Introduction

Sugarcane (*Saccharum officinarum* L.) is a glycophytic plant, belongs to family poaceae known to be cultivated in the tropical and subtropical regions worldwide. Brazil ranks first position in the world with respect to area (10.2 M ha) and production (768.67 MT) followed by India both in area (4.5 Mha) and production (348.44 MT) (FAOSTAT, 2016). Temperature range for its growth for different stages varies from 18° to 40°C. It has certain stages in its life cycle, where the application of water pays large dividends. Onset of tillering, elongation of internode and grand growth phase are the most crucial stage in a crop life cycle that need adequate amount of water (Srivastava and Rai, 2012). Salinity affect crop productivity by disturbing the plant basic phenomenon of growth and development like germination, vegetative and reproductive stages (Basalah, 2010 and Grewal, 2010, Granja et al., 2018). Salinity causes physical drought in soil and hinders the water uptake of plant leads to ionic toxicity, osmotic stress and nutrient
deficiency in plants from the soil (Shrivastava and Kumar, 2015). Basic problem behind salinity is, it decreases the soil osmotic potential by which sodium and chloride toxicity increases and water availability to plants decreases (Taiz et al., 2017; Simões et al., 2019). Several studies show that proline biosynthesis gene is induced on salinity stress that leads to its accumulation in plant (Simões et al., 2019), also it rapidly accumulates in plants subjected to water or osmotic stress and cold stress (Verslues et al., 2006). Green leaves are the site of its accumulation rather than nonphotosynthetic tissues of stressed plant (Perez-Perez et al., 2009). Normally, proline remain in low amount in plant and increased as salinity or other stress increases. We can say by this that plant that accumulate more proline in stressed condition are the tolerant and vice-versa. Up to 50 % decrease in crop yield is noticed with EC of 10.4 dSm⁻¹ (Santana et al., 2006; Granja et al., 2018). Essential nutrient are being taken by the plant roots in the form of soluble salts from the soil but its excessive accumulation inhibits the growth and development of plant. As per FAO 2015, a total of 800 MHz of land and 32 MHz of agricultural land are affected by salt stress.

With high evaporation and low precipitation rate crop plants in arid and semiarid zones are also getting affected by high salt stress (de AzevedoNeto et al., 2006; Ahmad et al., 2012). But in reality each climatic zone is more or less affected by the stress (Bhutta et al., 2004; Rengasamy, 2006). To held out against the salinity stress, plants amass the compatible solutes like proline, that reduces the cytoplasmic osmotic potential of plant cell, hence increase water absorption and scavenging reactive oxygen species (ROS) molecules (Qureshi et al., 2013; Pottosin et al., 2014; Gharsallah et al., 2016). In present study, we have investigated the proline content of plant under salinity and control condition for two consecutive years to observe the pattern if any change persists and also the effect on plant development.

**Materials and Methods**

Plant material were taken from Field laboratory and Experiment station of Sardar Vallabh Bhai Patel University of Agriculture & Technology Meerut, (U.P.), to which ten commercially used sugarcane varieties viz. Co 0118, Co 0238, Co 5011, CoLk 99270, CoS 8279, CoSe 8457, Co 5009, CoS 7250, CoPant 97222, Co 98014, were grown under two different levels of salinity, ECiw(Electrical conductivity of irrigation water) 10 dSm⁻¹ and ECiw 20 dSm⁻¹ along with the control with three replication in CRD (complete randomized design). EC of irrigation water was maintained by specific ratio of 3:1:2, of NaCl, Na₂SO₄, CaCl₂.2H₂O at the formative stage of plant and data for evaluation were taken at grand growth phase. Initial pH of soil was maintained at 6.2 and ECe (Electrical conductivity of the extract of a saturated soil paste) 1.39 dSm⁻¹.

**Proline content**

Proline content was estimated by the method of Bates et al., (1973).

**Reagents**

Aqueous sulfosalicylic acid (3% w/v)
Glacial acetic acid
Toluene

Acid ninhydrin reagent: 1.25 g of ninhydrin mixed with 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid.

**Procedure**

500 mg of fresh leaves were homogenized in 10 ml of aqueous sulfosalicylic acid (3%) and then centrifuge at 4000 rpm for 20 minutes. 2
ml of this aliquot was transferred into test tube and 2 ml of acid ninhydin reagent were added in each test tube. The mixture was heated on boiling water bath for 1 hours, after which reaction was terminated by placing the test tubes in ice box for cooling. Thereafter, the reaction mixture was shaken vigorously with 4 ml toluene and kept for 1 hr till the two layers formed. Chromatophore was thus extracted into toluene phase, (upper layer) was separated and its absorbance was measured at 520 nm using toluene as blank. L-Proline standard was used for quantification and the proline content in the sample was calculated using the formula.

Proline (mg/g fresh weight) = \[
\frac{36.2311 \times \text{O.D} \times \text{V}}{2 \times \text{W}}
\]

Where, \( W \) = Fresh weight of leaf in mg, \( \text{O. D.} \) = Optical density at 520 nm and \( \text{V} \) = total volume of extract in ml, \( 2 \) = Volume of aliquot taken for proline estimation in alkali and reduction of phosphomolybdic tungstate reagent by the tyrosine and tryptophan present in the treated protein.

**Statistical Analysis**

The data was subjected to statistical analysis using OPSTAT-1, SPSS (version 19.02), with significance at \( P \leq 0.05 \).

**Results and Discussion**

Osmotic stress and ion toxicity are the two prominent factors result due to NaCl stress. Normally, plant cells have higher osmotic pressure than to soil so it takes water and minerals from the soil but in case of salinity stress the osmotic potential of soil increases by the high aggregation of salt in the soil that makes plant unable to take water and essential minerals from the soil. This condition creates a condition of physiological drought in soil (Munnus et al., 2006; Bagum and Islam, 2015). In present study, we find that proline accumulate more in tolerant verities rather than susceptible plant when expose to salinity and its level further increases when we increase the EC of irrigation water respectively (Figure 1 and 2), control plant show less proline in their cell that signifies that plant under stress condition amass more proline in the cell as compared to non-stressed plant. Correlation of phenotypic traits with proline shows significant values of the mean pool data of two consecutive years (Table 1). Morphologically, plants show various symptoms under saline condition that truly proves the adverse effect of salinity on plant like plant growth reduction, decrease in length of internodes, cane girth and juice quality etc. (Hussain et al., 2004). Plants physiological and biochemical activity suffers due to disruption of anabolic and catabolic phenomenon (Corchete and Guerra, 1986; Torres-Schuman et al., 1989). Sugarcane plant has categorized as moderately sensitive towards salt stress (Shannon, 1997) and each plant or variety responds differently to salt stress due to their genotypic difference. Mahajan et al., (2013) examined the effect of salt stress on ten sugarcane genotypes viz., Co 94012, CoC 671, Co 740, CoM 0265, Co 86032, Co 9012, CoC 08026, CoM 08086, CoM 08011 and MS 08002, cultivated in three varying soil conditions viz., normal, saline and sodic soils, that were evaluated for the effect of salt stress on various factors like glycine betaine, proline, soluble protein contents, nitrate reductase activity and pyrroline-5-carboxylate synthase activity. The result revealed the increased accumulation of proline, glycine betaine, soluble protein and increased activity of pyrroline-5-carboxylate synthase activity in sodic soil can be used as biochemical markers for screening the efficient genotype of sugarcane for salt tolerance. Tolerant genotypes accumulate large amount of compatible solute that maintains the turgor pressure of cells eg.
glycinbetaine, free proline, sugar and polyols, above all, proline protects the cell from the ROS generated due to high salt induction (Jain et al., 2001). Some studies suggest that exogenous application of proline to stressed plant reduces the stress through ameliorating antioxidant activities and supressing sodium and chloride uptake with increase in potassium assimilation of plants (Heuer, 2010). In case of maize, on foliar spray plant growth and yield increases (Alam et al., 2016). In B. juncea plants antioxidant enzymes like catalase, peroxidase and superoxide dismutase activity increases with decrease in electrolyte leakage on applying proline (Wani et al., 2016; El Moukhtari et al., 2020). Anthony (1979), while investigating proline accumulation in eight species of marsh halophytes subjected to increasing salinity, found that plants accumulate proline only after attaining a threshold salinity level. Present study categorize the ten verities into tolerant varieties like CoPant 97222, CoS 7250, Co 98014 that accumulate more proline, moderate CoS 8279, Co 5011 and Co 5009 and susceptible varieties Co 0238, CoSe 8457 and Co 0118 in which is accumulation is less.

**Table 1** Correlation table based on the pool mean data of two consecutive years for phenotypic traits with proline under control and salinity, S1-10 dSm⁻¹ and S2-ECiw 20 dSm⁻¹ conditions for the year 2015-16 and 2016-17

|       | PH C | CG C | NTPH C | INPP C | LA C | INTL C | LAI C | PC C |
|-------|------|------|--------|--------|------|--------|-------|------|
| PH C  | 1    |      |        |        |      |        |       |      |
| S1    | 1    |      |        |        |      |        |       |      |
| S2    |      |      |        |        |      |        |       |      |
| CG C  |      | .635*|        |        |      |        |       |      |
| S1    |      | .682*|        |        |      |        |       |      |
| S2    |      | .750*|        |        |      |        |       |      |
| NTPH C|      |      | .547   |        |      |        |       |      |
| S1    |      |       | .766**|        |      |        |       |      |
| S2    |      |       | .813**|        |      |        |       |      |
| INPP C|      |      |        | .524   |      |        |       |      |
| S1    |      |       |        | .749*  |      |        |       |      |
| S2    |      |       |        | .841** |      |        |       |      |
| LA C  |      |      |        |        | .444 |        |       |      |
| S1    |      |       |        | .655*  |      |        |       |      |
| S2    |      |       |        | .690*  |      |        |       |      |
| INTL C|      |      |        |        | .737*|        |       |      |
| S1    |      |       |        | .742*  |      |        |       |      |
| S2    |      |       |        | .814** |      |        |       |      |
| LAI C |      |      |        |        | .104 |        |       |      |
| S1    |      |       |        | .477   |      |        |       |      |
| S2    |      |       |        | .698*  |      |        |       |      |
| PC C  |      |      |        |        | .882**|        |       |      |
| S1    |      |       |        | .673*  |      |        |       |      |
| S2    |      |       |        | .800** |      |        |       |      |

*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)
**Fig. 1** Proline estimation of ten sugarcane genotypes under control and salinity (10 dSm\(^{-1}\) and ECiw 20 dSm\(^{-1}\)) conditions for the year 2015-16

![Proline estimation graph](image1)

**Fig. 2** Proline estimation of ten sugarcane genotypes under control and salinity (10 dSm\(^{-1}\) and ECiw 20 dSm\(^{-1}\)) conditions for the year 2016-17

![Proline estimation graph](image2)

In conclusions due to salinity, water potential falls and solutes accumulate in the cell. Subsequently, the cell facilitates water towards it from the surrounding medium and stabilizes turgor pressure. Accumulation of K\(^+\), proline and sugar content enhances in tolerant line of sugarcane cultivar by the osmotic adjustment of leaf cells that reduces osmotic and leaf water potential of the tolerant plant as compared to sensitive plant. Being cytoplasmic solute, amino acid accumulates in the cell and its increase amount serves for osmotic adjustment under salinity. Accumulation of proline creates differences in osmotic potential that implies varietal difference in genetic level. Sugarcane is a perennial crop plant, if we discover the stage at which plant can adapt easily against stress it would be beneficial to screen out the genotypes for salinity tolerance. If one can understand the mechanism of sugarcane plant involve in physio-biochemical adaptation at different growth stages it will be profitable to enhance the cultivation of stress tolerant plant through genetic improvement strategies like biotechnological approach or conventional breeding techniques.
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Abbreviations

PH- plant height  
CG- cane girth  
NTPH- number of tillers per hill  
INPP- internode per plant  
LA- leaf area per plant  
INTL- intermodal length  
LAI- leaf area index  
PC- proline content

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