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Physiological characterization of cotton genotypes (*Gossypium herbaceum* L.) for salinity at seedling stage

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Abstract
Cotton important cash crop and is moderately tolerant to salinity but its yield is markedly affected due to poor germination and subsequent abnormal plant development under severe saline conditions. Therefore, cotton accessions need to be screened and evaluated for salt tolerance. The genus *Gossypium herbaceum* has benefits of biotic and abiotic stress resistance along with higher yield. The present study aimed to investigate the physiological response of desi cotton (*Gossypium herbaceum* L.) cultivars *viz*., GN.Cot-25, G.Cot-21, V-797 and GShv-907/13 under four different salt concentrations *viz*., 0.8 dS/m, 3 dS/m, 7 dS/m, 10 dS/m at seedling stage (30 DAS). The physiological traits *viz*., membrane stability (%), leaf relative water content (RWC) (%), chlorophyll content (μmol m^{-2}), photosynthesis rate (μmol CO_2 m^{-2} sec^{-1}), carotenoid content (mg g^{-1}), transpiration rate (mmol m^{-2} sec^{-1}) and stomatal conductance (μmol CO_2 m^{-2} sec^{-1}) were significantly reduced; while, surface wax content (μg/g of tissue) increased under different salt stress condition. However, GN.Cot-25 was able to maintain higher leaf relative water content (RWC), photosynthesis rate, chlorophyll content, carotenoid content, transpiration rate and stomatal conductance as compared to GShv-907/13 under of salt stress conditions whereas, the varieties, G.Cot-21 and V-797 showed moderate type of reduction for all parameters studied. Overall the study of physiological characterization proved to be effective in discriminating the desi cotton cultivars for salt tolerance.

Keywords: Salt stress, *Gossypium herbaceum*, cotton, physiological response

1. Introduction
Soil salinity has become a serious threat to agricultural productivity and a global challenge to sustainable agriculture and has been increasing over the time (Hossain *et al*., 2012) [11]. About 20% of world's cultivable land is adversely affected by soil salinity. Cotton is moderately tolerant to salinity with a salinity threshold of 7.7 dS/m (Chinnusamy and Zhu, 2005) [3] yet, soil salinity delays and decreases emergence of seedlings, cotton shoot growth and finally leads to reduction in seed cotton yield and fiber quality characteristics at moderate to high salinity levels (Khorsandi and Anagholi, 2009) [13]. The effects of salt stress on cotton can vary depending on growth stage, salt concentration and duration of salt treatment. Increased NaCl levels result in a significant decrease in root, shoot and leaf biomass as well as an increase in the root/shoot ratio in cotton (Meloni *et al*., 2001) [16]. Besides general stunting of plant growth, salinity causes several specific structural changes that disturb plant water balance. Salt stress affect photosynthesis activity and chlorophyll content in cotton due to restricted functioning of stomata (Saleh B., 2012) [23]. During seedling and vegetative stages, cotton plants can exhibit reduced stomatal conductance, transpiration rate, photosynthesis, water use efficiency and increased respiration rate (Higbie *et al*. 2010) [10].
Carotenoids are reported to play an important role in reactive oxygen species (ROS) scavenging, thereby protecting membranes from salt stress indicating that this trait could also serve as a useful indicator of NaCl stress in cotton (Zhang *et al*. 2014) [28]. Cell membrane stability has long been taken as an indicator of stress tolerance, so the study of extent of membrane leakage helps in better understanding of their relationship with salt tolerance in cotton. Leaf relative water content (RWC) is considered as an alternative measure of plant water status, reflecting the metabolic activity in plant tissues under salt stress.

Increasing salt concentration is detrimental to cotton and leads to cellular ion toxicity and imbalance of osmoregulation by competing or severely limiting the uptake of ions. Increased salinization warrants the development of salinity tolerant cotton genotypes for the sustained production. Desi cotton, especially *Gossypium herbaceum* is grown commercially in Gujarat, which is also having maximum salt affected area in the country. A simple, reliable criterion for evaluating salt tolerance of cotton is needed. The objectives of the present study was to characterize and compare the physiological responses of four desi cotton genotypes to determine a reliable measure of salt tolerance/susceptibility among genotypes in future screens for salt tolerance.

Materials and Methods

The seed of four desi cotton varieties of GN. Cot-25, G.Cot-21, V-797 and GShv-907/13 were collected from Main Cotton Research Station, Navsari Agricultural University, Surat, Gujarat. The varieties were grown in pre-constructed channels having four different EC concentration of Normal soil (0.8 dS/m) (Control; S_1), Normal soil + Saline Soil (1:1) (3 dS/m, S_2), Normal soil + Saline Soil (1:2) (7 dS/m, S_3), Saline soil (10 dS/m, S_4). The experiment was analysed as completely randomized design with factorial concepts (FCRD) with 3 replications and all parameters were analysed at 30 (DAS).

All physiological parameters were analyzed at 30 Days after Sowing (DAS) from leaves of cotton plants. Fresh upper leaf samples were collected for analysis and washed twice with tap water followed by deionized water. Leaves were analyzed in three repetitions for all the physiological estimation.

Cell membrane stability was measured according to the method of Premchandra *et al.* (1991) [20]. The relative water content (RWC) was estimated by the method of Barrs and Weatherly (1962) [2]. Surface wax content was estimated by standard method of Ebercon *et al.* (1977) [5]. Total chlorophyll content was measured with the help of optical chlorophyll meter (SPAD). Photosynthesis rate and gas exchange parameters like transpiration rate and stomatal conductance were measured with the help of InfraRed Gas Analyzer (Photosynthetic meter, Model CI-340, Handheld Photosynthetic system, CID-Bioscience). The carotenoids content was determined according to the method of Shabala *et al.* (1998) [24].

Results and Discussion

In recent years, the focus in screening has shifted towards examining specific physiological traits involved in salt tolerance because physiological traits are the key factors and useful tools for screening many samples in a short time and provide useful information about stress tolerance mechanisms, which could be useful to plant breeders for selecting and developing salt-tolerant genotypes. The physiological traits *viz*., membrane stability (%), leaf relative water content (RWC) (%), surface wax content (µg/g of tissue), chlorophyll content (µmol m^{-2}), photosynthesis rate (µmol CO_2 m^{-2} sec^{-1}), carotenoid content (mg g^{-1}), transpiration rate (mmol m^{-2} sec^{-1}) and stomatal conductance (µmol CO_2 m^{-2} sec^{-1}) were analyzed in desi cotton varities *viz*., GN.Cot-25, G.Cot-21, V-797 and GShv-907/13 at 30 DAS under salt stress.

Cell membrane stability has long been taken as an indicator of stress tolerance. The membrane stability (%) was found to be decreased with increase in salinity in all four desi cotton varieties. The highest membrane stability was recorded in GN.Cot-25 (86.32%) followed by G.Cot-21 (74.71%) and V-797 (71.94%) while, lowest membrane stability was observed in GShv-907/13 (60.85%) at highest salinity level (10 dS/m) as compared to control (Fig. 1). The reduction in membrane stability was also reported by Hassan *et al.* (2015) [8] in desi cotton (*Gossypium arboreum* L.) varieties, FDH 171 and FDH 786 under NaCl stress (100, 150 and 200 mM)

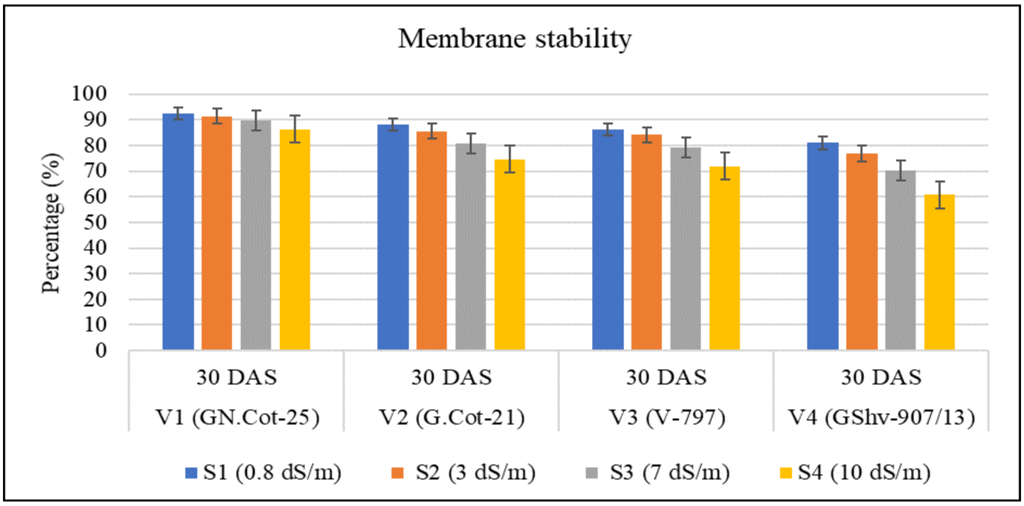

Fig 1: Membrane stability (%) of desi cotton varieties at salinity levels at 30 DAS (Vertical bar represents standard error of mean)

One of the prominent reasons for decreased in membrane stability (%) as the salinity increases was due to the peroxidation of lipids and loss of membrane integrity due to formation of reactive oxygen species (ROS). Plants possess antioxidant enzymes as well as antioxidant compounds to scavenge these ROS and antioxidant capacity of plants is directly related to their salt tolerance, so enhanced antioxidant system might be the reason for least decrease in membrane stability in GN.Cot-25 and the higher decrease in membrane stability of variety GShv-907/13 might reflects peroxidation of membrane lipids at higher salt concentration.

Extent of salt-induced effects on relative water content has been used as one of the vital water relation parameters for assessing degree of salt tolerance in plants (Siddiqi and Ashraf, 2008) [26]. RWC recorded reductions in all varieties subjected to different salinity stress. Highest RWC was recorded in GN.Cot-25 (78.01%) followed by G.Cot-21 (74.59%) and V-797 (73.15%) at higher salinity level (10 dS/m) as compared to control (0.8 dS/m) while, lowest RWC was observed in GShv-907/13 (67.40%) (Fig. 2). Similar kind of correlation between decrease in leaf relative water content against increasing salt stress level was observed by Hassan *et al*. (2015) [8] in desi cotton (*Gossypium arboreum* L.) varieties, FDH 171 and FDH 786 under NaCl stress (100, 150 and 200 mM) and Saleh (2012) [23] in four upland cotton (*Gossypium hirsutum* L.) varieties, Deir-Ezzor22, Niab78, Aleppo118 and Deltapine50 grown under non-saline conditions (control) and salt stress (200 mol m-3 NaCl) for 7 weeks. Higher decrease in RWC in GShv-907/13, moderate reduction in G.Cot-21 and V-797 compared to GN.Cot-25 suggested that GN.Cot-25 possess greater potential to maintain cellular osmosis than other varieties under salinity stress. Decline in leaf RWC under salt stress might be due to increased ion leakage (Munns, 2002) [18].

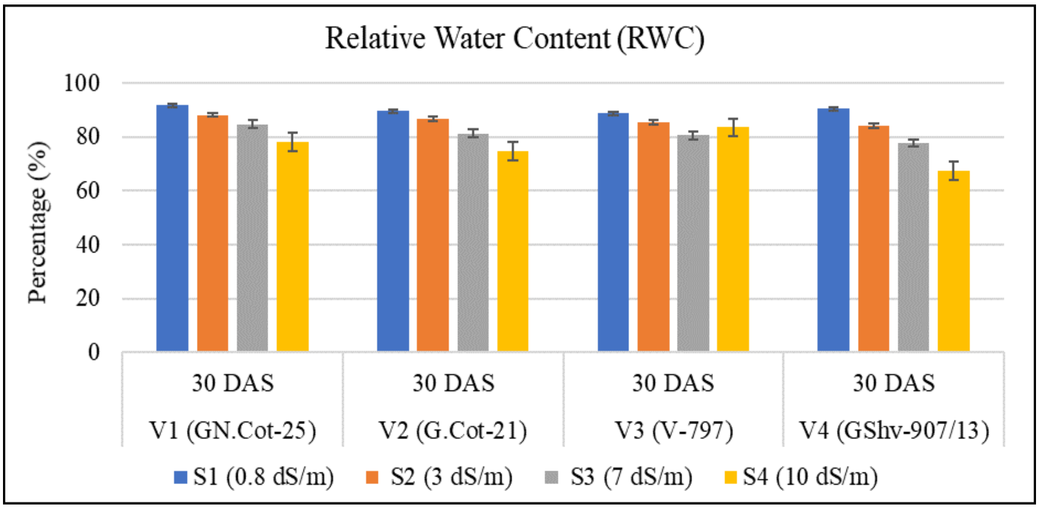

Fig 2: Relative water content (%) of desi cotton varieties at salinity levels at 30 DAS (Vertical bar represent standard error of mean)

Plants accumulate waxes on the surface of leaf to reduce residual transpiration in stress condition such as drought and salinity (Hasanuzzaman *et al*. 2017) [7]. Surface wax content was progressively increased with increase in salinity level in all varieties studied. As shown in Fig.3. the highest surface wax content was observed in GN.Cot-25 (35.64 µg/g of tissue) followed by G.Cot-21 (32.60 µg/g of tissue) and V-797 (31.84 µg/g of tissue) at the highest salinity level (10 dS/m) as compared to control while, lowest surface wax content was recorded in GShv-907/13 (27.11 µg/g of tissue). The similar kind of observation was reported by Ramani *et al*. (2018) [21] who screened eleven cotton genotypes against four different salinity levels and found that surface wax increased as the salt concentration increases and also reported that rate of increase in surface wax content was higher in tolerant genotypes as compared to susceptible genotypes. As per studies from model systems as well as crops, increased cuticular wax biosynthesis improves drought stress resistance. In rice, wheat, barley and sorghum, grain yield under water limiting conditions have positive correlation with wax content (Monneveux *et al*. 2004; Gonzalez and Ayerbe, 2010; Zhu and Xiong, 2013) [17, 6, 29]. Higher cuticular wax content is a promising trait for stress resistance conditions which was observed in GN. Cot-25 at 30 DAS.

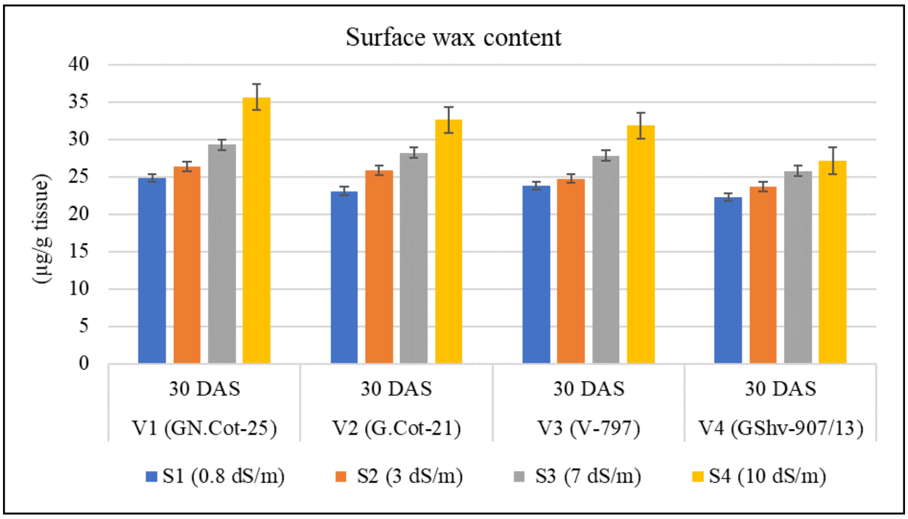

Fig 3: Surface wax content (µg/g of tissue) of desi cotton varieties at salinity levels at 30 DAS (Vertical bar represent standard error of mean)

The osmotic effect of salinity causes the reduction in intercellular CO_2 concentration and disintegration of photosystem which directly affects photosynthesis rate. Thus, salinity tolerance is related to the maintenance of net photosynthetic rate. Reduction in photosynthesis rate was detected across varieties with increase in salinity.

At the highest salinity level (10 dS/m) maximum leaf photosynthesis rate was noted in GN.Cot-25 (26.78 µmol CO_2 m^{-2} sec^{-1}) followed by V-797 (24.22 µmol CO_2 m^{-2} sec^{-1}) and

G.Cot-21 (20.55 µmol CO_2 m^{-2} sec^{-1}) whereas, lowest photosynthesis rate was recorded in GShv-907/13 (17.79 µmol CO_2 m^{-2} sec^{-1}) as compared to control (Fig. 4). Meloni *et al*. (2003) [15] found that net photosynthesis rate decreased in response to salt stress in two cotton cultivars, Guazuncho and Pora (hybrids between *Gossypium hirsutum /G. arboretum /G. raimondii*) and concluded that stomatal aperture limits leaf photosynthetic capacity in the NaCl-treated plants of both cultivars. Study of Desingh and Kanagaraj (2007) [4] on the photosynthesis rate of the two cotton varieties measured at different salt concentrations (50, 100 and 150 mM) varied significantly after 30 days of treatment. The photosynthetic rate decreased with increasing salinity in both cotton cultivars. Reduction in photosynthesis under salinity is unavoidable and it can be due to reduction in water potential.

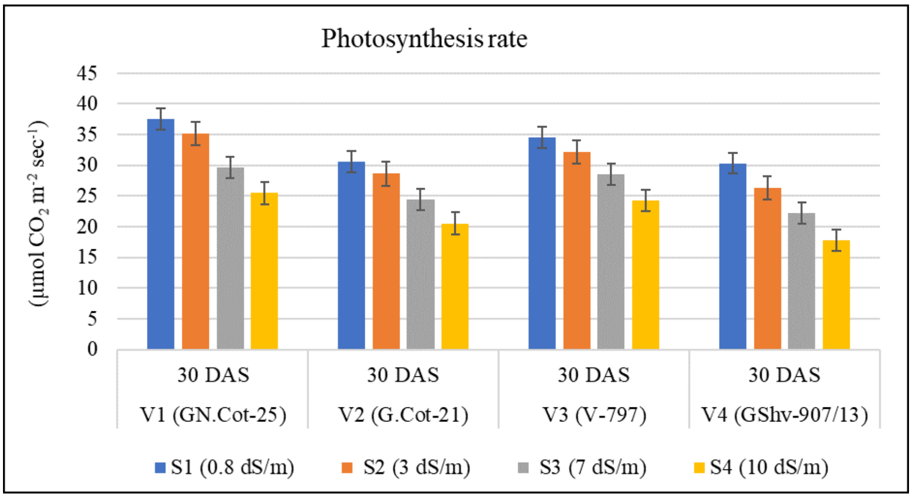

Fig 4: Photosynthesis rate (µmol CO_2 m^{-2} sec^{-1}) of desi cotton varieties at salinity levels at 30 DAS (Vertical bar represent standard error of mean)

Chlorophyll content of leaf is one of the essential physiological parameters, which influences photosynthesis and thus, indirectly associated with growth and yield of crop plants (Al-saady *et al.*, 2012) [1]. Chlorophyll degradation under saline condition is well-known phenomenon and had been reported in several plants. The chlorophyll content was decreased with increase in salinity in all four desi cotton varieties.

As shown in Fig. 5, at the salinity level (10 dS/m) highest chlorophyll content was observed in GN.Cot-25 (48.43 µmol m^{-2}) followed by G.Cot-21 (44.07 µmol m^{-2}) and V-797 (42.40 µmol m^{-2}) as compared to control (0.8 dS/m) while, lowest chlorophyll content was recorded in GShv-907/13 (30.80 µmol m^{-2}). The similar trend was reported by Saleh (2012) [23] who conducted a pot experiment to evaluate performance of four varieties of upland cotton (*G. hirsutum* L.) *viz.*, Deir-Ezzor22, Niab78, Aleppo118 and Deltapine50 grown under non-saline conditions (control) and salt stress (200 mol m^{-3} NaCl) for 7 weeks. Destruction of chlorophyll pigments by salt induced generation of ROS could be one of the reasons for loss of photosynthetic pigments (Saha *et al.* 2010) [22]. Less reduction of chlorophyll content in GN. Cot-25 compared to other varieties with increasing saline conditions suggests that GN.Cot-25 maintained high pigment composition in response to salinity stress which might contribute for high photosynthetic activity and thus, it is salt tolerant.

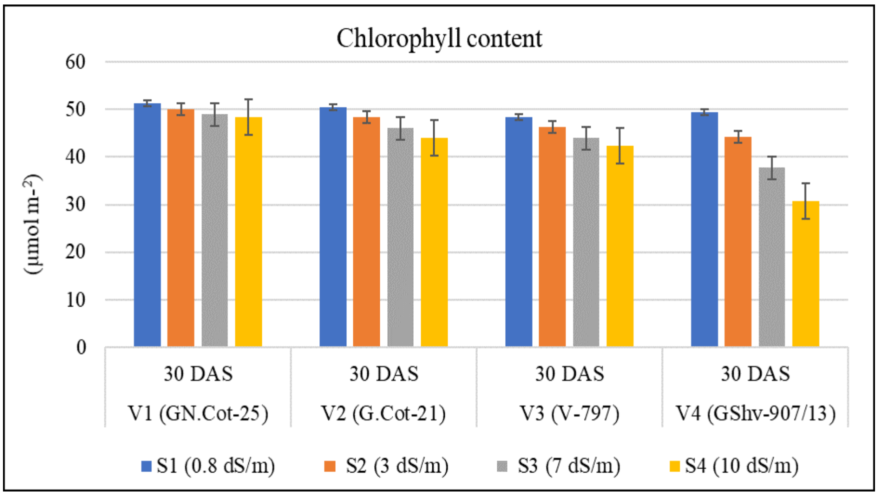

Fig 5: Chlorophyll content (µmol m^{-2}) of desi cotton varieties at salinity levels at 30 DAS (Vertical bar represents standard error of mean)

Carotenoids play an important role in ROS scavenging, thereby protecting membranes and photosynthetic apparatus from photo-inhibition from salt stress (Talukdar, 2012) [27]. Salt stress induces the modification of carotenoid concentration in salt sensitive plants and carotenoids decreases more slowly than chlorophylls under salt stress. The results showed that carotenoid content decreased with increase in salinity in all four desi cotton varieties.

At the salinity level (10 dS/m) highest carotenoid content was observed in GN.Cot-25 (0.87 mg g^{-1}) followed by G.Cot-21 (0.70 mg g^{-1}) and V-797 (0.69 mg g^{-1}) as compared to control while, lowest carotenoid content was recorded in GShv-907/13 (0.51 mg g^{-1}) (Fig. 6). Shah *et al*. (2011) [25] reported that cotton accessions FH-113, PB-899 and MNH-789 were found drought tolerant due to the high carotenoid content and CIM-506, FH-901 and CRIS-466 were found drought susceptible due to the low carotenoid content during water stress. Zhang *et al*. (2014) [28] also demonstrated that CCRI-79 (salt tolerant) and other salt-sensitive cotton cultivar, Simian 3 showed a decreasing trend of carotenoid content with increasing salt stress. Thus, carotenoid content could also serve as a useful indicator of salt stress in cotton.

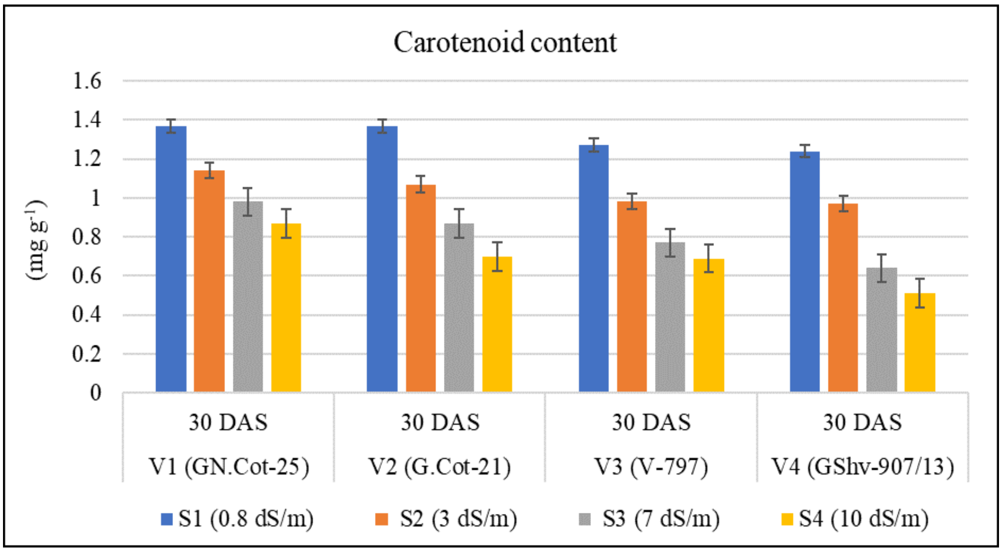

Fig 6: Carotenoid content (mg g^{-1}) of desi cotton varieties at salinity levels at 30 DAS (Vertical bar represents standard error of mean)

Transpiration rate is one of the gas exchange parameters which severely affected by salt stress. The decreased in transpiration rate was observed with increasing salinity level. At the salinity level (10 dS/m) higher transpiration rate was recorded in GN.Cot-25 (8.44 mmol m^{-2} sec^{-1}) followed by G.Cot-21 (5.54 mmol m^{-2} sec^{-1}) and V-797 (4.10 mmol m^{-2} sec^{-1}) as compared to while, lowest transpiration rate was recorded in GShv-907/13 (3.02 mmol m^{-2} sec^{-1}) (Fig. 7).

The ability of plants to maintain normal rates of transpiration under saline condition is an important indicator of salt tolerance, because transpiration is related to normal rates of CO_2 uptake for photosynthesis (Negrao *et al*. 2017) [19]. The least reduction in transpiration rate in GN.Cot-25 followed by G.Cot-21 and V-797 and maximum reduction in GShv-907/13 was recorded at highest salinity level. Thus, it can be concluded that GN.Cot-25 have tolerant mechanism to cope with the salinity stress which is absent in GShv-907/13. Similar kind of decrease in transpiration rate against salt stress was earlier reported by Maddan *et al*. (2013) [14] who observed that rate of transpiration decreased with increasing level of salinity in seven cultivars but the rate was much slower in resistant cultivar of cotton. The decreased in transpiration rate was also reported by Janagoudar (2002) [12] who studied six cotton genotypes against the salinity stress up to 14.8 dS/m and reported that transpiration rate was decreased with increase in salinity levels in all varieties but the tolerant varieties maintained higher transpiration rate at highest salinity levels as compared to susceptible varieties.

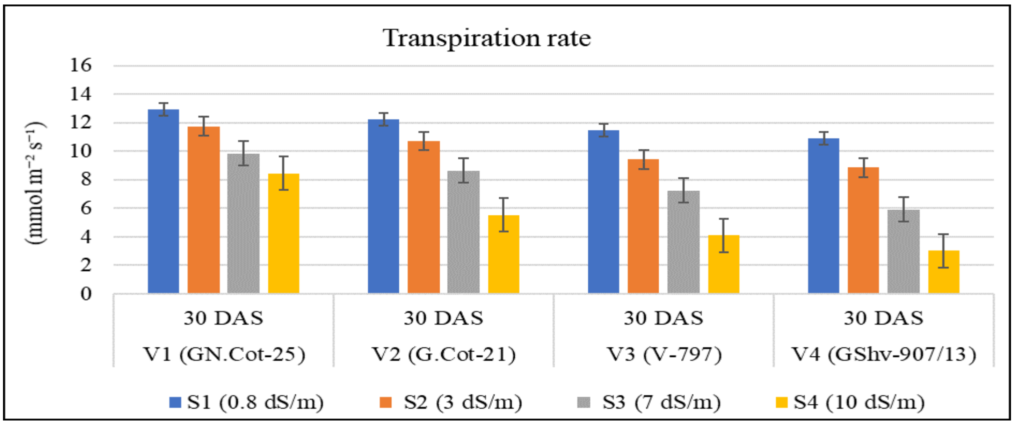

Fig 7: Transpiration rate (mmol m^{-2} sec^{-1}) of desi cotton varieties at salinity levels at 30 DAS (Vertical bar represents standard error of mean)

Stomatal conductance is also one of the gas exchange parameters like transpiration rate that severely affected by salt stress. Due to salt stress the decrease in stomatal conductance rate was observed as compared to normal conditions.

At 30 DAS, at the salinity level (10 dS/m) higher stomatal conductance was recorded in GN.Cot-25 (0.231 µmol CO_2 m^{-2} sec^{-1}) followed by G.Cot-21 (0.206 µmol CO_2 m^{-2} sec^{-1}) and V-797 (0.176 µmol CO_2 m^{-2} sec^{-1}) as compared to control (0.8 dS/m) while, lowest stomatal conductance was recorded in

GShv-907/13 (0.134 µmol CO_2 m^{-2} sec^{-1}) (Fig. 8). The least reduction in stomatal conductance was recorded in GN. Cot-25 followed by G.Cot-21 and V-797 and maximum reduction was observed in GShv-907/13. The slower decreased of stomatal conductance in GN. Cot-25 might be due to decline in leaf water content under salt stress as the water flow within plants is restricted at organ, tissue and cellular levels. This would lead to closing of stomata to conserve the water status in plants. Therefore, stomatal conductance is maintained under low water potential in resistant genotypes under high saline condition. Similar trend of decrease in stomatal conductance against salt stress was reported by Janagoudar, (2002) [12] who observed the effect of salt stress in six cotton genotypes and found that stomatal conductance was decreased with increase in salinity levels in all varieties but the tolerant varieties maintained higher stomatal conductance at highest salinity levels. Hassan *et al*. (2014) [9] also observed the gradual decrease in stomatal conductance under increase in salinity levels in two *G. arboreum* varieties FDH 171 and FDH 786.

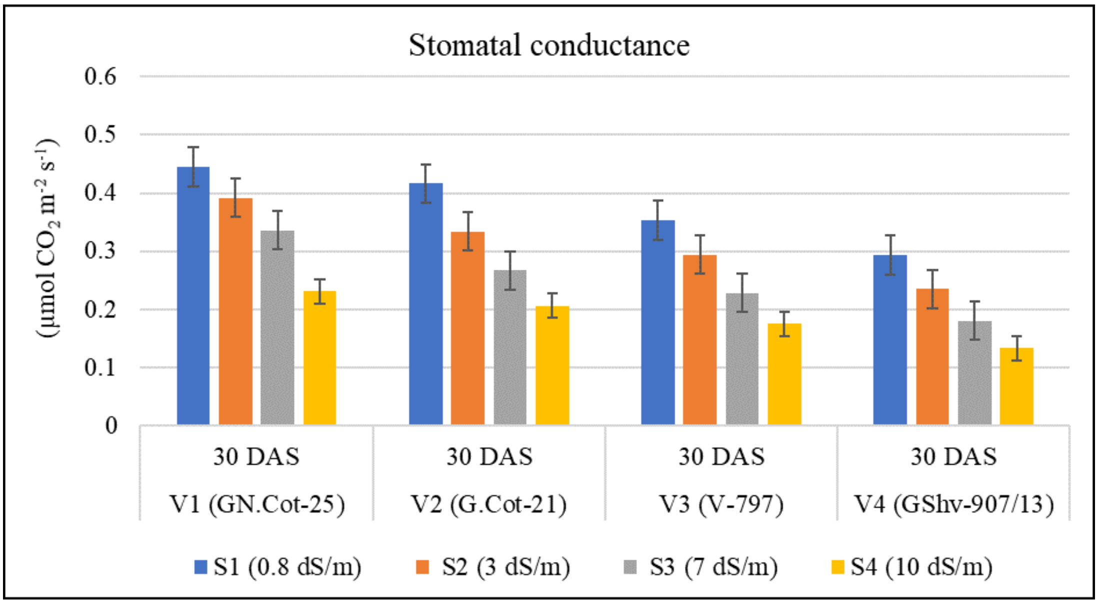

Fig 8: Stomatal conductance (µmol CO_2 m^{-2} sec^{-1}) of desi cotton varieties at salinity levels at 30 DAS (Vertical bar represents standard error of mean)

Conclusion

The results of this investigation showed that 'GN. Cot-25' cultivar had the highest tolerance to salt stress as it performed better in terms of all the character studied. This can be attributed to better gas exchange parameters and more efficient membrane homeostasis. Our results also implicate that physiological characters (Leaf photosynthesis rate, chlorophyll content, transpiration rate and stomatal conductance) can be assumed as good indicators of salt stress tolerance or susceptibility in cotton and these parameters are of immense important in large scale screening of genotypes for salt stress. Identification of salt tolerant cotton genotypes for salt affected areas can benefit the farming community through increased seed cotton yield and quality. Further these determinants should be analysed at different growth stages which can be helpful for a better understanding of the response of cotton crop plants towards salt stress.

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