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In vitro response of promising sugarcane varieties for salinity tolerance through callus culture

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Abstract
An investigation was carried out on two sugarcane varieties, *viz.*, Co 86032 and CoM 0265 at the Sugarcane Tissue Culture Laboratory of Main Sugarcane Research Station, Navsari Agricultural University, Navsari during 2014-2015 to obtain salt tolerant variants from calli of sugarcane (*Saccharum sp*.). Higher root length, shoot length and plantlet height were observed on MS medium (comprised of 0.5 mg/l NAA and 1 mg/l BAP) without NaCl in both genotypes. *In vitro* evaluation of plantlets in pot culture experiment for salt tolerance showed that, higher shoot: root ratio was observed in MS medium +3 mg/l 2, 4-D and 4 mg/l 2, 4-D + 2.00 % NaCl concentration in both the genotypes. Leaf number, leaf area, chlorophyll content and Na^+: K^+ ratio were decreased with increased the concentration of NaCl. In all the traits, the minimum value was observed when the medium treated with 2.5 % NaCl. Overall, Co 86032 gave better response in respect of all characters in the study as compared to CoM 0265.

Keywords: sugarcane, salinity, tissue culture, *in vitro*

Introduction
Salinity is one of the major abiotic stress, which greatly affects the sugarcane productivity and recovery. The soils with electrical conductivity of less than 4 dSm^{-1} are generally considered as salt-free, where almost all crops can be grown while the soil with EC range between 4-8 dsm^{-1} are generally considered as salty soil. Area near sea as well as improper irrigation practices and lack of drainage have generally led to accumulation of salts in the soil in concentrations that are harmful to the crops. Sugarcane is cultivated as a commercial crop in nearly 90 countries spread over the world. However, being a typical glycophyte, it exhibits stunted growth and poor yield under salinity**,** with its yield falling to 50% or even more of its true potential Subbarao and Shaw (1985) [29]. Besides this, salinity in root zone of sugarcane decreases sucrose yield through its effect on both biomass and juice quality Lingle and Wiegand (1997) [16]. About 7% of the world's total land area is affected by salt, as is a similar percentage of its arable land. In India, total salt affected soils are about 67.45 lakh ha, while in Gujarat it has spread in 22.22 lakh ha. Salinity becoming widespread in many regions, and may cause serious salinization of more than 50% of all arable land by the year 2050. Sugarcane being a glycophyte shows high sensitivity to salinity condition at various growth stages. The salts interfere with sugar production in two-fold manner, first by affecting growth rate and yield of the cane secondly by affecting the sucrose content of the stalk. The areas in which sugarcane were grown in Gujarat, of which, about 90 per cent area under south Gujarat condition. South Gujarat region is located near Arabian Sea, so some area of sugarcane cultivation affected by salt accumulated through sea water as well as improper irrigation practices. This situation decreased the production as well as area of sugarcane cultivation in south Gujarat region. The Main Sugarcane Research Station, Navsari Agricultural University, Navsari (Gujarat) has developed some varieties which performed better in salty situation but these varieties have not given consistent performance over years. The genotypes selected for present experiment are viz., Co 86032 (Co 62198 x CoC 671) is a high sugar, midlate maturing variety released from Coimbatore while CoM 0265 is a high yielding variety released from Padegaon, Maharashtra. It is becoming popular in south Gujarat region due to its productivity and consistent performance and covered near about 35 per cent of area of Gujarat but both the varieties are highly sensitive to salty situation. So, there is a felt need to develop resistant/ tolerant somaclones of these particular varieties. Heinz and Mee (1969) [13] and Barba and Nickle (1969) [4] put a corner stone for development of plantlets from sugarcane callus culture. Attempts were made through physical mutagenesis but fruitful results could not be achieved.

In this regard, callus culture is convenient tool for scientists to screen disease/salinity/drought resistant somaclones *in vitro* within a short period of time [Barba *et al* (1984); Naik and Vedamurthy(1997); Sundar *et al* (1999); Naik *et al* (2001) and Patel *et al* (2005)] [5, 18, 19, 21]. A variation observed among the plants regenerated from cells and tissues termed somaclonal variation has been considered a source of new plant genotype for crop improvement Brettell *et al* (1986) and Hadi and Bridgen (1996) [8, 12]. Somaclonal variation in combination with *in vitro* mutagenesis can be beneficial for the isolation of salinity and drought tolerant lines in a short duration employing *in vitro* selection Samad *et al* (2001) [25]. *In vitro* selection has been used for selection of salt tolerance Bressan *et al* (1985) and Rosas *et al* (2003) [7, 24]. Induction of callus and regeneration of plants using sugarcane varieties of India were reported elsewhere [Shukla *et al* (1994); Islam *et al* (1996) and Gosal *et al* (1998) [27, 14, 11]. In callus phase there are the maximum chances of mutation and somaclonal variation when plants regenerated from somatic embryos.

Materials and methods

The experiment was conducted at sugarcane tissue culture laboratory Main Sugarcane Research Station, Navsari Agricultural University, Navsari, Gujarat during 2014-2015. The genotypes *viz.,* Co 86032 and CoM 0265 are the experimental varieties. Meristem and leaf whorl explants of two varieties were collected from 5 month old grown plant from Main Sugarcane Research Station field. Cane tops with the growing apices were cut approximately 10 cm long and washed thoroughly in running tap water for 30 minutes. Outer sheaths of cane tops were removed by wiping the sheath with rectified spirit. The shoots were then washed with soap water (2 drops of Labonin into 250 ml of water) for about 5 to 6 minutes in a sterile 1 liter conical flask, followed by cleaning the materials with distilled water. The shoots were rinsed in 5 per cent sodium hypochlorite for 10 minutes. Then shoots were thoroughly rinsed in 70 per cent ethanol for 30 seconds followed by sterilize double distilled water for 4-5 times till ethanol was completely washed out from the surface of material. Surface sterilization was performed by using 0.1 per cent mercuric chloride solution. Shoots were shaken vigorously for 5 minutes. Then the container was taken to a laminar clean air station. They were rinsed 3 to 4 times with sterile double distilled water to remove all traces of chemicals. The isolation of shoot apex was done by carefully removing the 2-3 outer whorls of the developing leaves with the help of a sterile sharp blade. The second innermost and inner most whorls of developing leave cut in to small pieces of approximately one centimetre length with the help of a sterile sharp blade and utilized as explant for callus induction on MS medium supplemented with different concentrations of 2,4-D. After 4 weeks, embryogenic calli were separated from the explants and transferred to MS media supplemented with different levels of NaCl (0, 0.5, 1, 1.5, 2 and 2.5 %) during serial subculture (in a step-wise manner). Cultures were grown in 100 ml glass jars containing 25 ml of culture medium closed with aluminum foil caps. Plantlets were regenerated and then rooted after 3 to 4 weeks of transfer of high healthy callus on regeneration and root medium, that is, MS medium of the same composition as earlier mentioned, but with special hormones and none 2, 4-D in a growth chamber under long-day conditions (16/8 h light/dark cycle) at a temperature of 25 ± 2°C and relative humidity of 60 to 70%. Light was provided by white fluorescent tubes (60 W, photon flux density 50 µmol/m^2/s^1). The best and healthy plantlets were selected as tolerant somaclonal variants for the next evaluations.

Observation recorded during Experiment

Length of regenerated shoots from callus developed on different treatments medium, measured in centimetre. Root length of *in vitro* plantlets was measured in centimetre. These observations were recorded at the 30 day after inoculation on rooting media.

Plant height (cm) was recorded under secondary hardening. Total chlorophyll in leaves was determined by DMSO (Dimethylsulphoxide) method described by Arnon, 1949. The leaves from plants selected from each treatment were used for the estimation of leaf area after 60 days of planting. Leaf area was measured by leaf area meter (Model LI3000, LI-COR, USA) and expressed as cm^2. Total number of green leaves on the plant from each treatment were counted at 60 days after planting and recorded. Shoot root ratio was estimated by dry weight basis. The potassium and sodium contents were estimated by flame photometer (Jenway PFP 7, ELE Instrument Co. Ud.) method and expressed as ratio on the basis of dry weight.

Statistical analysis

The data generated from the various *in vitro* experiments were subjected to statistical analysis in Factorial Completely Randomized Design (FCRD). Transformation of data was carried out prior to statistical analysis as suggested by [Steel and Torrie (1981)] [28].

Result and discussion

Shoot length (cm)

Co 86032

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum shoot length was observed in T_1 (9.03 cm) followed by T_2 (8.10 cm), T_3 (7.50 cm) and T_4 (7.27 cm) while, plantlets developed from leaf whorl callus, maximum shoot length was observed in T_7 (9.13 cm) followed by T_8 (8.27 cm), T_9 (7.63 cm) and T_{10} (7.10 cm). The minimum shoot length was observed in T_6 (5.70 cm) followed by T_{12} (5.80 cm) and T_5 (6.20 cm). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum shoot length was observed in T_{13} (8.97 cm) followed by T_{14} (7.97 cm), T_{15} (7.20 cm) and T_{16} (7.07 cm) while, plantlets developed from leaf whorl callus, maximum shoot length was observed in T_{19} (8.60 cm) followed by T_{20} (7.87 cm), T_{21} (7.23 cm) and T_{22} (7.17 cm). The minimum shoot length was observed in T_{18} (5.37 cm) followed by T_{24} (5.63 cm) and T_{17} (6.43 cm).

CoM 0265

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum shoot length was observed in T_1 (8.40 cm) followed by T_2 (8.10 cm), T_3 (7.90 cm) and T_4 (7.60 cm) while, plantlets developed from leaf whorl callus, maximum shoot length was observed in T_7 (8.20 cm) followed by T_8 (8.17 cm), T_9 (7.53 cm) and T_{10} (7.50 cm). The minimum shoot length was observed in T_{12} (5.97 cm) followed by T_6 (6.00 cm). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum shoot length was observed in T_{13} (8.10 cm) followed by T_{14} (7.87 cm), T_{15} (7.60 cm) and T_{16} (7.43 cm) while, plantlets developed from leaf whorl callus, maximum shoot length was observed in T_{19} (8.03 cm) followed by T_{20} (7.90 cm), T_{21} (7.53 cm) and T_{22} (7.33 cm). The minimum

shoot length was observed in T_{18} (5.87 cm) followed by T_{24} (6.03 cm).

The plantlets regenerated from meristem and leaf whorl callus supplemented with MS medium + 3 mg/l 2, 4-D observed very little differences in shoot length but the shoot length were gradually decreased with increase in salt concentrations. Similar trends were observed in plantlets developed using MS medium + 4 mg/l 2, 4-D. The control showed maximum shoot length in both the genotypes under both conditions. These results are agreement with [Akhtar *et al* (2003); Wahid and Ghazanfar (2006) and Ather *et al* (2009)] [3, 32, 2]. The minimum shoot length was observed in plantlets treated with 2.5 % NaCl. The saline solution may be at water potential of -24 bar, the value for sea water, or higher. Plants challenged by this magnitude of water potential develop through medium, so the leaves are unable to meet transpirational demands, and wilt and desiccate.

Root length (cm)

Co 86032

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum root length was observed in T_1 (3.07 cm) followed by T_2 (2.87 cm), T_3 (2.80 cm) and T_4 (2.53 cm) while, plantlets developed from leaf whorl callus, maximum root length was observed in T_7 (3.00 cm) followed by T_8 (2.70 cm), T_9 (2.53 cm) and T_{10} (2.47 cm). The minimum root length was observed in T_{12} (1.53 cm) followed by T_6 (1.73 cm). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum root length was observed in T_{13} (2.83 cm) followed by T_{14} (2.73 cm), T_{15} (2.57 cm) and T_{16} (2.57 cm) while plantlets developed from leaf whorl callus, maximum root length was observed in T_{19} (2.80 cm) followed by T_{20} (2.57 cm), T_{21} (2.53 cm) and T_{22} (2.37 cm). The minimum root length was observed in T_{18} (1.43 cm) followed by T_{24} (1.47 cm).

CoM 0265

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum root length was observed in T_1 (3.03 cm) followed by T_2 (2.80 cm), T_3 (2.57 cm) and T_4 (2.37 cm) while, plantlets developed from leaf whorl callus, maximum root length was observed in T_7 (3.00 cm) followed by T_8 (2.67 cm), T_9 (2.50 cm) and T_{10} (2.40 cm). The minimum root length was observed in T_6 and T_{12} (1.27 cm). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum root length was observed in T_{13} (3.10 cm) followed by T_{14} (2.83 cm), T_{15} (2.80 cm) and T_{16} (2.40 cm) while, plantlets developed from leaf whorl callus, maximum root length was observed in T_{19} (2.73 cm) followed by T_{20} (2.70 cm), T_{21} (2.60 cm), T_{22} (2.53 cm) and T_{23} (2.53 cm). The minimum root length was observed in T_{18} (1.43 cm) followed by T_{24} (1.30 cm).

The plantlets regenerated using meristem and leaf whorl callus supplemented with MS + 3 mg/l 2, 4-D observed very little differences in root length but it gradually decreased with the increase in the salt concentration. Similar trends were observed when plantlets developed from both explants derived callus which supplemented with 4 mg/l 2, 4-D. The control showed maximum while minimum root length was observed when the medium supplemented with 2.5 % NaCl in both the genotypes. These results are agreement with [Akhtar *et al* (2003) and Shomeili *et al* (2011)] [3, 26]. According to Bhatnagar *et al* (2008) [6] roots were the first plant organs which affected by salt stress and most sensitive once. Salinity can rapidly inhibit root growth and hence the capacity for uptake of water and essential mineral nutrients from the soil [Neumann (1997)] [20].

Plantlet height (cm)

Co 86032

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum plantlet height was observed in T_1 (37.60 cm) followed by T_2 (36.53 cm), T_3 (32.47 cm) and T_4 (30.97 cm) while, plantlets developed from leaf whorl callus, maximum plantlet height was observed in T_7 (37.30 cm) followed by T_8 (36.60 cm), T_9 (31.67 cm) and T_{10} (31.67 cm). The minimum plantlet height was observed in T_{12} (24.20 cm) followed by T_6 (24.50 cm). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum plantlet height was observed in T_{13} (37.53 cm) followed by T_{14} (36.90 cm), T_{15} (32.70 cm) and T_{16} (32.43 cm) while, plantlets developed from leaf whorl callus, maximum plantlet height was observed in T_{19} (37.53 cm) followed by T_{20} (36.80 cm), T_{21} (32.60 cm) and T_{22} (31.00 cm). The minimum plantlet height was observed in T_{24} (24.57 cm) followed by T_{18} (24.73 cm).

CoM 0265

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum plantlet height was observed in T_1 (32.37 cm) followed by T_2 (29.43 cm), T_3 (28.43 cm) and T_4 (27.20 cm) while, plantlets developed from leaf whorl callus, maximum plantlet height was observed in T_7 (32.10 cm) followed by T_9 (29.37 cm), T_9 (28.60 cm) and T_{10} (27.13 cm). The minimum plantlet height was observed in T_{12} (21.07 cm) followed by T_6 (21.17 cm). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum plantlet height was observed in T_{13} (31.83 cm) followed by T_{14} (29.10 cm), T_{15} (27.40 cm) and T_{16} (27.00 cm) while, plantlets developed from leaf whorl callus, maximum plantlet height was observed in T_{19} (31.50 cm) followed by T_{20} (28.53 cm), T_{21} (27.47 cm) and T_{22} (27.07 cm). The minimum plantlet height was observed in T_{24} (20.63 cm) followed by T_{18} (20.53 cm).

The plantlets regenerated from both explant callus supplemented with MS + 3 mg/l 2, 4-D and 4 mg/l 2, 4-D showed little differences in plantlets height in both the genotypes. The decrease in plantlet height in both the genotypes gradually with increasing the levels of NaCl and the minimum shoot length was observed in plantlets treated with 2.5 % NaCl. Similar results were noticed by [Shomeili *et al* (2011)] [26].

Shoot: Root ratio

Co 86032

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum shoot: root ratio was observed in T_5 (2.30) followed by T_4 (2.20), T_3 (2.00) and T_1 (1.60) while, plantlets developed from leaf whorl callus, maximum shoot: root ratio was observed in T_{11} (2.30) followed by T_{10} (1.90), T_9 (1.90) and T_7 (1.53). The minimum shoot: root ratio was observed in T_6 (1.27) followed by T_2 (1.33), T_{12} (1.50), and T_8 (1.50). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum shoot: root ratio was observed in T_{17} (2.43) followed by T_{16} (2.23), T_{15} (2.00) and T_{14} (1.93) while, plantlets developed from leaf whorl callus, maximum shoot: root ratio was observed in T_{23} (2.47) followed by T_{22} (2.13), T_{21} (1.83) and T_{20} (1.73). The minimum shoot: root ratio was

observed in T_{24} (1.30) followed by T_{19} (1.33), T_{18} (1.47), and T_{13} (1.47).

CoM 0265

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum shoot: root ratio was observed in T_5 (2.13) followed by T_4 (1.70), T_1 (1.40) and T_3 (1.33) while, plantlets developed from leaf whorl callus, maximum shoot: root ratio was observed in T_{11} (2.07) followed by T_{10} (2.03), T_9 (1.57) and T_7 (1.53). The minimum shoot: root ratio was observed in T_{12} (1.17) followed by T_6 (1.23), T_2 (1.27), and T_1 (1.40). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum shoot: root ratio was observed in T_{17} (1.97) followed by T_{16} (1.73), T_{15} (1.43) and T_{14} (1.37) while, plantlets developed from leaf whorl callus, maximum shoot: root ratio was observed in T_{23} (2.07) followed by T_{22} (1.63), T_{21} (1.57) and T_{19} (1.40). The minimum shoot: root ratio was observed in T_{24} (1.17) followed by T_{20} (1.23), T_{18} (1.23), T_{14} (1.23) and T_{13} (1.47).

The plantlets regenerated using meristem and leaf whorl callus supplemented with MS + 3 mg/l 2, 4-D observed very little differences in shoot: root ratio. Similar trend was observed in meristem and leaf whorl callus supplemented with MS + 4 mg/l 2, 4-D. The control showed maximum shoot: root ratio in both the genotypes but after increasing the level of NaCl the shoot: root ratio was decreased gradually. The increase in value of the shoot: root dry weight ratio at high NaCl concentrates indicates that roots were affected positively by salinity than shoots. These results are agreement with [Akhtar *et al* (2003) and Shomeili *et al* (2011)] [3, 26].

Leaf Number

Co 86032

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum leaf number was observed in T_1 and T_2 (6.40) followed by T_3 (6.27), T_4 (6.07) and T_5 (5.80) while, plantlets developed from leaf whorl callus, maximum leaf number was observed in T_7 (6.13) followed by T_8 (5.83), T_9 (5.60) and T_{10} (5.60). The minimum leaf number was observed in T_{12} (4.60) followed by T6 (4.80). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum leaf number was observed in T_{13} (6.20) followed by T_{14} (6.00), T_{15} (5.40) and T_{16} (5.20) while, plantlets developed from leaf whorl callus, maximum leaf number was observed in T_{19} (5.60) followed by T_{20} (5.53), T_{21} (5.30), T_{22} (5.00) and T_{23} (5.00). The minimum leaf number was observed in T_{24} (4.03) followed by T_{18} (4.60).

CoM 0265

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum leaf number was observed in T_1 (7.43) followed by T_2 (6.77), T_3 (6.47) and T_4 (6.27) while, plantlets developed from leaf whorl callus, maximum leaf number was observed in T_7 (6.93) followed by T_8 (6.80), T_9 (6.57) and T_{10} (6.27). The minimum leaf number was observed in T_{12} (5.60) followed by T_6 (5.70). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum leaf number was observed in T13 (6.83) followed by T_{14} (6.70), T_{15} (6.40) and T_{16} (6.20) while, plantlets developed from leaf whorl callus, maximum leaf number was observed in T_{19} (6.63) followed by T_{20} (6.30), T_{21} (6.13) and T_{22} (5.90). The minimum leaf number was observed in T_{18} (4.67) followed by T_{24} (4.80).

Higher number of leaves were observed in control conditions (0 % NaCl) while, minimum number of leaves were observed when plantlets were treated with 2.5 % NaCl in both the genotypes. Similar result was observed by [Shomeili *et al* (2011)] [26] as the increasing concentrations of NaCl, leaf number was decrease. As the leaf provides the platform for photosynthesis, leaf area indicates the strength of the source of a crop. Photosynthesis and dry matter production of a plant is proportional to the amount of leaf area on the plant [Padmathilake *et al* (2007)] [22].

Leaf Area (cm^2 $plant^{-1}$)

Co 86032

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum leaf area was observed in T_1 (52.43) followed by T_2 (46.45), T_3 (42.28) and T_4 (32.72) while, plantlets developed from leaf whorl callus, maximum leaf area was observed in T_7 (50.64) followed by T_8 (47.11), T_9 (42.03) and T_{10} (33.86). The minimum leaf area was observed in T_{12} (30.35) followed by T_6 (30.74). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum leaf area was observed in T_{13} (50.27) followed by T_{14} (44.22), T_{15} (43.16) and T_{16} (42.20) while, plantlets developed from leaf whorl callus, maximum leaf area was observed in T_{19} (49.52) followed by T_{20} (46.19), T_{21} (43.21) and T_{22} (31.78). The minimum leaf area was observed in T_{24} (28.30) followed by T_{18} (28.98).

CoM 0265

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum leaf area was observed in T_1 (37.66) followed by T_2 (36.45), T3 (35.31) and T_4 (33.65) while, plantlets developed from leaf whorl callus, maximum leaf area was observed in T_7 (37.81) followed by T_8 (35.67), T_9 (33.71) and T_{10} (33.44). The minimum leaf area was observed in T_{12} (28.49) followed by T_6 (28.53). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum leaf area was observed in T_{13} (37.42) followed by T_{14} (36.35), T_{15} (34.73) and T_{16} (33.26) while, plantlets developed from leaf whorl callus, maximum leaf area was observed in T_{19} (37.40) followed by T_{20} (35.48), T_{21} (34.33) and T_{22} (33.24). The minimum leaf area was observed in T_{24} (28.22) followed by T_{18} (28.54).

Higher leaf area was observed in control conditions (0 % NaCl) while, minimum leaf area was observed when plantlets were treated with 2.5 % NaCl in both the genotypes. Similar result was observed by [Wahid and Ghazanfar (2006); Shomeili *et al* (2011) and Wahid (2004)] [32, 26, 31].

Chlorophyll content (mg g^{-1})

Co 86032

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum chlorophyll content was observed in T_1 (5.60) followed by T_2 (5.30), T_3 (5.13) and T_4 (4.77) while, plantlets developed from leaf whorl callus, maximum chlorophyll content was observed in T_7 (5.57) followed by T_8 (5.37), T_9 (5.33) and T_{10} (4.63). The minimum chlorophyll content was observed in T_{12} (3.67) followed by T6 (3.70). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum chlorophyll content was observed in T_{13} (5.47) followed by T_{14} (5.23), T_{15} (5.20) and T_{16} (4.57) while, plantlets developed from leaf whorl callus, maximum

chlorophyll content was observed in T_{19} (5.27) followed by T_{20} (4.80), T_{21} (4.80) and T_{22} (3.83). The minimum chlorophyll content was observed in T_{24} (3.33) followed by T_{18} (3.37).

CoM 0265

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum chlorophyll content was observed in T_1 (4.60) followed by T_2 (4.47), T_3 (4.50) and T_4 (3.90) while, plantlets developed from leaf whorl callus, maximum chlorophyll content was observed in T_7 (4.50) followed by T_8 (4.30), T_9 (4.00) and T10 (3.33). The minimum chlorophyll content was observed in T_{12} (2.70) followed by T_5 (2.77). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum chlorophyll content was observed in T_{13} (4.47) followed by T_{14} (4.27), T_{15} (4.10) and T_{16} (3.40) while, plantlets developed from leaf whorl callus, maximum chlorophyll content was observed in T_{19} (4.37) followed by T_{20} (4.20), T_{21} (3.70) and T_{22} (3.03). The minimum chlorophyll content was observed in T_{18} (2.50) followed by T_{24} (2.57), T_{23} (2.60) and T_{22} (3.03).

The result showed that the plantlets regenerated using meristem and leaf whorl callus supplemented with MS + 3 mg/l 2, 4-D and MS + 4 mg/l 2, 4-D observed less differences in chlorophyll content in both the genotypes. The control showed maximum chlorophyll content in both the genotypes but after increasing the level of NaCl the chlorophyll content was decreased gradually. These results are agreement with [Wahid and Ghazanfar (2006) and Shomeili *et al* (2011)] [32, 26]. The minimum chlorophyll content was observed in plantlets treated with 2.5 % NaCl. The rate of salt accumulation in shoots of salt tolerant plants can be determined by the rate of transpiration. Transpiration rate generally tend to decline with increasing rhizospheric salinity in both sensitive and tolerant plants [Michael *et al* (1997)] [17]. It might be due to salt accumulation in the mesophyll which reduced somatal aperture [Flowers and Yeo (1995)] [10]. Chlorophyll concentration can be used as a sensitive cellular metabolic state, thus, its decrease signifies toxicity in tissues due to accumulation of ions [Don *et al* (2010)] [9].

Na^+: K^+ ratio

Co 86032

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum Na^+: K^+ ratio was observed in T_1 (0.82) followed by T_2 (0.81), T_3 (0.57) and T_4 (0.32) while, plantlets developed from leaf whorl callus, maximum Na^+: K^+ ratio was observed in T_7 (0.82) followed by T_8 (0.79), T_9 (0.57) and T_{10} (0.31). The minimum Na^+: K^+ ratio was observed in T_6 (0.13) followed by T_{12} (0.14). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum Na^+: K^+ ratio was observed in T_{13} (0.83) followed by T_{14} (0.78), T_{15} (0.56) and T_{16} (0.34) while, plantlets developed from leaf whorl callus, maximum Na^+: K^+ ratio was observed in T_{19} (0.85) followed by T_{20} (0.83), T_{21} (0.56) and T_{22} (0.39). The minimum Na^+: K^+ ratio was observed in T_{24} (0.14) followed by T_{18} (0.17).

CoM 0265

The plantlets developed from meristematic callus, MS supplemented with 3 mg/l 2, 4-D, maximum Na^+: K^+ ratio was observed in T_1 (0.53) followed by T_2 (0.40), T_3 (0.38) and T_4 (0.33) while, plantlets developed from leaf whorl callus, maximum Na^+: K^+ ratio was observed in T_7 (0.54) followed by T_8 (0.43), T_9 (0.42) and T_{10} (0.31). The minimum Na^+: K^+ ratio was observed in T_6 (0.13) followed by T_{12} (0.15), T_5 (0.18) and T_{11} (0.20). The plantlets developed from meristematic callus, MS supplemented with 4 mg/l 2, 4-D, maximum Na^+: K^+ ratio was observed in T_{13} (0.41) followed by T_{14} (0.36), T_{15} (0.27) and T_{16} (0.26) while, plantlets developed from leaf whorl callus, maximum Na^+: K^+ ratio was observed in T_{19} (0.47) followed by T20 (0.46), T_{21} (0.32) and T_{22} (0.29). The minimum Na^+ K^+ ratio was observed in T_{18} (0.11) followed by T_{17} (0.12), T_{23} (0.15) and T_{24} (0.15).

Higher Na^+: K^+ ratio was observed in control conditions (0 % NaCl) while, minimum Na^+ K^+ ratio was observed when plantlets were treated with 2.5 % NaCl in both the genotypes. These findings are in agreement with Shomeili *et al* (2011); Ashraf *et al* (2007) and Karpe *et al* (2012) [26, 1, 15].

Table 1: Response of sugarcane cv. Co 86032 and CoM 0265 to salt stress

Tr. No	Treartments	Shoot lenght		Root length		Plantlet height		Shoot : Root ratio		Leaf Number		Leaf Area		Chlorophyll content		Na^+ : K^+ ratio	
		Co 86032	CoM 0265	Co 86032	CoM 0265	Co 86032	CoM 0265	Co 86032	CoM 0265	Co 86032	CoM 0265	Co 86032	CoM 0265	Co 86032	CoM 0265	Co 86032	CoM 0265
T_1	$A_1B_1C_1$	9.03	8.4	3.07	3.03	37.6	32.37	1.6	1.4	6.4	7.43	52.43	37.66	5.6	4.6	0.82	0.53
T_2	$A_1B_1C_2$	8.1	8.1	2.87	2.8	36.53	29.43	1.33	1.27	6.4	6.77	46.45	36.45	5.3	4.47	0.81	0.4
T_3	$A_1B_1C_3$	7.5	7.9	2.8	2.57	32.47	28.43	2	1.33	6.27	6.47	42.28	35.31	5.13	4.5	0.57	0.38
T_4	$A_1B_1C_4$	7.27	7.6	2.53	2.37	30.97	27.2	2.2	1.7	6.07	6.27	32.72	33.65	4.77	3.9	0.32	0.33
T_5	$A_1B_1C_5$	6.2	7.43	2.3	2.1	30.37	24.6	2.3	2.13	5.8	6.23	32.64	32.75	4.63	3.63	0.24	0.18
T_6	$A_1B_1C_6$	5.7	6.03	1.73	1.27	24.5	21.17	1.27	1.23	4.8	5.7	30.74	28.53	3.7	3.37	0.13	0.13
T_7	$A_1B_2C_1$	9.13	8.2	3	3	37.3	32.1	1.53	1.53	6.13	6.93	50.64	37.81	5.57	4.5	0.82	0.54
T_8	$A_1B_2C_2$	8.27	8.17	2.7	2.67	36.6	29.37	1.5	1.33	5.83	6.8	47.11	35.67	5.37	4.3	0.79	0.43
T_9	$A_1B_2C_3$	7.63	7.53	2.53	2.5	31.67	28.6	1.9	1.57	5.6	6.57	42.03	33.71	5.33	4	0.57	0.42
T_{10}	$A_1B_2C_4$	7.1	7.5	2.47	2.4	31.67	27.13	1.9	2.03	5.6	6.27	33.86	33.44	4.63	3.33	0.31	0.31
T_{11}	$A_1B_2C_5$	6.47	7.4	2.27	2.3	30.53	24.67	2.3	2.07	5.4	6.07	33.78	31.83	4.37	2.77	0.28	0.2
T_{12}	$A_1B_2C_6$	5.8	5.97	1.53	1.27	24.2	21.07	1.5	1.17	4.6	5.6	30.35	28.49	3.67	2.7	0.14	0.15
T_{13}	$A_2B_1C_1$	8.97	8.1	2.83	3.1	37.53	31.83	1.47	1.37	6.2	6.83	50.27	37.42	5.47	4.47	0.83	0.41
T_{14}	$A_2B_1C_2$	7.97	7.87	2.73	2.83	36.9	29.1	1.93	1.23	6	6.7	44.22	36.35	5.23	4.27	0.78	0.36
T_{15}	$A_2B_1C_3$	7.2	7.6	2.57	2.8	32.7	27.4	2	1.43	5.4	6.4	43.16	34.73	5.2	4.1	0.56	0.27
T_{16}	$A_2B_1C_4$	7.07	7.43	2.57	2.4	32.43	27	2.23	1.73	5.2	6.2	42.2	33.26	4.57	3.4	0.34	0.26
T_{17}	$A_2B_1C_5$	6.43	7.1	2.2	2.37	30.47	24.73	2.43	1.97	5	5.83	30.52	32.55	4.1	3.2	0.24	0.12
T_{18}	$A_2B_1C_6$	5.37	5.87	1.43	1.43	24.73	20.53	1.47	1.23	4.1	4.67	28.98	28.54	3.37	2.5	0.17	0.11
T_{19}	$A_2B_2C_1$	8.6	8.03	2.8	2.73	37.53	31.5	1.33	1.4	5.6	6.63	49.52	37.4	5.27	4.37	0.85	0.47
T_{20}	$A_2B_2C_2$	7.87	7.9	2.57	2.7	36.8	28.53	1.73	1.23	5.53	6.3	46.19	35.48	4.8	4.2	0.83	0.46
T_{21}	$A_2B_2C_3$	7.23	7.53	2.53	2.6	32.6	27.47	1.83	1.57	5.3	6.13	43.21	34.33	4.8	3.7	0.56	0.32
T_{22}	$A_2B_2C_4$	7.17	7.33	2.37	2.53	31	27.07	2.13	1.63	5	5.9	31.78	33.24	4.47	3.03	0.39	0.29
T_{23}	$A_2B_2C_5$	6.53	7.07	2.27	2.53	30.57	24.53	2.47	2.07	5	5.43	31.35	32.29	3.83	2.6	0.25	0.15
T_{24}	$A_2B_2C_6$	5.63	6.03	1.47	1.3	24.57	20.63	1.3	1.17	4.03	4.8	28.3	28.22	3.33	2.57	0.14	0.15
SEM±	A	0.016	0.018	0.013	0.015	0.035	0.017	0.015	0.012	0.026	0.021	0.052	0.031	0.02	0.024	0.002	0.002
	B	0.016	0.018	0.013	0.015	0.035	0.017	0.015	0.012	0.026	0.021	0.052	0.031	0.02	0.024	0.002	0.002
	C	0.023	0.032	0.022	0.026	0.06	0.029	0.026	0.021	0.037	0.036	0.09	0.053	0.035	0.042	0.004	0.004
	A × B	0.028	0.026	0.018	0.022	0.049	0.024	0.021	0.017	0.046	0.03	0.074	0.043	0.029	0.034	0.003	0.004
	B × C	0.04	0.045	0.031	0.037	0.085	0.041	0.037	0.03	0.065	0.051	0.127	0.075	0.05	0.059	0.005	0.006
	A × C	0.04	0.045	0.031	0.037	0.085	0.041	0.037	0.03	0.065	0.051	0.127	0.075	0.05	0.059	0.005	0.006
	A × B × C	0.057	0.064	0.044	0.053	0.121	0.058	0.052	0.042	0.091	0.073	0.18	0.106	0.07	0.084	0.007	0.009
CD @ 5%	A	0.047	0.052	0.036	0.043	0.099	0.048	0.043	0.035	0.075	0.06	0.148	0.087	0.058	0.069	0.006	0.007
	B	0.047	0.052	0.036	0.043	0.099	0.048	0.043	0.035	0.075	0.06	0.148	0.087	0.058	0.069	0.006	0.007
	C	0.081	0.091	0.063	0.075	0.171	0.083	0.074	0.06	0.13	0.103	0.256	0.151	0.1	0.119	0.01	0.012
	A × B	0.066	0.074	0.051	0.061	0.14	0.067	0.06	0.049	0.106	0.084	0.209	0.123	0.082	0.097	0.008	0.01
	B × C	0.115	0.128	0.089	0.106	0.242	0.117	0.104	0.085	0.184	0.146	0.362	0.214	0.142	0.168	0.014	0.017
	A × C	0.115	0.128	0.089	0.106	0.242	0.117	0.104	0.085	0.184	0.146	0.362	0.214	0.142	0.168	0.014	0.017
	A × B × C	0.162	0.181	0.125	0.15	0.343	0.165	0.147	0.121	0.26	0.207	0.512	0.302	0.2	0.238	0.02	0.024
CV %		1.36	1.49	3.15	3.8	0.65	0.37	4.93	4.8	2.89	2.03	0.79	0.55	2.6	3.93	2.54	4.85

A_1= 3 mg/l of 2, 4-D, A_2 = 4 mg/l of 2, 4-D, B_1 = Meristem, B_2 = Leaf Whorl, C_1 = 0 % NaCl, C_2 = 0.5 % NaCl, C_3 = 1 % NaCl, C_4 = 1.5 % NaCl

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