Based Genome Analysis of *in Vitro* Selected Putative Drought Tolerant Plants

Genomic analysis of *in vitro* selected putative drought tolerant plants for investigating genetic stability and variability was performed using RAPD approach. PCR of *in vitro* selected putative drought tolerant plants and parent genotype CPF-248 was performed using three highly polymorphic RAPD primers that were selected based on the information derived from genetic diversity analysis (our unpublished data). The data revealed that IPSV1 plant showed different DNA banding pattern and polymorphism in comparison with parent genotype (CPF-248) but IPSV2 was similar to DNA profile as parent genotype (plates 5a and 5b).

Physiological Analysis

Subsequent to genomic analysis, IPSV1, IPSV2 and parent genotype (CPF-248) were then subjected to physiological analyses. Thus, different physiological parameters viz., leaf water potential, leaf osmotic potential, leaf turgor pressure, stomatal conductance, CO_2 assimilation rate, transpiration rate, water use efficiency and relative leaf water content were investigated.

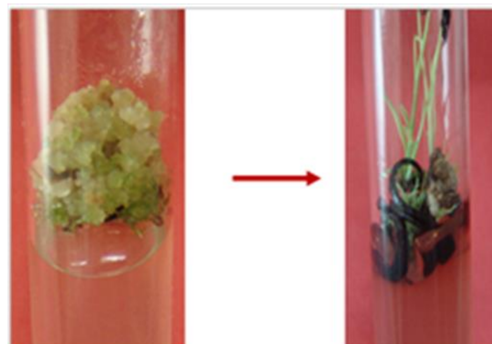

Plate 2: *In vitro* regeneration response of 35 days old calli induced on CIM3 and shifted on RM1

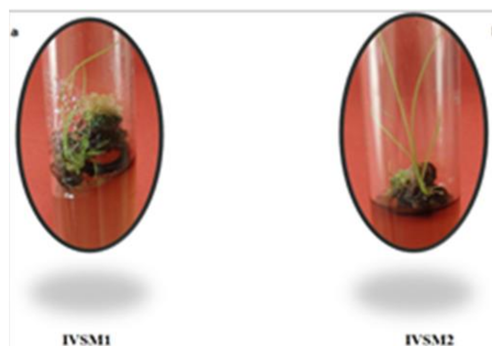

Plate 3: *In vitro* selection of putative drought tolerant somaclonal variant. (a) IVSM1 (7% PEG) (b) IVSM2 (8% PEG)

Plate 4: Acclimatization of *in vitro* selected drought tolerant plants

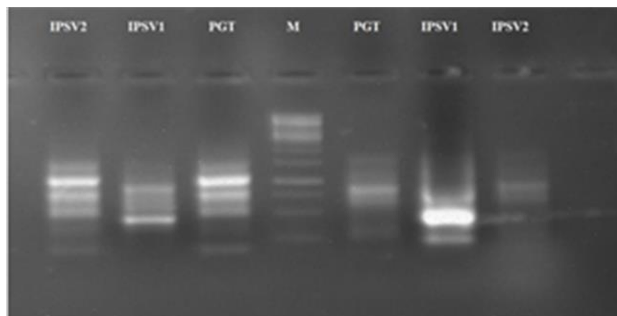

Plate: 5a: (RAPD) (PCR) analysis (GL L-01, GL Decamer L-08) of parent genotype (PST) and *in vitro* selected putative somaclonal variants (IPSV)

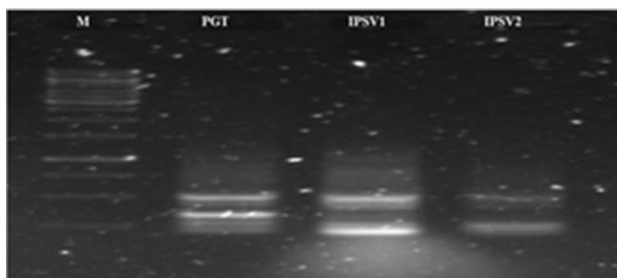

Plate: 5a: (RAPD) (PCR) analysis (GL Decamer K-07) of parent genotype (PST) and *in vitro* selected putative somaclonal variants (IPSV)

More leaf water potential (less negative) was observed in IPSV1 as compare to parent genotype. However, IPSV2 showed its water potential value which is less negative than the parent genotype and more negative than IPSV1 (Table 5). Similar results were obtained in case of leaf osmotic potential (Table 6) and leaf turgor pressure (Table 7).

Stomatal conductance (mol m^{-2} s^{-1}), Net CO_2 assimilation rate (A) (µmolCO_2m^{-2} s^{-1}) and transpiration rate (mmol H_2O m^{-2} s^{-1}) were observed to be higher in IPSV1 than IPSV2 as compared to parent genotype (Table 8, 9 and 10). WUE and RWC were also higher in IPSV1 as compared to IPSV2 and parent plant (Table 11 and 12).

Table 4: *in vitro* selection media (IVSM) to be used

IVSM 1	IVSM 2	IVSM 3	IVSM 4
RM 1 + 7% PEG	RM 1 + 8% PEG	RM 1 + 9% PEG	RM 1 + 10% PEG

Table 5: Leaf Water Potential (-MPa)

Parent Genotype	IPSV1	IPSV2
-1.67	-0.857	-0.960

Table 6: Leaf Osmotic Potential (MPa)

Parent Genotype	IPSV1	IPSV2
-1.86	-1.16	-1.22

Table 7: Leaf Turgor Pressure (MPa)

Parent Genotype	IPSV1	IPSV2
0.19	0.303	0.260

Table 8: Stomatal Conductance (mol m^{-2} s^{-1})

Parent Genotype	IPSV1	IPSV2
0.20	0.29	0.26

Table 9: Net CO_2 assimilation rate (A; µmol CO_2 m^{-2} s^{-1})

Parent Genotype	IPSV1	IPSV2
24.04	37.99	30.28

Table 10: Transpiration rate (E; mmol H_2O m^{-2} s^{-1})

Parent Genotype	IPSV1	IPSV2
1.49	1.79	1.55

Table 11: Water use efficiency (A/E)

Parent Genotype (CPF-248)	IPSV1	IPSV2
16.134	21.223	19.535

Table 12: Leaf relative water contents (RWC)

Parent Genotype	IPSV1	IPSV2
0.730	0.872	0.786

Discussion

Amongst ten most cultivated crops of the world, sugarcane is an imperative cash crop grown in tropical and subtropical areas. Sugarcane is a major contributor to sugar industry thereby adding the GDP of the country. There are several biotic and abiotic stresses, which are affecting the yield and quality parameters of this crop. As sugarcane is a water loving crop, so the scarcity of water in terms of drought stress is the major edaphoclimatic factor causing severe yield losses (Dedemo *et al*., 2013; Wang *et al*., 2015). High ploidy level and vegetative propagation due to various barriers in its sexual reproduction are the basic loopholes of sugarcane breeding. Hence biotechnological approaches are becoming the hope in improving this complex crop (Suprasanna *et al*., 2011).

The induction of somaclonal variations for improving varieties has been proved as successful biotechnological tool for the improvement of varieties (Yang *et al*., 2010; Krishna *et al*., 2016). For somaclonal variation study, sugarcane genotype CPF-248 was selected on the basis of data provided by Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan due to its agronomic features.

This is actually newly approved variety with good agronomic features in terms of high yield potential, excellent sugar recovery over standard varieties SPF-245 and HSF 240 (Ali *et al*., 2013; Tabassum, 2013) but it has low tolerance for abiotic stresses particularly drought and cold stresses according to information provided by Sugarcane Research Institute, AARI. But in this research study, we focused on and addressed drought stress. Because this genotype has more importance in terms of cane yield and sugar recovery over HSF-240 that is a drought tolerant genotype. So, this study was designed to enhance drought tolerance in this genotype.

In this study five callus induction media were used with varying concentrations of 2, 4-D (5.75 mg/L, 6.75 mg/L, 7.75 mg/L, 8.75 mg/L, 9.75 mg/L). The embryogenic and fragile calli were obtained from the callus induction media supplemented with 7.75 mg/L of 2, 4-D. Various scientists such as Sadat *et al*. (2011); Goel *et al*. (2010) and Ather *et al*. (2009) observed that 3 mg/L of 2, 4-D induced maximum calli. Ramanand *et al*., 2006 observed better results for one of the genotype when callus inducing media was supplemented with 4 mg/L of 2, 4-D. MS media supplemented with 2.5 mg/L 2, 4-D also proved to be good in callus induction as reported by Bahera and Sahoo (2009). Similarly, Tahir *et al*., 2011 observed the best percentage of callus induction in media supplemented with 4 mg/L of 2, 4-D. In this study the higher concentrations of 2, 4-D are used to induce the genetic variations. No genetic variability was detected in calli grown on media containing 2, 4-D from 1−5 mg/L as reported by Sweby *et al.* (1994) and Ijaz *et al*. (2012). The effect of age of calli on regeneration as reported by Ijaz *et al*. (2012) is also significant that could contribute in genetic variability. In this study 35 day old calli on CIM3 showed the better regeneration capacity.

Regeneration media supplemented with BAP (3.25 mg/L) and casein hydrolysate (1.75 mg/L) (RM1) showed the better results than RM2 and RM3. Shoot regeneration has been reported on regeneration media supplemented with 0.5 mg/L BAP (Gill *et al*., 2004), 0.25 mg/L BAP (Ijaz *et al*., 2012), 1 mg/L BAP (Gopitha *et al*., 2010; Sadat *et al*., 2011). An enhanced regeneration capacity has been observed by using even higher rate of BAP viz, 1.5 mg/L and 2 mg/L by Khan *et al*. (2009) and Bahera and Sahoo (2009), respectively.

For the assessment of genetic variability, different genetic markers are being used. The use of RAPD markers is preferred by the researchers because it is a cheaper and quick method and can amplify very low quantities of DNA (Soniya *et al*., 2001). To assess the genetic variability, three RAPD primers were used viz, GL Decamer K-07 (AGCGAGCAAG), GL Decamer L-01 (GGCATGACCT), GL Decamer L-08 (AGCAGGTGGA). These showed high polymorphism in our other genetic diversity experiment. IPSV1 (*in vitro* selected putative somaclonal variant 1) gave different DNA banding pattern than parent plant while, IPSV2 (*in vitro* selected putative somaclonal variant 2) gave the similar genetic profile as that of parent. Hapsoro *et al*. (2015) also used the RAPD markers to detect the similarities and differences among 38 sugarcane varieties of different origin. Ijaz *et al*. (2012), Rao *et al.* (2014) and Seema *et al.* (2014) also used the RAPD primers for detection of genetic variability.

Genetic variations are translated into morphological and physiological variations. The efficient and comprehensive knowledge about the physiological parameters especially in terms of water relations and gas exchange attributes can help in selection of drought tolerant and water ease efficient crops (Endres *et al*., 2010). So, in this study, physiological analyses were performed to investigate the drought tolerance of IPSV1 and IPSV2 in comparison with parent plant. The stomatal conductance has a positive correlation with photosynthetic activity and transpiration rate (Azevedo *et al*., 2004; Liberato *et al*., 2006; Endres *et al*., 2010). The result of this study showed similarity with the study of these researchers. The IPSV1 with greater stomatal conductance showed higher photosynthetic rate. The higher value of water use efficiency is also correlated with the higher values of photosynthetic rate proving IPSV1 is more drought tolerant as compared to the other two plants. Water stress requires the plants to maintain their physiological and metabolic activities for their survival under stress conditions which in turn required the maintenance of turgor by balancing the inlet and outlet of water (Medeiros *et al.*, 2013). IPSV1 maintained its higher water potential as compared to the other two plants showing its ability of good osmoregulation.

Conclusion

This research study is an effort to present the significance of somaclonal variations in improving the plant genotypes against drought stress,

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References

Ali, Z., M. Afzal, A. Ghaffar, N. Fiaz, M. ul Hassan, G.M. Wains, M.A. Mudassir and M.A. Sarwar, 2013. CPF 248: *Recently Approved Sugarcane Variety for Punjab*. http://www.psst.org.pk

Ather, A., S. Khan., A. Rehman and M. Nazir, 2009. Optimization of the protocols for callus induction, regeneration and acclimatization of sugarcane cv. Thatta-10. *Pak. J. Bot*., 41: 815−820

Azevedo, N.A.D., J.T. Prisco. J.E. Filho, C.F. de Lacerda, J.V. Silva, P.H.A. de Costa and E.G. Filho, 2004. Effects of salt stress on plant growth, stomatal response and solute accumulation of different maize genotypes. *Braz. J. Plant Physiol.,* 16: 31−38

Bahera, K.K. and S. Sahoo, 2009. Rapid in vitro micropropagation of sugarcane (*Saccharum officinarum* L. cv-Nayana) through callus culture. *Nat. Sci.,* 7: 1−10

Bakker, H., 1999. *Sugarcane Cultivation and Management.* Kluwer Academic/Plenum Publishers, New York, USA

Carr, M.K.V. and J.W. Knox, 2011. The water relations and irrigation requirements of sugar cane (*Saccharum officinarum*): a review. *Exp. Agric*., 47: 1−25

Christenhusz, M.J.M. and J.W. Byng, 2016. The number of known plants species in the world and its annual increase. *Phytotaxa,* 261: 201−217

Dedemo, G.C., F.A. Rodrigues, P.G. Roberto, C.B. Neto, S.C. França and S.M. Zingaretti, 2013. Osmoprotection in sugarcane under water deficit conditions. *Plant Stress*, 7: 1−7

Endres, L., J.L.D. Souza, I. Teodoro, P.M. Marroquim, C.M.D. Santos and J.E. de Brito, 2010. Gas exchange alteration caused by water deficit during the bean reproductive stage. *Rev. Bras. de Engenharia Agríc. Ambiental,* 14: 11−16

Gill, N.K., R. Gill and S.S. Gosal, 2004. Factor Enhancing Somatic Embriogenesis and Plant Regeneration in Sugarcane (*Saccharum officinarum* L.). *Ind. J Biotechnol.,* 3: 119−123

Goel, Y., V.P. Singh, M. Lal and M.L. Sharma. 2010. *In vitro* morphogenesis in leaf sheath explants of sugarcane hybrid var. CoS 99259. *Sug. Technol*., 12: 172−175

Gopitha, K., L. Bhavani and J. Senthilmanickam, 2010. Effect of the different auxins and cytokinins in callus induction, shoot, root regeneration in sugarcane. *Int. J. Pharm. Biol. Sci*., 1: 1−7

Hapsoro, D., A.W. Hayane, S.D. Utom, N. Sriyani and Y. Yusnita, Y. 2015. Genetic diversity among sugarcane (*Saccharum officinarum* L.) genotypes as shown by randomly amplified polymorphic DNA (RAPD). *Agrivita*, 37: 247−253

Ijaz, S., I.A. Rana, I.A. Khan and M. Saleem, 2012. Establishment of an in vitro regeneration system for genetic transformation of selected sugarcane genotypes. *Genet. Mol. Res.*, 11: 512−530

Khan, S.A., H.A.M.I.D. Rashid, M.F. Chaudhary, Z, Chaudhry, Z. Fatima, S, Siddiqui and M. Zia, 2009. Effect of cytokinins on shoot multiplication in three elite sugarcane varieties. *Pak. J. Bot*., 41: 1651−1658

Krishna, H., M. Alizadeh, D. Singh, U. Singh, N. Chauhan, M. Eftekhari and R.K. Sadh, 2016. Somaclonal variations and their applications in horticultural crops improvement. *Biotechnology*, 6: 1−18

Liberato, M.A.R., J.F.C. Gonçalves, L.R. Chevreuil, A.R.N. Junior, A.V. Fernandes and U.M.S. Junior, 2006. Leaf water potential, gas exchange and Chlorophyll a fluorescence in acariquara seedlings (*Minquartia guianensis* Aubl.) under water stress and recovery. *Braz. J. Plant Physiol*., 18: 315−323

Medeiros, D.B., E.C.D, Silva, R.J.M.C. Nogueira, M.M. Teixeira and M.S. Buckeridge, 2013. Physiological limitations in two sugarcane varieties under water suppression and after recovering. *Theor. Exp. Plant Physiol.*, 25: 213−222

Palotta, M.A., R.D. Graham., P. Langridge, D.H.B. Sparrow and S.J. Barker, 2000. RFLP mapping of manganese efficiency of barley. *Theor. Appl. Genet*., 101: 1100−1108

Ramanand, N., N. Kureel, N. Subhanand, M. Lal and S.B. Singh, 2006. Plantlet regeneration through leaf callus culture in sugarcane. *Sugar. Technol*., 8: 85−87

Rao, V.P., R. Chaudhary, S. Singh, R.S. Sengar and V. Sharma, 2014. Assessment of genetic diversity analysis in contrasting sugarcane varieties using random amplified polymorphic DNA (RAPD) markers. *Afr. J. Biotechnol*., 13: 3736−3741

Sadat, S., M.S. Hoveize, M. Mojadam and S.K. Marashi, 2011. The study of callus induction and regeneration potential of sugarcane, varieties SP70-1143 and CP76-331. *World Appl. Sci. J.,* 13: 1106−1111

Scholander, P., E. Bradstreet, E. Hemmingsen and H. Hammel, 1965. Sap Pressure in Vascular Plants: Negative hydrostatic pressure can be measured in plants. *Science*, 148: 339−346

Seema, N., S.I.A. Khan, Raza, S. Yasmeen, S. Bibi and G.S. Nizamani, G. 2014. Assessment of genetic variability in somaclonal population of sugarcane. *Pak. J. Bot.*, 46: 2107−2111

Soniya, E.V., N.S. Banerjee and M.R. Das, 2001. Genetic analysis of somaclonal variation among callus derived plants of tomato. *Curr. Sci*., 80: 1213−1215

Suprasanna, P., V.Y. Patade, N.S. Desai, R.M. Devarumath, P.G. Kawar, M.C. Pagariya, A. Ganapathi, M. Manickavasagam and K.H. Babu, 2011. Biotechnological developments in sugarcane improvement: an overview. *Sugar. Technol.*, 13: 322−335

Sweby, D.L., B.I. Huckett and F.C. Botha, 1994. Minimizing somaclones variation in tissue cultures of sugarcane. *Proc. S. Afr. Sugar. Technol. Assoc*., 68: 46−50

Tabassum, I., 2013. *CPF-248- Fresh and Medium Maturing Cane Variety*. http://technologytimes.pk

Tahir, S.M., K. Victor and S. Abdulkadir, 2011. The Effect of 2, 4-Dichlorophenoxy Acetic Acid (2, 4-D) Concentration on Callus Induction in Sugarcane (*Saccharum officinarum*). *Nig. J. Basic Appl. Sci.*, 19: 213−217

Venkataramana, S., P.N.G. Rao and K.M. Naidu, 1986. The effects of water stress during the formative phase on stomatal resistance and leaf water potential and its relationship with yield in ten sugarcane varieties. *Field Crops Res*., 13: 345−353

Wang, Y., L. Lin and H. Chen, 2015. Assessing the economic impacts of drought from the perspective of profit loss rate: a case study of the sugar industry in China. *Nat. Hazards Earth Syst. Sci.,* 15: 1603−1616

Wang, J., B. Roe, S. Macmil, Q. Yu, J.E. Murray, H. Tang, C. Chen, F. Najar, G. Wiley, J. Bowers and M.A.V. Sluys, M.A., 2010. Microcollinearity between autopolyploid sugarcane and diploid sorghum genomes. *BMC Genom.*, 11: 261

Yang, W.R., Q.X. Zhang, H.T. Pan and M. Sun, 2010. In vitro regeneration of Lilium tsingtauense Gilg. and analysis of genetic variability in micropropagated plants using RAPD and ISSR techniques. *Propag. Ornam. Plants*, 10: 59−66

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