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Research Article

Rapid assessment of water severe stress tolerant cotton (*Gossypium hirsutum* L.) genotypes based on seedling and morph-physiological traits at two moisture levels

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Abstract: Cotton is an important crop of almost countries. It is backbone of textile industry. Water severe stress possess the most important constraint to plant survival and crop productivity. It is responsible for shedding of small squares on large scale, resulting in a decrease in flowering. Therefore, the main objective of this study was to assess the response of some cotton genotypes at seedlings stage against water severe stress taken from State Key Laboratory of Cotton Biology-Anyang, CAAS, China. The experiment was conducted under Randomized Complete Block Design with three replications. The treatment of the experiment included two factors (control 100% and severe stress 50%). After 55 days, fourteen morph-physiological traits (Transpiration Rate, CO2 assimilation, Stomatal Conductance, Chlorophyll Content, Leaf Temperature, Photosynthesis Rate, New Leaf Area, Stem Diameter, New Internode, Plant Height, Shoot Fresh Weight, Shoot Dry Weight, Root Fresh Weight and Root Fresh Weight) were recorded at seedling stage and compared under control and water severe stress. It was found that highly significant for all traits under treatment levels, reflected the effects of water variability on the traits. Cluster analysis also grouped the genotypes according to their response to water severe stress, detected that the genotypes Zhong 1476, Deltapine 14 and Zhong 915 were the higher tolerant genotypes under water severe stress. These genotypes may be selected and exploited to improve water severe tolerance for the cotton crop breeding programs which have a significant impact to minimize the adverse effects of drought on cotton in an easy and non-expensive way.

Key words: Cotton, water stress, morpho-physiological traits, Tolerance.

Introduction

Cotton (*Gossypium hirsutum* L.) is the most important fiber crop, providing half of the global fiber requirement. Cotton seed contains 21% oil and 23% protein, both of which are of relatively high quality. Cotton seed oil also used in products such as soap, margarine, emulsifiers, cosmetics, pharmaceuticals, rubber, and plastics (J. H. Zonta *et al.*, 2017; Mvula *et al.*, 2018; Akbar and Hussain, 2019 and Iftikhar *et al.*, 2019).

Water plays an important role in the production of plants. In every part of the world, it is the factor for agricultural crops in the initiation of growth, subsequent maintenance of developmental process throughout the plant's life. (Lisar *et al.*, 2012; Noorka and Haidery, 2011; Shafi *et al.*, 2012) stated that water is the core medium for carrying metabolites, nutrients and molecule in all physiological processes of plants. Water stress is the most prevalent abiotic constraint that causes for yield reduction in agricultural production (Robin *et al.*, 2003; Ahmad *et al.*, 2010 and Elsayed *et al.*, 2019) which creates difficulties in completing normal physiological functions by lowering plant water potential and turgor (Lisar *et al.*, 2012). It affects also the carboxylation, photosynthetic efficiency

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which alters the whole plant growth and mechanisms, electron transport chain and ultimately yield by decreasing total biomass production, net yield and grain filling (Bibi Amir et *al.,* 2014 and Iftikhar *et al.,* 2019). Leaves per plant, leaf dry weight, shoot dry weight, stem dry weight, ion accumulation, carbon fixation, chlorophyll contents and leaf to stem ratio decreases with increase in drought stress severity (Rahbarian *et al*., 2011; Hajibabaee *et al*., 2012; Bibi Amir *et al.,* 2014 and Elsayed *et al.,* 2019). Water severe stress at earlier stages of plant growth reduces the root and shoot dry mass, net biomass, sodium and potassium ion concentration and suppresses the chlorophyll a, chlorophyll b and total chlorophyll but differ in different genotypes of the same specie (Ebrahim, 2012; Bibi Amir *et al*., 2014). So, Plants can overcome this stress by dehydration avoidance or by dehydration tolerance (Blum, 2005). Thus, the reactions of plants to water stress at different levels depending upon the intensity and duration of stress as well as kind of plant and its growth stage (Jaleel *et al*., 2008).

Seedling trait is still an important aspect of crop breeding programme, since the final stand of a crop depends on seedling characteristics, because of breeding for water stress requires continuous efforts primarily through the efficient screening techniques and knowledge of genetic mechanism governing heritable parameters. (Noorka *et al.,* 2013). Genetically, equivalent cotton plant populations, when subjected to water deficit show reduction in yield of up to 50% if compared to those that have been irrigated (Brito *et al*., 2011; Mvula *et al.,* 2018 and Iftikhar *et al.,* 2019).

Physiological traits linked with water severe tolerance in cotton have strong relationship with yield parameters. For example, photosynthetic rate which significantly decreases with the imposition of water stress, can be effectively used for germplasm screening under water severe condition. Since, the response of germplasm with genetic variability may exhibit differential response under normal and water severe conditions, regular screening of emerging germplasm need to be carried out for better adaptability and sustainable production Akbar and Hussain (2019).

A number of studies around the scientific world have been done at early growth stages in cotton to evaluate the genetic relationship among various seedling traits in cotton under various water stress levels (Wajid*et al.,* 2011 and Bibi Amir et *al.,* 2014). Thus, it was deduced that plants have a variation in their response to water stress genetically, even close relative genotypes within a specie. There are many works were done in cotton under water stress. Basal *et al.,* (2005) reported that root characteristics play an important role in determining the response of plants to water severe stress and that decreases shoot growth rate, plant height and yield. Iqbal *et al.,* (2011) found that the differing measurement of root and shoot lengths of cotton seedlings reflected variability among varieties/lines to the adverse effect of water stress. Ali *et al*., (2011) reported that the information about significant among the traits is important for initiation of any breeding programme because it provides a chance for selection of desirable genotypes with desirable traits. Therefore, the investigation was carried out to understand the morpho-physiological responses of cotton genotypes tested according to the standard procedure toward water stress at seedling stage used (100%= Control condition) and (50%= Severe stress).

Materials and Methods

The experiment was conducted from July to August 2018 in the State Key Laboratory of Cotton Biology, CAAS glasshouse with three repeats completely randomized block design. Seeds of all 22 genotypes tested (Table 1) were collected from the same organization. Seedling grown in pots of 30 cm high and 15 cm diameter size with 2 kg soil of air dried. The treatment levels were two levels 100% as control condition, watered daily to keep soil at field capacity with 500 ml of water per pot and 50% as severe stress condition with 250 ml of water per pot after the emergence of the three true leaves of plant Mvula *et al.,* (2018).

Measurement of physiological parameters, photosynthesis, CO_2 assimilation, transpiration rate, stomatal conductance, leaf temperature was taken on the most recently emerged, full expanded and well exposed leaves under bright light using an Infrared Gas Analyzer (CIRAS 1-PPSystem, Stortfield, Hitchin, Herts, UK). The chlorophyll content parameters was determined by SPAD meter (502 Plus, Spectrum Technologies, Plainfield, IL, USA). Stem diameter was measured on the shoot which was earlier separated from the root using a ruler from the middle of the lower first and second node of the plants (Mvula *et al.,* 2018), and new internode was measured as well. After the first time of flowering stage (55 days), plants were harvested, then spread on filter paper for measurement of root length (cm) and shoot length (cm) by meter. Plants were cut into roots and shoots to record fresh root weight (g) and fresh shoot weight (g), the roots and shoots dried for 72h at 60°C to evaluate dry shoot weight (g) and dry root weight (g) by electric balance for the 10 plants from each replicate for all genotypes and water stress levels. Weight of each plant (dry root weight + dry shoot weight) after oven drying was recorded after weighting on electric balance to obtain total biomass.

The mean performance of all traits were subjected to statistical analysis used Software packages of NCSS-2019 to test the differences among genotypes tested. Relationships among genotypes and traits was also performed in the form of heatmap hierarchical cluster NCSS-2019.

Table 1. List of evaluated cotton genotypes.

No.	Genotypes	No.	Genotypes
1	Xinluzao 45	**12**	Bo 425
2	Deltapine 14	**13**	Ken N27-3
3	Zhong 915	**14**	Xinluzao 30
4	Zhongmiansuo 49	**15**	Xinluzao 32
5	Xinluzao24	**16**	Xinluzhong 19
6	Xinluzao 27	**17**	Xinluzhong 21
7	Xinluzao 33	**18**	Xinluzhong 22
8	Xinluzhong 27	**19**	Huiyuan 717
9	Xinluzhong 40	**20**	Jinken 148-39
10	Xinluzhong 47	**21**	J 521
11	Zhong 1476	**22**	YHM 28

Results

Analysis of variance (Table 2) showed highly significant for all traits under treatment levels, therefore the effects of water variability on the traits were found significant. On the other side were found non-significant for the interaction. Regarding to the mean performance of morph-physiological traits their values were decreased with water sever stress level except leaf temperature and chlorophyll content traits as compared to control condition (Figure 1).

Table 2. Combined analysis of variances (Mean Squares) for Morpho-physiological traits regarding to considered cotton genotypes.

S.O.V.	D.F.	TR	CO2	SC	Ch C	LT	Ph R
Treat	1	9.49**	42993.30**	0.023**	415.28**	331.23**	431.65**
Genotypes	21	0.11**	2791.69	0.00	51.08**	0.02	1.09
Treat x Genotypes	21	0.09	1824.82	0.00	9.38	0.55	0.74
Error	88	0.06	2324.27	0.00	11.23	0.59	1.07

For highly significant at p<0.01; **SOV (Source of variation); **D.F.** (Degrees of Freedom); **TR** (Transpiration Rate); **CO2** assimilation; **SC** (Stomatal Conductance); **Ch C** (Chlorophyll Content); **LT** (Leaf Temperature) and **Ph R** (Photosynthesis Rate).

Con. Table 2:

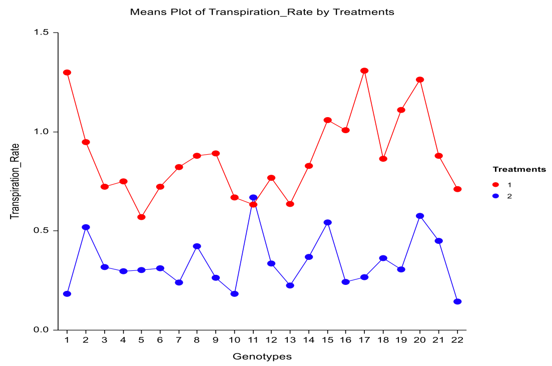
S.O.V.	D.F.	NLA	SD	NI	PH
Treat	1	2812.47**	38.29**	50.44**	8759.62**
Genotypes	21	60.67	0.21	1.38**	176.03**
Treat x Genotypes	21	24.81	0.09	0.26	6.39
Error	88	78.16	0.21	0.24	17.63

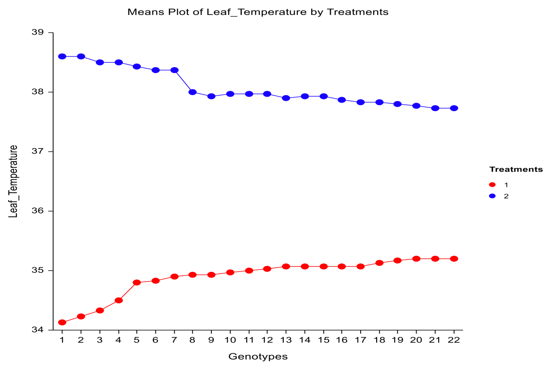
For highly significant at p<0.01; **SOV (Source of variation); **D.F.** (Degrees of Freedom); **NLA** (New Leaf Area); **SD** (Stem Diameter); **NI** (New Internode); **PH** (Plant Height).

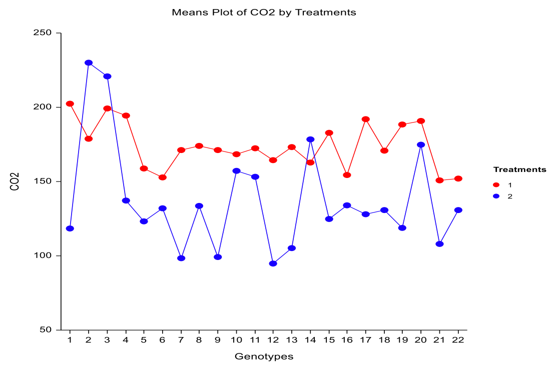
Con. Table 2:

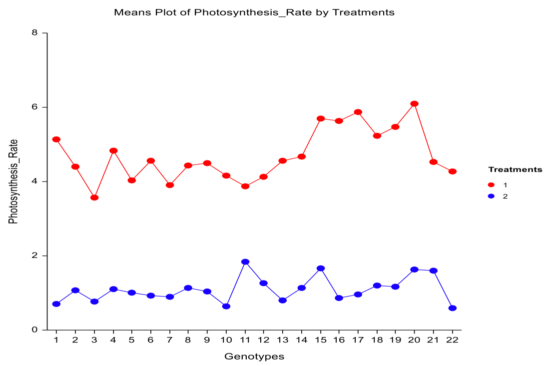
S.O.V.	D.F.	SFW	SDW	RFW	RDW
Treat	1	270.61**	9.17**	13.36**	0.87**
Genotypes	21	2.48	0.11	0.56	0.02
Treat x Genotypes	21	0.99	0.03	0.2	0.01
Error	88	1.94	0.08	0.47	0.02

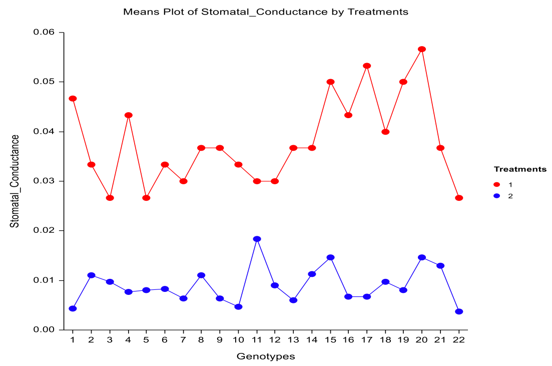
For highly significant at p<0.01; **SOV (Source of variation); **D.F.** (Degrees of Freedom); **SFW** (Shoot Fresh Weight); **SDW** (Shoot Dry Weight); **RFW** (Root Fresh Weight) and **RDW** (Root Fresh Weight).

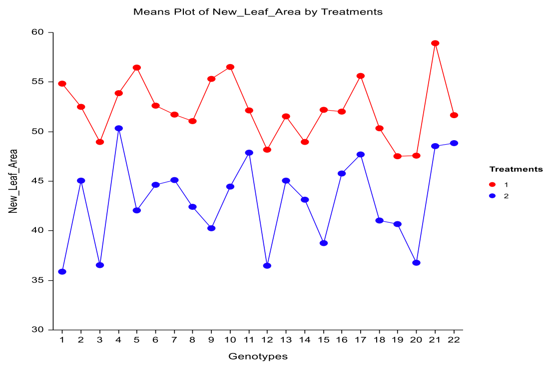


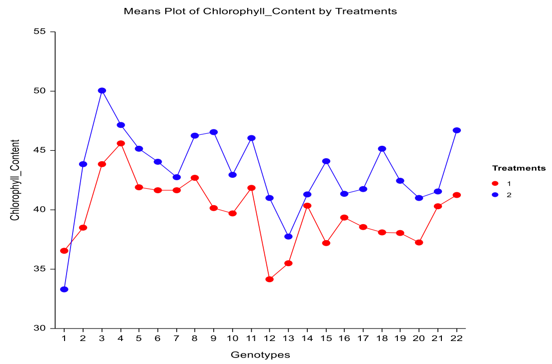


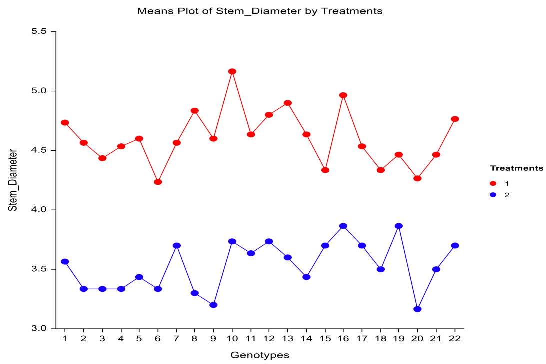












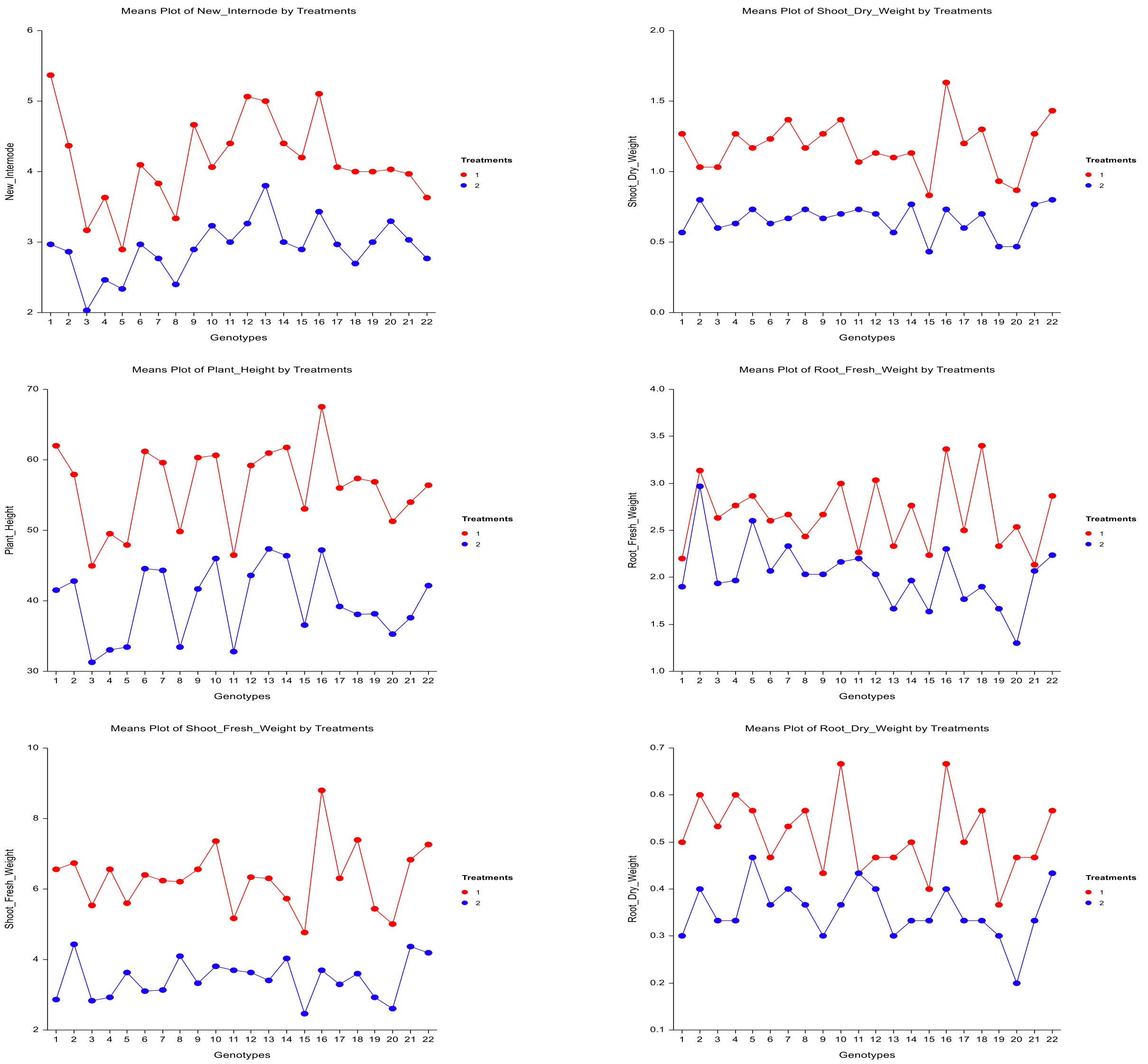


Figure 1. Plots showing means and standard errors per genotype for each trait evaluated under treatments: 1(red color) = (100%= Control condition) and 2(blue color) = (50%= Severe stress).

Table 3. Cluster analysis grouping cotton genotypes tested with morpho-physiological traits.

Cluster	Columns (Variables)
1	Transpiration Rate, CO2 assimilation, Stomatal Conductance, Photosynthesis Rate
2	Chlorophyll Content, New Leaf Area, Leaf Temperature, Stem Diameter, Shoot Fresh Weight, Root Fresh Weight, Shoot Dry Weight, Root Dry Weight.
3	Plant Height, New Internode
Cluster	**Rows (Genotypes)**
1	1, 9, 12, 13, 14, 16, 21
2	2, 3, 4, 5, 6, 7, 8, 10, 11, 18, 22
3	15, 17, 19, 20

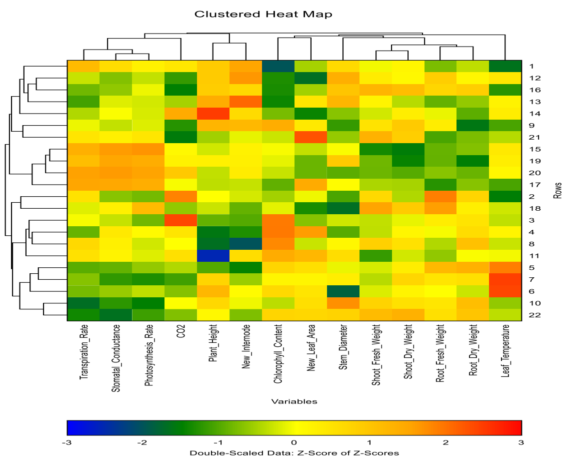


Figure 2. Heatmap hierarchical cluster analysis showing three clusters based on response of water severe stress for the 22 considered cotton genotypes.

The results of normal conditions showed that the genotype (Xinluzao 45) was the best performance genotype to transpiration rate, CO2 assimilation and new internode traits. Moreover, the genotype (Jinken 148-39) was the higher genotype toward transpiration rate and stomatal conductance traits. Furthermore, the genotype (Xinluzhong 19) was the excellent genotype for the traits plant height, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight. Meanwhile, the best genotypes in performance toward the traits chlorophyll content, leaf temperature, photosynthesis rate, new leaf area, stem diameter were the genotypes Zhongmiansuo 49, YHM 28, Jinken 148-39, J 521, Xinluzhong 47, respectively.
Concerning to the results of water severe stress the genotype (Zhong 1476) was the best performance genotype to the traits transpiration rate, stomatal conductance and photosynthesis rate. Moreover, the genotype (Deltapine 14) was the excellent for CO2 assimilation, leaf temperature, shoot fresh weight and root fresh weight traits. Meanwhile, genotype (Zhong 915) was the good performance genotype for the chlorophyll content trait. While, the best genotypes toward the traits new leaf area, stem diameter, new internode, plant height, shoot dry weight and root dry weight were Zhongmiansuo 49, Xinluzhong 19 and Huiyuan 717, Ken N27-3, Ken N27-3 and Xinluzhong 19, Deltapine 14 and YHM 28, Xinluzao 24, respectively.

Heatmap hierarchical cluster analysis (Euclidean distance) was carried out and three cluster (columns) were detected according to the different response to water severe stress (Table 3 Figure 2). The cluster one involved the traits (transpiration rate, CO2 assimilation, stomatal conductance and photosynthesis rate). Meanwhile, the cluster two included the traits (chlorophyll content, new leaf area, leaf temperature, stem diameter, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight) and cluster three presented (plant height and new internode) traits. In addition, each cluster contain genotypes in groups (rows) that are related in their response to water severe stress and corresponding in response for cluster traits, the cluster one contains the genotypes (Xinluzao 45, Xinluzhong 40, Bo 425, Ken N27-3, Xinluzao 30, Xinluzhong 19 and J 521). Furthermore, the cluster two collected the genotypes (Deltapine 14, Zhong 915, Zhongmiansuo 49, Xinluzao24, Xinluzao 27, Xinluzao 33, Xinluzhong 27, Xinluzhong 47, Zhong 1476, Xinluzhong 22 and YHM 28). On the other hand, the cluster three involved (Xinluzao 32, Xinluzhong 21, Huiyuan 717 and Jinken 148-39) genotypes.

Discussion

Analysis of variance showed significant differences among the treatment levels for all traits. It indicated that the treatments have influenced the genotypes for all the traits. On the other hand, there are no significant in interaction reflected no variation of the traits in the studied set of cotton genotypes. These results indicated that genotypes presented in this study have narrow genetic makeup toward traits. Genetic variation under drought and controlled conditions suggested the importance of root and shoot parameters in breeding programs (Bibi *et al.,* 2012; Maruti and Katageri, 2015; Zafar and Azhar, 2015 and Mvula *et al.,* 2018).

Growth is influenced by various internal and external factors besides its genetic makeup and is an important tool for assessing crop productivity in various crops. Root characteristics especially root length is important for a plant to have comparatively well-established above-ground parts by exploiting the available water. Root related parameters contributed more to the plant growth and survival. As good root growth is necessary for establishment of crop stand and root helps in uptake of water and mineral elements necessary for plant growth and plays role in water severe tolerance (Brunner *et al.,* 2015 and Iftikhar *et al.,* 2019). Water severe stress reduced growth in different characters of cotton plant (Khalid *et al.,* 2011). It also shifts the normal development process of a plant. Stress tolerant plants tries to adjust their solute concentration to adjust their solute-water balance in the cell to maintain cell's turgor pressure (Snowden *et al.,* 2014). Under the treatment level 50% water severe stress, overall growth reduction was observed as compared to the control treatment however some genotypes i.e. Zhong 1476, Deltapine 14 and Zhong 915 showed better growth. These genotypes recorded good transpiration rate, stomatal conductance, photosynthesis rate, CO2 assimilation, leaf temperature, shoot fresh weight, root fresh weight and chlorophyll content. These traits helped them survive better under water severe stress. Basal *et al.,* (2005) reported that root related traits are more important for water severe stress tolerance due to help plants to uptake water and nutrients essential for its growth. In addition, roots helps to maintain water balance by uptake of water from the soil. Hence deep root system favors plant to cope water severe stress Iftikhar *et al.,* 2019.

Higher chlorophyll contents indicated that photosynthetic rate will be higher due to improve in water absorption, higher root volume, higher root length and weight which indicated that crop productivity may be enhanced under water severe stress conditions and accumulation of organic compounds enhanced as well (Ebrahim, 2012 and Bibi Amir *et al.,* 2014). Ahmed *et al.,* (2002) found that water severe stress decreased the CO2 assimilation rate. Zlatve *et al.,* (2004) also suggested that decreasing CO2 assimilation under water severe stress may be related to restriction of CO2 diffusion into the leaf, and also inhibition of biochemical processes such as ATP synthase. Moreover, higher shoot length reflected efficiency of seedling ability of photosynthesis and accumulation of organic compounds was much higher. Furthermore, Fresh shoot weight inferred that the moisture contents were higher in the seedlings and stored organic compounds that help seedlings to withstand under water severe stress. Besides, higher dry shoot weight, fresh and dry root weight and leaf area indicated that the seedlings showed higher photosynthetic rate as chlorophyll contents were higher and water absorption was also higher due to greater length and volume of roots referred that the storing ability of organic compounds in shoot was higher that may help seedling to withstand and survive under water severe stress. These results are in harmony with Ali *et al.,* 2012; Ebrahim, 2012 and Bibi Amir et *al.,* 2014.

Average linkage method showed cluster of 22 genotypes. The points closest to each other are gathered in one cluster because distance between them is small as compared to others. This method proved an efficient and precise to screen large plant population. (Noorka and Ihsan, 2007) have used cluster analysis in wheat germplasm to group different wheat genotypes based on various characteristics under water stress.

Conclusion

As a conclusion, three genotypes presented in this work more tolerant to water severe stress which might play a significant role for the incorporation of water severe stress tolerance genes to improve production in local farming systems and under ecological farming, or even in commercial types. Traditionally, the development of such cultivars has been hampered by the complex nature of water severe stress adaptation, genotype × environment interactions and the difficulty of having an effective drought screening method (Elsayed *et al.,* 2019). Moreover, the parameters measured in this work have allowed a first rapid and low-cost screening to select genotypes with the best behavior in early stages of plant growth in order to further assess fruit yield and quality in the future. Therefore, herein we report a useful and straightforward set of traits for water severe tolerance screening. Using our results and those of the studies cited herein, had high genetic potential for excellent performance under water severe stress condition by selection intensity for drought tolerance which may be used in succeeding research programmes.

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