Novel Hydroponic Design For Screening Cotton Genotypes For Salinity Tolerance

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ABSTRACT: A laboratory experiment was conducted to test different hydroponic designs for screening cotton accessions for salinity tolerance. The study was aimed to find out a repeatable, reproducible and reliable hydroponic design in which large sample size could be screened in one go. Six *arboreum* genotypes viz: LD1026, LD1027, LD1019, LD1037, LD1029 and LD949 were screened for salinity tolerance via four different hydroponic designs at EC 18 and 21 dS/m and were compared with the national check, RAHS-14. The glass experiments and the thermocol tray design produced large variability in the survival percentage due to small sample size (4-6 seeds/replication) and cross bred nature of cotton. However, a novel design consisting of wooden trays which could hold large sample size gave more accurate and repeatable results and hence the same is being recommended for hydroponic screening of cotton genotypes. Maximum survival percentage was observed with LD949 (~40 %) followed by LD1037 (~39.5%) and least was in LD1019 (~4%) and LD1029 (~5%) at 21 dS/m. The growth parameters recorded at 15 DAG corroborated the survival data. The national check RAHS-14 recorded a survival percentage of 35% at 21 dS/m. Based on this study, LD949 and LD1037 proved better than RAHS-14 for salinity tolerance under hydroponic conditions.

Key words: Cotton, hydroponics, salinity, screening, survival percentage, growth parameters

Several environmental factors adversely affect plant growth and development and final yield performance of a crop. Drought, salinity, nutrient imbalances (including mineral toxicities and deficiencies) and extremes of temperature are among the major environmental constraints to crop productivity worldwide. It has been estimated that 20% of world's cultivable land is adversely affected by soil salinity. In India about 8.6 mha (Pathak, 2000) of land area is affected by soil salinity. Salt affected soils occur in the states of Uttar Pradesh, Gujarat, West Bengal, Rajasthan, Punjab, Maharashtra, Haryana, Orissa, Delhi, Kerala and Tamil Nadu. Almost 2.8 million hectares of salt-affected soils are present within the Indo-Gangetic alluvial plain occupying parts of Punjab, Haryana, Uttar Pradesh, Delhi, Bihar and Rajasthan states (Abrol *et al*, 1971). Out of the total 577.86 km^2 area of land use/land cover of Punjab, 9.96 km^2 ie 1.72% is severely salt affected soil and 45.63 km^2 ie 7.90 % is moderate salt affected soil. Salinity is one of the most important abiotic stresses, limiting cotton production. India contributes 19% of the total cotton produced in the world. The productivity of cotton in India is least being 0.5 tons/ha as compared to 1.2 tons/ha in China and 0.9 tons/ha in U.S.A (Anonymous, 2009). There are 20-30% losses in cotton production mainly due to abiotic stresses (Bhute *et al*, 2012). Nine per cent of the

country's area under cotton cultivation is in Punjab. In Punjab water logging, salinity and alkalinity problems have arisen in South Western districts which also constitute the cotton belt of Punjab (PRSC, 2010). Out of these 49% are saline, 43% sodic and 8% saline-sodic. The twin problems of waterlogging and salinization in South-west Punjab are broadly attributed to the depressional location of the area coupled with the lack of proper drainage system, poor percolation because of impervious clay strata and constant seepage from Rajasthan Feeder Canal & Sirhind Feeder Canal (Mihir, 2013). The groundwater in many parts of southwest Punjab contains high concentration of dissolved salts with electrical conductivity varying from 2 to 7 dS/m (Shakya and Singh, 2010). Although cotton is moderately tolerant to salt, with a salinity threshold of 7.7 dS/m (Maas, 1990; Chinnusamy and Zhu, 2005) yet, soil salinity delays and reduces emergence of seedlings, decreases 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). However, a substantial variation in tolerance to salinity among cotton cultivars has been reported (Ashraf, 2002). All the same it would be better to screen cotton genotypes for salinity tolerance.

It is generally accepted that the germination and seedling stage of plant cycle is more sensitive to salinity than adult stage (Lianes *et al*, 2005). So the screening for salinity tolerance should be performed at seedling stage. Different methods for screening can be adopted. Extreme spatial and temporal variability in soil salinity makes screening of genotypes for salinity tolerance under field conditions unfeasible (Richards, 1983; Ibrahim, 2003). Solution culture screening/hydroponic system has been reported as a more sensitive, rapid and reliable method for screening cultivars for high salt tolerance. In the present study, the effort has been laid to construct a low priced, novel and reusable hydroponic design for hydroponic for screening cotton genotypes for salinity tolerance.

MATERIALS AND METHODS:

The experiment was conducted in the Biochemistry Department of Punjab Agricultural University, Ludhiana. Six *Gossypium arboreum* genotypes were procured from Cotton section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Salt tolerant *Gossypium herbaceum* RAHS-14 was provided by University of Agricultural Sciences, Dharwad. The design for hydroponics included transparent plastic glasses, thermocol glasses, thermocol trays and wooden trays.

Germination of seeds: The genotypes were screened hydroponically for salt tolerance by the modified method of Hemphill *et al* (2006). Uniform sized seeds of each genotype were surface-sterilized in 0.1% mercuric chloride for 10 min, followed by washing thoroughly with distilled water. For this, seeds were germinated on filter paper in Petri dishes at room temperature (25-28˚C) for 3 days. The seedlings were then transplanted to the hydroponic medium (half strength Hoagland solution) of electrical conductivity (EC) 18 and 21 dS/m along with control (EC < 2 dS/m) in separate trays.

Tranfer to hydroponic designs: a) Disposable transparent glasses of capacity 200 ml were filled with approximately 180 ml of half strength Hoagland solution of EC 18 and 21 dS/m and

control followed by covering the glasses with a net to hold the germinated cotton seedling. The survival percentage was recorded 15 DAG (days after germination). Each glass contained 4-6 seeds of each genotype and there were 4 replications (glasses) for each genotype. The solution in the glasses was changed twice a week and the EC was checked regularly with a hand EC meter.

b) Thermocol glasses of 200 ml capacity were filled with 180 ml of half strength Hoagland solution of EC 18 and 21 dS/m followed by covering with net on the top. The control consisted of half strength Hoagland solution of EC < 2 dS/m. The germinated cotton seedlings were transferred from the petri dishes on net over the glasses. Each glass contained 4-5 germinated seeds and each glass constituted one replication with 4 replications per genotype. The EC was maintained throughout the experiment and solution in the glasses was changed at regular intervals. The survival percentage was recorded 15 DAG.

c) A plastic tray (30 cm × 45 cm) was taken and a thermocol sheet of 2 cm thickness was cut viz-a-viz plastic tray size and equal sized holes (each of radius 1 cm) were made at equal distances using a red hot iron rod. The corners of the sheet were curved with hot knife so that the sheet fits properly and tightly into the tray. Each thermocol tray consisted of eight rows with four holes in each row. Net was fixed on one side of the thermocol tray (the side that faced the Hoagland solution). The fully stretched net was firmly attached to the thermocol with fevicol and drawing pins. The fevicol was applied on both the sides of the thermocol tray at all the places including the space between two consecutive holes and was kept for 2 days to dry. Theses trays were then kept over the plastic trays filled with 6 l half strength Hoagland solution of EC 18 and 21 along with control (EC < 2 dS/m). The germinated cotton seedlings were kept in the holes (@ 5 per hole) touching the nutrient solution. This means that a single tray can hold 8 genotypes in quadruplicate each and each replication having 5 seeds or 16 genotypes in duplicate. EC and pH was checked regularly.

A real buy novel method (Wooden frames): Wooden frames were designed with two parts, an outer frame (40 cm × 26 cm) and an inner frame (35cm × 25 cm) which helped fixing the net. The net of suitable size, slightly bigger than the outer frame, was cut to be kept over the outer frame. After stretching the net from all sides, the inner frame was put in place to fix the net (Fig 1). The inner frame contained six partitions (for demarcating the different genotypes), one for each genotype and each partition can hold capacity of 40-50 cotton seeds. The plastic tray was filled with 5 l half strength Hoagland solution of EC 18 and 21 dS/m along with control (< 2 dS/m). The outer frame had four legs (of height 4 cm) in the corners to support the frame and to keep the net just touching the solution. The cotton seedlings were transferred to the wooden tray for the further growth hydroponically The EC was maintained by changing the solution twice a week.

Growth parameters: Survival percentage and growth parameters (root/ shoot length and weight) were recorded 15 DAG (days after germination).

RESULTS AND DISCUSSION:

Screening of a large number of genotypes for salinity tolerance is a cumbersome process. Greater sample size implies the greater accuracy of the experiment. Cotton germplasm has hardly been screened for salinity tolerance and shows a large variation within seeds of same genotype because of its cross bred nature. So, sample size and easy handling of the experiment is essential for the quick and accurate result attainment.

Comparative performance of seven cotton genotypes at three different salinity levels was observed by Ibrahim *et al*, 2007 in thermopore sheets floating on half strength Hoagland's nutrient solution in 200 l capacity iron tubs lined with polythene sheet. B-284 and FH-945 were found tolerant against NaCl stress. Dogan *et al*, 2012 transferred the young cotton plants after germination into sterile jars with MS (250 ml) containing different levels of NaCl for one month and observed a decrease in stem length and fresh weight with increasing salinity levels. Cotton seedlings showed more susceptibility to salt stress in hydroponic cultures than to MS medium (Sattar *et al*, 2010). Hemphill *et al* (2006) suggested a screening method for salt tolerance in cotton in which cut, disposable pipet tips were used to hold the germinated seedlings in place within the pastic trays. Expensive cylindrical PVC tubes filled with cylindrical black polycarbonate pellets in a series of 50 l tubs was used as a hydroponic screening method against salinity in barley (Tavakkoli *et al*, 2012). This implies that large number of methods were previously available for screening hydroponically but there was a financial constraint in our laboratory which made us devise a method which is cheap and reusable. Solution culture selection approach can be helpful for the screening of cotton genotypes for salt tolerance, if using physiological traits and criteria (Akhtar *et al*, 2010).

Comparing the hydroponics designs:

Washing thoroughly with detergent and distilled water make the transparent and thermocol glasses reusable. No special preparation is required for them. Just the glasses and the net is all that was required. But, the plastic glasses allowed light to enter into them from sides due to their transparency. Light favors algal growth thereby interfering with plant development. It also disfavours the natural conditions where the roots are in dark environment. So, the thermocol glasses were preferred over them which reduced the penetration of light. But screening of 150 genotypes means a total of 1800 glasses (twelve glasses of every genotype, 4 each at EC 18, 21 dS/m and control). Decanting the old solution and putting fresh solution into such a large number of glasses is a time consuming task. Further, each replication does not have the same micro-environment, putting limitation to result accuracy. So, the tray method should be preferred over the glass method.

The thermocol tray provides the same microenvironment to all the replications thereby reducing the error probability. The change of the solution can be done for eight genotypes simultaneously meaning the requirement of approximately 60 trays in total for 150 genotypes at two different ECs and control. Though the environment given to different replications is same, but sometimes, the fevicol gets dissolved in solution which losen the net and the seeds of contrasting genotypes from different holes may get mixed hence ruining the experiment. Along with this, they have the limitation of being used only once due to the algal growth at the bottom side and cutting of net

for taking the plants out easily without damaging the roots. The fevicol mixed in the Hoagland solution can also interfere with the plant growth. The thermocol tray is fixed over the plastic tray with pressure. This may lead to a physical disturbance to seeds during the change of the solution. Wooden trays are free of all the limitations mentioned above. No problem of mixing of contrasting genotypes is there. The legs at the corners provide the support to keep them on the shelf during the change of the solution. Sample size taken in this case is the largest (50) which increases the chances of surity and accuracy. Washing with detergent and cleaning with ethanol makes them reusable.

The wooden trays designed will be specific for a crop depending on the size of the seed. These are highly suitable to screen out a large number of cotton genotypes with greater sample size. If made in the right way, can prove to be the real buy and easy to handle hydroponics design, giving repeatable results. There is variability in survival percentage of six *arboreum* genotypes with less sample size (4-6 seeds/replication), hence giving false results. The data of survival percentage of different *arboreum* accessions show that greater sample size gives using wooden trays give similar results.

Different genotypes under salinity conditions adapt very differently from one another. A plant's first line of defense is in its roots. The physical growth parameters such as root, shoot length and fresh weight have shown to contribute more towards salt tolerance of crop at early growth stages and can be used as a selection criteria for salt tolerance (Mane *et al* 2010, Shonjani 2002). Sattar *et al* (2010) observed a 50 % reduction in survival percentage of cotton genotype FDH-786 at 300 mM salt concentration with 0% survival at 500 mM and beyond upto 1000 mM in hydroponic cultures.

The breeding in cotton has not been done for salinity tolerance. Therefore a large variation was seen among the genotypes of cotton. The results in Table 1 show a variation in survival percentage with small sample size in glasses and thermocol tray while the wooden trays with large sample size gave similar results.
Maximum survival percentage was observed with LD949 followed by LD1037 and least was in LD1019 and LD1029. RAHS-14 showed least decrease in root/shoot length and weight. This implies that LD949 and LD1037 are highly salinity tolerant while LD1037 and LD1026, LD 1019 and LD1029 are moderately and least salinity tolerant.

ACKNOWLEDGEMENT:

The authors express their sincere gratitude to DST INSPIRE, NEW DELHI for providing financial support for carrying out the experiment.