

Root Biomass and Water Use Efficiency Determine Biomass Accumulation under Drought Stress: Implications for Identifying Donor Parents for Breeding for Abiotic Stress Tolerance in Cotton

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ABSTRACT

Water stress is the most yield limiting factor in field crops, including cotton. This study aimed to determine drought-tolerant genotypes. 158 cotton (*Gossypium hirsutum* L.) germplasm accessions were grown in a "field root phenotyping structure". Our results showed significant genotypic variability in root dry weight (RW), root volume (RV), root length (RL) and carbon isotope discrimination ($\Delta^{13}\text{C}$) among cotton accessions. Based on differing root dry weight and carbon isotope discrimination, cotton accessions were classified into four groups. We found that different groups had different biomass accumulated and group-I with high RW and low $\Delta^{13}\text{C}$ showed highest biomass produced compared with other groups. Therefore, genotypes belonging to group-I might be better than other genotypes belonging to other groups under drought stress. To assess this hypothesis, six genotypes were selected from group-I (three) and group-II (three) and raised in containers under well-watered (100% field capacity) and water limited (55% field capacity) conditions. Drought stress reduced root traits, leaf area, total dry matter in both groups. However, genotypes belong to group-I accumulated high total dry matter under water stress, mainly due to maintain high photosynthetic rate, transpiration rate and carboxylation efficiency. Results emphasize the relevance of selecting drought-tolerant genotypes by choosing genotypes with a large root system coupled with a low $\Delta^{13}\text{C}$.

Keywords: cotton, dual stable isotope ratios, water stress, water use efficiency

Abbreviations: A, assimilation rate; Ci, internal CO₂ concentration; CWT, cumulated water transpired; DAS, days after sown; $\Delta^{13}\text{C}$, carbon stable isotope discrimination; ET, evapo-transpiration; E, evaporation; Fc, field capacity; gs, stomatal conductance; LAD, leaf area duration; MTR, mean transpiration rate; NAR, net assimilation rate; $\Delta^{18}\text{O}$, oxygen stable isotope enrichment; T, transpiration rate; VPD, vapor pressure deficit; WUE, water use efficiency

INTRODUCTION

Cotton is considered the first commercial crop in many countries especially in India where it occupies the largest area globally. However, majority of cotton cultivation in India (> 70%) is under rain fed condition (www.indiastat.com). In the 20th century the global surface temperature has increased 0.74°C and likely to rise by 6.4°C in the 21st century (IPCC 2009) that will lead to further increase demand for water to meet crop evo-transpiration demand. Under this scenario, cotton will be exposed to moisture and heat stress more frequently thus, will affect its morphological as well as physiological characters which result in reduced biomass and yield (Pace *et al.* 1999; Pettigrew 2004; Ennahli and Earl 2005; Levi *et al.* 2009). A number of different morpho-physiological traits have been suggested as important to maintain high productivity under drought stress in cotton (Ball *et al.* 1994; Xu *et al.* 1995; Kumar and Singh 1998; Kasperbauer 1999; Pace *et al.* 1999; AbouKheir *et al.* 2012).

Basal *et al.* (2005) showed that root parameters can be used as reliable selection criteria for drought tolerance in upland cotton (*Gossypium hirsutum* L.). Increased root length under drought stress helps plants to get water from deeper soil layer (Ludlow and Muchow 1990; Ball *et al.* 1994; Prior *et al.* 1995; Pace *et al.* 1999) that contributes substantially positively in biomass production and crop performance (Passioura 1983; Blum 2009). In wheat, increased

grain yield was observed in varieties capable to harness extra soil water from deeper soil profile after anthesis (Kiregaard *et al.* 2007). Similarly, deeper root maize genotype accompanied with high transpiration had better shoot dry biomass produced (Hund *et al.* 2009). Therefore, there is relevance of breeding for high root traits to achieve better productivity under water limited conditions (Reynolds *et al.* 2007; Reynolds and Tuberosa 2008). In addition, many physiological traits have vital role to cope drought stress effects. The most important one is water use efficiency (WUE) which is defined as the amount of biomass produced per unit water transpired. It is a key determinant of plant productivity under limited water supply (Passioura 1986, 1996). As a result of discovery that plants discriminate against the heavy carbon isotope (O'Leary 1981) and establishment of the theory linking carbon isotopic discrimination ($\Delta^{13}\text{C}$) with WUE, scientists starts using $\Delta^{13}\text{C}$ as surrogate to WUE in crops (Ismail and Hall 1992, 1993; White *et al.* 1996; Ashok *et al.* 1999; Boominathan 2001; Sheshshayee *et al.* 2003; Condon *et al.* 2004; Bindumadhava *et al.* 2005; Impa *et al.* 2005; Stiller *et al.* 2005; Xu *et al.* 2007; Seibt *et al.* 2008; AbouKheir *et al.* 2010, 2012).

In this study we hypothesized that cotton accessions selected for low $\Delta^{13}\text{C}$ (high WUE) with high root dry weight will produce more total dry matter under well water and moisture stress conditions.

MATERIALS AND METHODS

Experiment I: Assess the genetic variability in few root traits and WUE using special constructed 'Field Root Phenotyping Structure'

A total of 158 of diverse cotton (*Gossypium hirsutum* L.) germplasm accessions (Appendix 1) obtained from Aukur Seeds Pvt. Ltd, Nagpur, Maharashtra, India, were used in this experiment. These germplasm accessions were raised in specially constructed structure called as 'Field Root Phenotyping Structure' with dimensions 1.5 m tall, 3 m wide and 20 m long (Fig. 1), built using cement bricks at field research unit in Department of Crop Physiology, University of Agricultural Sciences, Bangalore, India. Soil was mixed with farm yard manure at 15 t.ha⁻¹ and filled in these structures then compacted to mimic the real field conditions. Cotton plants planted in rows and an exact plant population was maintained. The plants were provided with adequate nutrients through fertilizers at the rate of 100:50:50 NPK per hectare. This approach provides the near natural condition for phenotyping. Thus, the measurements of the root traits from plants grown in such root structures would be very accurate. At the end of the experiment (110 days after sowing-DAS), the brick walls were dismantled with care and the soil washed away using a strong jet of water. The roots were separated carefully from soil particles then root length (RL, in cm, by measuring the root length from base of the plant to the tip of the longest root), root volume (RV, in ml, by water displacement method) and root dry weight (RW, in g) were recorded. In addition, shoot (leaf and stem) dry weight was also recorded (SW in g) to compute total dry matter (TDM) produced by plant.

Carbon isotope ratio was determined using Isotope Ratio Mass Spectrometer (IRMS; Delta-plus, Thermofinnigan, Bremen, Germany) interfaced with an elemental analyzer (NA1112, CarloErba, Italy) via a continuous flow device (ConFlo-III, Thermofinnigan, Bremen, Germany). A composite leaf sample, comprising of the 10 mature leaves representing all positions of the plant canopy, were harvested and oven dried for 3 days at 70°C and homogenized to fine powder with a ball mill. Six replications from each cotton line were analyzed for $\delta^{13}C_{1b}$ with an analytical uncertainty of less than 0.1‰. Carbon isotope discrimination ($\Delta^{13}C$ expressed in per mil-‰) was computed as follows, assuming the isotopic composition of atmospheric air ($\delta^{13}C_a$) to be -8‰ relative to Pee Dee Belemnite (international standard) (Farquhar *et al.* 1989):

$$\Delta^{13}C = \{ \delta^{13}C_a - \delta^{13}C_{1b} \} / \{ 1 + (\delta^{13}C_{1b}/1000) \}$$

Experiment II: Assessment of the response of root traits, WUE and associated physiological traits to water stress in selected cotton accessions

Based on experiment 1, three cotton germplasm accessions have been selected from group-I as high RW and low $\Delta^{13}C$ (Ca/H-155, Ca/H-168, Ca/H-19) and three from group-II as low RW and high $\Delta^{13}C$ (Ca/H-51, Ca/H-216, sahana). These selected genotypes were examined under water limited condition. Seeds from these genotypes were sown in carbonised rubber containers with a capacity to hold 50 kg of soil mixed with farmyard manure in a ratio of 3:1 and have soil water holding capacity of around 20%. The crop was raised without any moisture stress till 35 DAS. Two different soil water status at 100% field capacity (FC, well watered) and 55% FC (drought stress) treatments were maintained. The soil surface was covered with 0.5 kg plastic pieces to minimize evaporation. The side walls of the containers were covered with reflective insulation to prevent excessive heating from direct sunlight. The containers with plants were placed under a rain out shelter which was moved over the experimental area during night and rain episodes. The containers were arranged in Completely Randomized Design (CRD) with eight replications for each water regimes. The WUE and associated physiological traits were measured as per Udayakumar *et al.* (1998) and Cernusak *et al.* (2008). Briefly, the method involves weighing the containers on a daily basis using a mobile weighing device during the experimental period from 35 to 85 DAS. During the experimental period, each



Fig. 1 Specially constructed field root phenotyping structure to assess genetic variability in root traits in a large number of accessions.

container was weighed daily and the amount of water lost during each measuring cycle was replaced, bringing the containers back to their initial weight with respect to soil water status. Total plant water use over experimental period (ET) was computed. In addition to the containers containing plants, four control containers without plants were also deployed to estimate evaporation (E) from the soil surface independently of plant transpiration. Total water transpired by plant (Cumulative Water Transpired -CWT) over experimental period was calculated as the difference between ET and E. At the start (35 DAS) and at the end (85 DAS) of the experiment, biomass of leaf, stem and root and leaf area were determined. The initial data was recorded in four replications whereas the remaining containers (six replications) were used to record the final data (at 85 DAS) for each water regime. WUE was computed from the ratio of biomass produced during the experimental period (50 days) to CWT and expressed in g biomass per liter water transpired. Leaf Area Duration (LAD) over the experimental period. $LAD = \{LA1 + LA2\} / 2 \times 50$ days, where LA1 and LA2 were leaf area at the beginning (35 DAS) and end (85 DAS) of the experiment respectively. The Net Assimilation Rate (NAR) is a time averaged measure of photosynthetic rate which was computed as the ratio of biomass produced during the experimental period to LAD.

Photosynthesis measurements

Experiments were conducted with six selected cotton accessions to determine the effect of different CO₂ concentration and light intensity in assimilation rate (A). Gas exchange of the youngest fully expanded leaf was measured using a portable photosynthesis system (Li-6400, Li-COR Inc., Lincoln, Nebraska, USA). Leaf cuvette was set at photosynthetic photon flux density (PPFD) of 1500 mmol.m⁻².s⁻¹ and temperature of 28°C. For measurements of A vs. C_i (A/C_i curves), the [CO₂] in the leaf cuvette was set at 11 levels (350, 250, 150, 100, 50, 450, 600, 800, 1000, 1250 and 1500 ppm). Similarly, Leaf cuvette was set at CO₂ concentration at 370 ppm and temperature of 28°C. For measurements of A vs.

Table 1 Root length, root volume, root dry weight, shoot dry weight, root to shoot ratio, leaf area, total dry matter, $\Delta^{13}\text{C}$, and specific leaf area among 158 cotton germplasm accessions when grown in “Field Root Phenotyping Structure” for 110 DAS.

	RL	RV	RW	SW	R/S	LA	TDM	$\Delta^{13}\text{C}$	SLA
Mean	72.44	40.39	9.80	124.42	0.09	0.78	143.54	20.08	150.13
Min	24.76	8.56	0.49	16.76	0.004	0.05	22.62	17.71	48.67
Max	139.03	94.81	26.39	358.54	0.266	2.21	396.3	22.2	292.41
SD	10.42	10.67	2.94	27.04	0.01	0.18	35.27	0.13	20
CV	14.39	26.43	30.01	21.73	8.20	23.36	24.57	0.66	13.32
CD at 5%	24.07	24.66	6.79	62.46	0.0163	0.4219	81.46	0.3	0.0046

$\Delta^{13}\text{C}$: Carbon stable isotope discrimination, LA: leaf area, RW: root dry weight, RL: root length, R/S: root to shoot ratio, RV: root volume, SLA: specific leaf area, TDM: total dry matter.

light intensity (light response curves), the light intensity in the leaf cuvette was set at 11 levels (1250, 1000, 750, 500, 250, 150, 100, 50, 1500, 1750, 2000 $\text{mmol.m}^{-2}.\text{s}^{-1}$). Then, the A, g_s , C_i and other gas exchange parameters were recorded.

The analysis of variance as per Federer (1977) was carried out for different characters in order to assess the variability among the cotton germplasm accessions. ANOVA was calculated by using augmented design1 programme designed in AgriStat software package. Means were separated using the Waller-Duncan test after it was determined that there was a significant difference at the 5% level for the F value.

RESULTS

Experiment 1

A diverse set of 158 cotton germplasm accessions were raised in ‘Root Field Structure Phenotyping’ and a significant genetic variability in root parameters was noticed (Table 1). Cotton accessions used in this investigation showed 5- and 11-fold variation in RL and RV, respectively (Table 1). Ca/H-269 showed highest root length (139.03 cm.plant^{-1}) and Ca/H-153 showed highest root volume (94.81 cm^3). The amount of RW produced over an experimental period of 110 days varied from as low as 0.49 g.plant^{-1} in Ca/H-8 to as high as 26.39 g.plant^{-1} in Ca/H-19 (Table 1). Likewise, SLA and LA showed significant genetic variability among cotton germplasm accession used in this experiment (Table 1). Results revealed a significant correlation between LA with RW (Table 2).

Similarly, shoot weight showed significant genetic variability and ranged from 16.76 to 358.5 g.plant^{-1} (Table 1). A significant genotypic variability in root to shoot ratio (R/S) was noticed among cotton accessions (Table 1) and it showed significant correlation with LA and TDM (data not shown). The TDM computed by adding up the biomass of constituent plant parts including root biomass, also varied significantly among cotton germplasm accessions with an average 143.54 g.plant^{-1} (Table 1). A significant relationship between TDM with RW ($r = 0.74$) and LA ($r = 0.82$) was noticed (Table 2).

The variation in $\Delta^{13}\text{C}$ was significantly varied from 17.71 to 22.20‰ in Ca/H-190 and Ca/H-253 respectively, with a mean of 20.08‰ (Table 1). There was no discernible relationship ($r = 0.012$) between $\Delta^{13}\text{C}$ and TDM (Table 2).

Based on RW coupled with $\Delta^{13}\text{C}$ cotton accessions were classified into four groups: first, genotypes have high RW with low $\Delta^{13}\text{C}$ (group-I). The second group contains genotypes have low RW coupled with high $\Delta^{13}\text{C}$ (group-II). The third group contains genotypes have high RW with High $\Delta^{13}\text{C}$ (group-III) and finally, the fourth one contains genotypes have low RW with low $\Delta^{13}\text{C}$ (group-IV). Table 3 illustrates the significant variation between groups and group-I showed high TDM accumulated and high growth rate (ROG) 1.74 g.day^{-1} .

Experiment 2

To assess the response of contrasting lines to different soil moisture regimes. Six cotton accessions, three Ca/H-155, Ca/H-168 and Ca/H-19 belong to group-I and other three Ca/H-51, Ca/H-216 and Sahana belong to group-II, were

Table 2 Correlation coefficients among root dry weight, leaf area, TDM and $\Delta^{13}\text{C}$ for 158 cotton germplasm accessions when grown in “Field Root Phenotyping Structure” for 110 DAS.

	RW	LA	TDM	$\Delta^{13}\text{C}$
RW	1.00			
LA	0.50 **	1.00		
TDM	0.74**	0.82**	1.00	
$\Delta^{13}\text{C}$	0.03	-0.06	0.01	1.00

$\Delta^{13}\text{C}$: Carbon stable isotope discrimination, LA: leaf area, RW: root dry weight, TDM: total dry matter.

Table 3 Root length, root volume, root dry weight, $\Delta^{13}\text{C}$, total dry matter, and rate of growth among four groups.

	Group-I	Group-II	Group-III	Group-IV
RL	78.46 a	69.93 b	79.04 a	65.06 b
RV	50.08 a	33.06 b	48.78 a	32.42 b
RW	13.37 a	7.32 b	13.12 a	6.68 b
$\Delta^{13}\text{C}$	19.41 a	20.59 b	21.09 c	19.43 a
TDM	191.86 a	114.74 c	166.32 b	105.32 d
ROG	1.74 a	1.04 c	1.68 b	1.01 d

$\Delta^{13}\text{C}$: Carbon isotope discrimination, RL: root length, RW: root dry weight, RV: root volume, TDM: total dry matter. Means within columns followed by the same letter are not significantly different according to Fisher's *t*-test. Group-I: High root biomass + low $\Delta^{13}\text{C}$ (High WUE), Group-II: Low root biomass + high $\Delta^{13}\text{C}$ (low WUE), Group-III: high root biomass + high $\Delta^{13}\text{C}$ (low WUE), Group-IV: low root biomass + low $\Delta^{13}\text{C}$ (High WUE).

grown under two different water regimes.

Significant genetic variation was found in LA and LW under watered and water stressed treatment. LW was reduced under moisture stress but the rank of genotypes for LW was similar in both treatments (Table 4). Total dry biomass, accumulated during experimental period between 35 to 85 DAS, varied from 87.70 g.plant^{-1} in Ca/H-51 to 159.61 g.plant^{-1} in Ca/H-168 under well water conditions while under moisture stress TDM was reduced and ranged from 80.06 g.plant^{-1} (Ca/H-51) to 123.21 g.plant^{-1} (Ca/H-19) (Table 4). The group-I genotypes belonging to group-I had significantly higher TDM than genotypes belonging to group-II under both water treatment.

Significant genetic variation in RW and RV was noticed in both treatments (Table 5). The group-I had greater ($p = 0.05$) RV than group-II in well water treatment. Under water deficit, RV had decreased more in group-II except in Ca/H-51 (Table 5).

The group-I genotypes exhibited 35% ($p = 0.05$) as much root dry weight as the group-II genotypes under watered and water stressed treatments (Table 5). The RW decreased in both group-I and group-II genotypes under water stress.

Table 6 illustrates the significant genetic variation in WUE, NAR and CWT among the both groups' genotypes. An increased in WUE under drought stress was observed. Genotypes belong to group-I had higher WUE ($p = 0.05$) than group-II in both treatments. Net assimilation rate ranged from 7.29 g.cm^{-2} (Ca/H-216) to 11.27 g.cm^{-2} (Ca/H-155) under well watered condition and decreased under drought stress treatment (Table 6). NAR, under drought condition, had reduced in both groups genotypes except in Ca/H-51 but group-I type had maintained higher NAR than

Table 4 Leaf area, leaves dry weight and total dry matter in well watered (WW) and water stressed (WS) plants of group I and group II genotypes at the end of 85 DAS.

Genotypes	LW (g.plant ⁻¹)		TDM (g.plant ⁻¹)		LA (cm ²)		
	WW	WS	WW	WS	WW	WS	
Group-I	Ca/H- 155	94.01 a	71.23 a	153.62 a	122.82 a	7914.71 a	6001.92 a
	Ca/H- 168	94.63 a	61.06 b	159.61 a	106.98 b	7030.52 b	4928.78 c
	Ca/H- 19	82.91 b	72.75 a	142.78 b	123.21 a	6457.19 c	5578.97 b
	Mean	90.52	68.35	152.01	117.67	7134.14	5503.22
	SD	6.60	6.36	8.53	9.26	734.27	540.57
Group-II	Ca/H- 51	51.86 e	47.03 d	87.70 d	80.06 d	4138.76 e	3346.18 e
	Ca/H- 216	70.55 c	56.24 c	111.34 c	92.33 c	6308.96 c	4611.43 c
	Sahana	64.95 d	52.59 c	114.34 c	91.07 c	4818.97 d	3773.79 d
	Mean	62.45	51.95	104.46	87.82	5088.90	3910.47
	SD	9.59	4.64	14.59	6.75	1109.99	643.60

LA: leaf area, LW: leaf weight, TDM: total dry matter.

Means within columns followed by the same letter are not significantly difference at p = 0.05 according to Waller-Duncan CD.

Table 5 Root volume and root dry weight in well watered and water stressed plants of group I and group II genotypes at the end of 85 DAS.

Genotypes	RV (ml.plant ⁻¹)		RW (g.plant ⁻¹)		
	WW	WS	WW	WS	
Group-I	Ca/H- 155	102.00 a	93.75 a	11.49 a	11.83 a
	Ca/H- 168	96.67 a	90.00 ab	11.96 a	10.36 a
	Ca/H- 19	86.67 b	81.67 b	12.29 a	11.34 a
	Mean	95.11	88.47	11.91	11.18
	SD	7.78	6.18	0.40	0.75
Group-II	Ca/H- 51	53.33 d	53.00 d	8.12 b	8.47 b
	Ca/H- 216	69.00 c	60.00 cd	9.28 b	8.90 b
	Sahana	71.00 c	63.75 c	8.82 b	8.49 b
	Mean	64.44	58.92	8.74	8.62
	SD	9.67	5.46	0.58	0.24

RV: root volume, RW: root weight.

Means within columns followed by the same letter are not significantly difference at p = 0.05 according to Waller-Duncan CD.

group-II. The CWT reduced in group-I genotypes from 22 to 31% under stress compared to well water condition. On the other hand, the reduction in group-II genotypes was from 3.7 to 20.8% under drought stress compare to well water condition.

CO₂ and light response curves

To further investigation the effect of stress on photosynthetic machinery among the group-I and group-II of genotypes, CO₂ response and light response curves were generated for both well watered and water stressed plants (Figs. 2, 3).

The initial slope of the CO₂ response curve (dA/dC_i) often considered as an indication of carboxylation efficiency, did not differ significantly between the group-I and group-II categories under well watered condition. The

dA/dC_i showed a marked reduction in water stressed plants. The dA/dC_i decreased to 0.159 for group-I type while it decreased to 0.141 for group-II type (Table 7), suggesting that stress effect on carboxylation efficiency was more severe in group-II genotypes. Further, the maximum carbon assimilation rate (A_{max}) did not differ significantly between stressed and control but the C_i at which A_{max} was attained differed significantly. The A_{max} was reached at 1000 ppm for control plants where as it was 1250 ppm in group-II under moisture stress treatment. However, there is no difference in C_i at which A_{max} in group-I. These results suggested that the stress levels significantly affected carboxylation process as well as CO₂ diffusion (Table 7).

Similar trends were observed with the light response curves. The light composition point (LCP), an induction of the minimum light required to achieve positive carbon gain, was comparable among the well watered treatment of both group-I and group-II (Table 7). The LCP marginally increased under stress for group-I whereas the group-II required significantly higher light energy to initiate positive carbon fixation (Table 7). The maximum photosynthetic rate (A_{max}) at saturation light intensity was comparable among well watered plants of both groups. However, water stress had greatest effect on light utilization only in group-II. The greater efficiency estimated from the initial slope of light response curve was significantly less for the group-II (Table 7).

DISCUSSION

Among of several stresses that affect crop growth and productivity, drought is perhaps the most prominent stress. Significant developments have been achieved in understanding the physiology of drought resistance and developing physiological screening techniques for drought resistance which reduce time in breeding programs (Ludlow and

Table 6 Water use efficiency, net assimilation rate and cumulative water transpired in well watered and water stressed plants of group I and group II genotypes at the end of 85 DAS.

Genotypes	WUE (g.L ⁻¹)		NAR (g.cm ⁻²)		CWT (L. plant ⁻¹)		
	WW	WS	WW	WS	WW	WS	
Group-I	Ca/H- 155	4.91 ab	5.00 b	11.27 a	8.32 bc	33.04 a	25.13 a
	Ca/H- 168	4.98 a	5.30 a	10.11 b	9.16 a	30.65 ab	20.99 b
	Ca/H- 19	5.05 a	5.36 a	9.27 c	8.96 ab	29.78 b	23.03 ab
	Mean	4.98	5.22	10.22	8.81	31.16	23.05
	SD	0.07	0.19	1.00	0.44	1.69	2.07
Group-II	Ca/H- 51	4.71 bc	4.75 c	7.45 d	8.60 b	17.46 e	16.81 c
	Ca/H- 216	4.44 d	4.97 bc	7.29 d	6.86 d	25.44 c	20.75 b
	Sahana	4.57 cd	5.18 ab	8.69 c	7.73 c	22.21 d	17.58 c
	Mean	4.57	4.97	7.81	7.73	21.70	18.38
	SD	0.13	0.22	0.77	0.87	4.02	2.09

CWT: cumulative water transpired, NAR: net assimilation rate, WUE: water use efficiency.

Means within columns followed by the same letter are not significantly difference at p = 0.05 according to Waller-Duncan CD.

Table 7 CO₂ compensation point (CCP), light compensation point (LCP), maximum photosynthetic rate (A_{max}), internal CO₂ Concentration at A_{max} (C_i@A_{max}), light intensity at A_{max} (PPFD @A_{max}), initial slope of CO₂ response curve (dA/dC_i) and initial slope of light response curve (dA/dPPFD).

CO ₂ response curve					
Groups	Water regimes	CCP	A _{max}	C _i @A _{max}	dA/dC _i
Group-I	WW*	130	87.36	1000	0.228
	WS	150	86.00	1000	0.159
Group-II	WW	125	85.52	1000	0.192
	WS	195	85.06	1250	0.141

Light response curve					
Groups	Water regimes	LCP	A _{max}	PPFD @A _{max}	dA/dPPFD
Group-I	WW	110	26.64	1500	0.045
	WS	120	27.57	1500	0.044
Group-II	WW	110	27.02	1500	0.044
	WS	150	24.00	1750	0.038

* WW: well watered, WS: water stressed.

CCP:CO₂ compensation point, LCP: light compensation point, A_{max}: maximum photosynthetic rate, C_i@A_{max}: internal CO₂ concentration at A_{max}, PPFD @A_{max}: light intensity at A_{max}, dA/dC_i: initial slope of CO₂ response curve, dA/dPPFD: initial slope of light response curve.

Muchow 1990; Blum 1998). It is opined that under drought stress, genotypes capable of harness more water from the deep soil profile by good root biomass and producing high biomass per unit amount of harnessed water are high productivity. In present investigation, a significant genotypic variation in root traits and biomass assessed in 158 cotton germplasm accessions (Table 1). The variation in these traits was 5-, 52- and 17-fold in RL, RW and TDM, respectively. Earlier studies, showed root traits like tap root weight, root to shoot ratio and rapidity of root system development in cotton are relevant traits for drought tolerance thereby enhancing biomass and yield (Cook 1985; Yadav *et al.* 1997; Pace *et al.* 1999; Sinclair and Muchow 2001; Li *et al.* 2005). In this study, significant relationship ($r = 0.74$) between RW and TDM was observed among 158 cotton germplasm accessions (Table 2). This confirms earlier reports that big root system genotypes accumulate more biomass and thus yield (Kiregaard *et al.* 2007; Hund *et al.* 2009).

Since the major challenge is development of technologies allowing maintained or even increase production with minimum water use (Zeigler and Puckridge 1995). Thus, to achieve this goal, the efficiency of productive water use needs to be increased. The relationship between WUE and $\Delta^{13}C$ has been well established in many crops (Saranga *et al.* 1998; Boominathan 2001; Rao *et al.* 1995; Sheshshayee *et al.* 2003; Condon *et al.* 2004; Bindumadhava *et al.* 2005; Impa *et al.* 2005; Stiller *et al.* 2005; Xu *et al.* 2007; Seibt *et al.* 2008; AbouKheir *et al.* 2010, 2012). Therefore, $\Delta^{13}C$ can be used as surrogate for WUE in crops. A significant genetic variability in $\Delta^{13}C$ among cotton accessions was noticed (Table 1). As WUE in upland cotton is under stomatal control (AbouKheir *et al.* 2010) so increase WUE is generally associated with reduction in transpiration therefore, decrease in TDM accumulation. We hypothesized that combination of high WUE with high water mining through good root biomass will result in high TDM. Based on this assumption, cotton accessions were classified into four groups (Table 3). There was no significant between group-I and group-III in root traits however, the biomass produced by group-I genotypes were higher compare with group-III genotypes. This is due to higher efficiency in producing more biomass per unit water (WUE). Therefore, root biomass was not enough alone to produce highest biomass. Genotypes belong to groups I and IV did not show any significant genotypic variability in $\Delta^{13}C$, however, group-IV has lower TDM produced due to increasing WUE by reducing transpiration. Blum (2009) showed that water use through good root system but not WUE is the only impor-

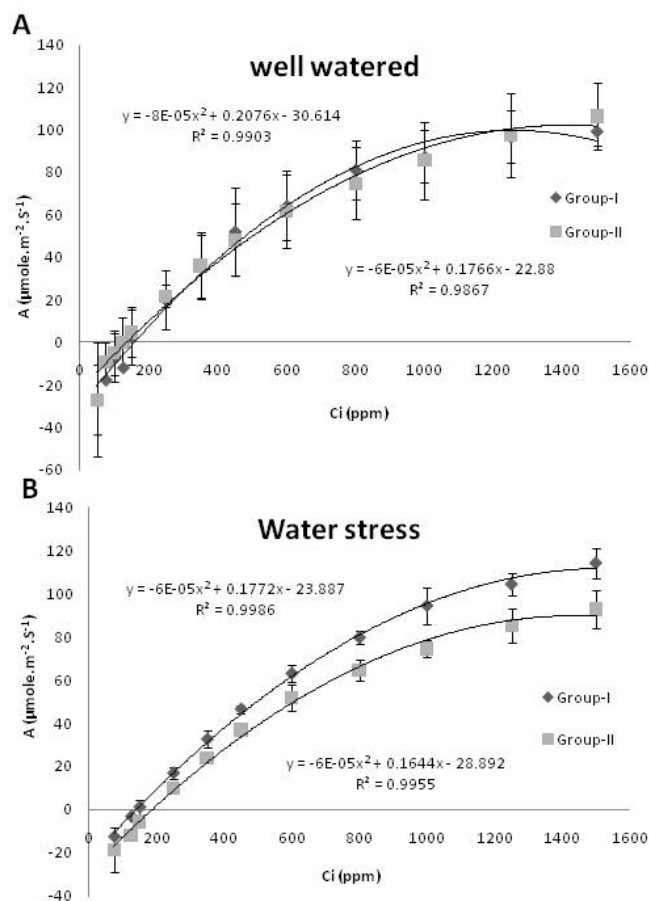


Fig. 2 Typical curves of net CO₂ assimilation (A) as a function of intercellular CO₂ concentration (C_i) in average of group I and II under well-watered (A) and moisture stress (B) treatments. Error bars represent the standard deviation of mean.

tant factor to improve biomass production. In our study, the genotypes that have a combination of both high root biomass and high WUE (low $\Delta^{13}C$) are more effective in biomass production under well watered condition. The genotypes with high root and high WUE can be considered as “capacity type” and are the most preferable set of genotypes for crop improvement. These genotypes by virtue of a great chloroplast capacity to fix carbon would be the ones most effectively use water for growth (Blum 2009). On the other hand, the conductance types though are water savers, are poor producers of biomass and yield. Recently, a simple stable isotopes based approach was developed to identify such “capacity type” while $\Delta^{13}C$ is well established as a surrogate for WUE, Sheshshayee *et al.* (2005, 2011) provide experimental evidence for the use of oxygen isotope environment as an accurate measure of transpiration and stomatal conductance. Using a “dual isotopes” approach, AbouKheir *et al.* (2010) developed a simple technique for identifying the capacity types.

Here we hypothesize that a genotype with high root biomass and high WUE will maintain better performance and growth under drought stress. To verify performance of these genotypes under drought stress condition, three cotton accessions were selected from each group-I (Ca/H-155, Ca/H-168, Ca/H-19) and group-II (Ca/H-51, Ca/H-216, Sahana) examined under two different water regimes in container experiment.

Significant genetic variation was noticed among two cotton groups in RV, RW and WUE. As expected, group-I genotypes had higher RW, RV and WUE than group-II genotypes under well watered treatment (Tables 5, 6). Drought stress reduced root dry weight and volume were reduced under drought stress in both groups (Table 5) this result confirm earlier reports (Malik *et al.* 1979; Taylor

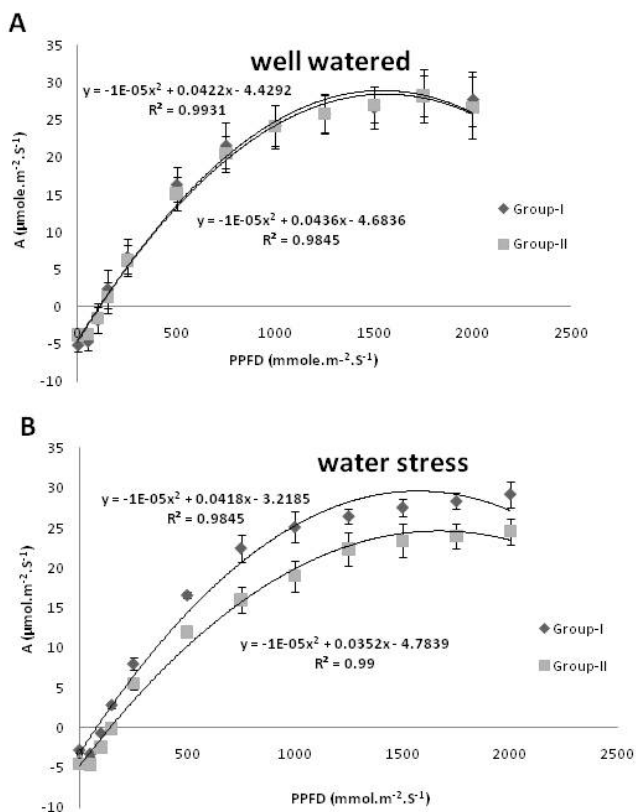


Fig. 3 Typical curves of net CO₂ assimilation (A) as a function of light intensity (PPFD) in average of group I and II under well-watered (A) and moisture stress (B) treatments. Error bars represent the standard deviation of mean.

1983; Ball *et al.* 1994; Xiong *et al.* 2006; Kulkarni and Phalke 2009). This decline in root biomass is mainly due to reduction in root thickness in response to drought that may be a common response in cotton (McMichael and Quisenberry 1991; Fernández *et al.* 1996). In contrary, an increase in tap root length under moisture stress without any change in cotton tap root dry weight was also reported (Pace *et al.* 1999). Group-I genotypes had significantly higher RW and RV under both water regimes compared to group-II genotypes (Table 5).

Cotton canopy development is highly sensitive to drought stress which causes to reduction in number of leaves (Krieg and Sung 1986) and/or inhibit LA (Rosenthal *et al.* 1987). Drought stress treatment reduced LA lead to smaller leaf size thus reduction in leaf dry weight (Table 4). The group-I genotypes maintained high LA and thus LW compared with group-II genotypes under stress.

Surprisingly, a higher reduction in TDM was recorded in group-I (22.59%) than group-II (15.93%), this may be due to group-I accessions have experienced drought stress much earlier than group-II accessions by maintaining higher transpiration under water stress condition (Table 6). Although lines belong to group-I have high root biomass, the limited soil size in pot almost eliminated the advantages of high root biomass to harness more water from deeper soil profiles. However, group-I still have high biomass under both water regimes due to have more active photosynthetic leaves that associated with accumulating more biomass (Gerika *et al.* 1996).

A reduction in photosynthesis under drought stress was noticed (Table 6; Figs. 2, 3) which is similar to that reported earlier (McMichael and Hesketh 1982; Marani *et al.* 1985; Turner *et al.* 1986; Genty *et al.* 1987; Ephrath *et al.* 1990; Faver *et al.* 1996; Deeba *et al.* 2012). This reduction could be due to decrease CO₂ availability caused by diffusion limitations through the stomata and the mesophyll (Flexas *et al.* 2004, 2007) or the alterations of photosynthetic metabolism (Lawlor and Cornic 2002). This study

documented that group-I genotypes have higher photosynthetic rate at any given CO₂ concentration or light intensity (PPFD) under moisture stress (Figs. 2B, 3B) therefore, group-I has increased WUE under moisture stress (Table 6) by maintaining relatively high transpiration (Table 6) associated with a high photosynthesis rate (Table 6; Figs. 2B, 3B) compare with group-II genotypes. Table 7 illustrates that group I has high carboxylation efficiency examined by light and CO₂ response curve.

In conclusion, it is clear that association between high water mining through big root biomass coupled with high WUE that lead to produce higher biomass under well watered and limited water conditions and reduce the penalty of breeding for either WUE or big root system alone under limited water condition. Group-I genotypes capable to accumulate more biomass thus more yield due to maintain high photosynthetic rate and continuous supply water from deeper soil profile under drought stress. However, it is important to examine group-I genotypes in field under irrigated and rain fed conditions.

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