Bangladesh J. Sugarcane, **24-27** : *144-147* *July, 2005*

SCIENTIFIC NOTE

Evaluation of Tissue Culture Derived Sugarcane Clones Under Water-Logging Stress

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Received on 31.8.2003

Key words : Sugarcane, Tissue culture, water-logging stress, tolerant somaclones.

In Bangladesh, yield of sugarcane is very poor due to various reasons which vulnerability of sugarcane to water stress such as flood, water-logging and drought are most important (Miah *et. al.*, 1993). Estimate shows that about fifty percent sugarcane is planted on lands where water remains stagnant for longer period or become submersed during monsoon flood resulting in very poor yield (Miah, *et al.,* 1993). Sugarcane varieties built-in superior tolerance to water-logging and flood stress is crying need to ensure reasonable yield. Conventional breeding method is being practised to develop water-logging tolerance in sugarcane varieties which takes very long time. Tissue culture techniques for correction of varietal defects might be a shorter and alternate method for varietal improvement of sugarcane against water-logging stress. Development of salt tolerant sugarcane plants through tissue culture was reported by Tanvir *et al.* (2001). Evaluation of drought tolerant in tissue culture derived sugarcane clones was reported elsewhere (Anon., 1995). The present investigation was undertaken to evaluate water-logging tolerance of tissue culture derived sugarcane clones.

Callus induction and plants regeneration were obtained from sugarcane variety Isd 16 using unexpanded leaf slices as explant following the method of Krisnamurthi (1987). Explants were sterilized in 0.1 percent mercuric chloride solution for 15 minutes followed by 3-4 times washing with sterilized distilled water before placement in the flask containing callus induction medium. Media for callus and shoot induction were prepared following the method of Heinz et. al. (1990) and culture medium for root induction as suggested by Larkin (1982). Modified MS (Murashige and Skoog, 1962) medium supplemented with 4.0 mg/l 2,4-D; 5.0 mg/l IAA + 2.0 mg/l Kn + 20. g/l sucrose and 5.0 mg/l NAA + 70.0 g/l sucrose for callus induction, shoot and root generation respectively. The flasks were kept in a dark room at 25 to 28 0C for callus development. For shoot and induction the flasks were kept at 20 0 C under 400 W Philips HPL lamps 12 hours in light and 12 hours in darkness (Krisnamurthi and Tlaskal, 1974).

After successful developments of shoots and roots, plantlets were transplanted to small pots containing sterilized soil. Forty tissue culture derived plants were retransplanted to big pot following the method Hossain *et al.* (1995). The plants in big pots were allowed to grow for a period of 125 days. Then the pots with plants were transferred into concrete water tanks (360 cm. X 200 cm X 90 cm) containing water that subjected the to 45 cm constant water-logging condition. The plants were allowed to grow under induced water-logging stress condition in concrete tank for 120 days.

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Data on visual tolerance rating based on leaf greenness of plants, days to initiation of primordial water-root, and cane elongation rate were collected during water-logging stress period; number and length of primordial water-root, number and weight of cane and brix reading were recorded at harvest after 120 days of inundation.

The results of the experiment have been presented in the table 1 and 2. it is revealed from the table 1 that clones TC-008, TC-009, TC-018 and TC-046 were found to be highly tolerant to water-logging stress having tolerance rating 1. Rest of the clones were found to be tolerant to water-logging stress and showed tolerance rating 2. The donor variety Isd 16 showed intolerant reaction having tolerance rating 4. The highest number and length of primordial water roots were obtained in highly tolerant clones TC-009 and TC-008 respectively. Rahman and Alam (1985) studied eleven clones including three commercial varieties of sugarcane under water-logging condition. They reported that clones significantly differed in their ability to produced primordial water-roots which was considered as an important adaptation mechanism to water-logging stress. Primordial water-roots are used to take dissolve oxygen in water. Among tissue culture derived clones tolerant clones (TC-005 and TC-019) started to initiate primordial water-root production earlier than highly tolerant clones (TC-008, TC-009, TC-018 and TC-046). This indicate that tolerance clones were affected earlier than highly tolerant clones and started to initiate water-root utilized dissolve oxygen in water to minimize water-logging stress condition. However, donor variety Isd 16 and clones TC-002 did not follow the same mechanism. The highest number of cane per pot was obtained in the clones TC-008 and TC-018 followed by clones TC-009. The higher weight per cane was recorded in the clones, which produced higher number of cane per pot. Perhaps due to having highly tolerance to water-logging stress these three clones (TC-008, TC-018 and TC-009) were able to maintain the growth during water-logging stress conditions. The tolerance rating and elongation rate of cane was negatively correlated. This result corroborated with the results of Miah et. al., (1993). Beside, morphological adaptation plant might have anatomical, physiological and biochemical adaptations. Brix reading of all tissue culture derived clones were higher than the donor variety Isd 16. Higher brix readings were recorded in clones having highly tolerance rating. This was possibly due to protection of sugar degradation by their tolerance mechanism under water-logging condition. Variation in stalk length, number and weight of cane, and sugar yield in tissue culture derived sugarcane clones was also reported by Purohit (2000).

All tissue culture derived clones are different regarding tolerance rating, primordial water-root production, growth and yield over donor variety. These are due to somaclonal variation in tissue culture derived clones. Genetic variability develops spontaneously during tissue cultures (Purohit, 2000). The terms somaclonal variation was introduced by Larkin and Scowcroft (1981). Variant clones obtained from tissue culture of variety Isd 16 highly tolerant to tolerant against water-logging stress. The present results sought for further field trial for release as varieties. The highly tolerant and tolerant cloned would be used as breeding materials for further development of water-stress tolerant clones and varieties also.

Table 1. Tolerance rating and production of primordial water-roots by tissue culture derived clones and their donor variety grown under induced water-logging condition.

Clone / Variety	Tolerance rating (1-5)*	Days to initiation of primordial water-root	Average number of primordial water root per node	Average length of primordial water root (cm)
TC-002	2	5	5.0	16.3
TC-005	2	3	6.2	19.5
TC-008	1	4	6.0	25.0
TC-009	1	5	6.5	21.8
TC-018	1	4	6.3	22.1
TC-019	2	3	5.8	17.5
TC-046	1	4	5.8	18.9
lsd 16 (Donor)	4	7	4.9	16.2

* Tolerance rating scale (1-5) is based on greenness of plant and other data collection, where 1= Highly tolerant, 2 = Tolerant, 3 = Moderately tolerant, 4 = In tolerant, 5 = Highly intolerant.

Table 2. Elongation rate, number of cane per pot, weight of cane per pot and brix reading of tissue culture derived clones in comparison with donor variety subjected to water-logging stress.

Clone / Variety	Elongation rate of cane (cm / day)	Number of cane per pot	Weight of cane per pot (kg)	Brix
TC-002	0.50	4	2.2	17.4
TC-005	0.65	4	2.0	21.0
TC-008	0.20	6	3.5	22.4
TC-009	0.43	5	3.0	21.8
TC-018	0.18	6	3.7	21.0
TC-019	0.58	3	1.6	20.6
TC-046	0.45	4	2.1	22.4
lsd 16 (Donor)	0.71	3	1.5	15.0

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