Evaluation of Phenotypic Variability for the Water Use and Water Use Efficiency Based on Physiological Determinants in Cotton (*Gossypium hirsutum* L.)

Ehab Abou Kheir

Department of Biotechnology, Sweida Research Center, General Commission of Scientific Agricultural Research Sweida, Syria

ABSTRACT

A significant area of cotton production are located in limited rainfed areas. Being a commercially important crop, improvement of drought tolerance has received increasing attention in crop improvement programs. Currently, the emphasis is to identify and assess variability in specific traits of roots, water use efficiency (WUE), and other physiological traits that have relevance in enhancing drought stress tolerance. The objective of this study to assess the phenotic variability of some drought tolerant traits and establishing the use of carbon and oxygen stable isotopic signature as surrogate to WUE and transpiration respectively.

Thirteen advanced breeding lines (F7) of cotton raised in containers holding 30 kg soil. After seedling establishment, 50 days after sowing (DAS) at the field facility of Department of Crop Physiology, University of Agricultural Sciences, Bangalore, India, physiological parameters influencing WUE and biomass accumulation during vegetative phase of crop development (50- 80 DAS) were assessed. Significant genotypic variation was observed in canopy cover (Leaf Area Duration), total dry biomass (TDM) and cumulative total water transpired (CWT). TDM accumulated showed a significant association with leaf area and CWT but not with WUE. Based on the relationships of WUE with physiological traits such as Mean Transpiration and Net Assimilation Rates, WUE of the examined cotton genotypes was under a strong stomatal control. Results of the stable carbon and oxygen isotopes ratios further revealed a lesser inference of chloroplast capacity on WUE. It is demonstrated that cotton lines with higher chloroplast capacity can still be identified from “conductance types” lines. Through, the uses of a dual isotope approach as useful selectable strategy in yield improvement in cotton.

Key Words: Carbon isotope discrimination, Cotton, Oxygen isotope enrichment, Water use efficiency.

INTRODUCTION

Cotton is an important commercial crop that drives the textile industry besides being the second largest oilseed crop in the world. Cotton is cultivated in more than seventy countries from tropical to subtropical regions in the world (Yu and Kohel, 1999). Although selection for high absolute yield was a successful strategy for crop improvement, the narrow variability in yield *per se* among improved cultivars, and the low heritability for yield indicate that this strategy may not provide the required breeding advantage (Blum, 2009). It is suggested that constitutive sub component traits need to be put together into a single genetic background to achieve improved productivity through a novel trait based breeding strategy.

A large number of adaptive as well as constitutive traits have been identified and are being proposed for exploitation in crop improvement for water limited conditions (Richards, 1996; Miflin, 2000; Collins *et al*., 2008). From the agronomic point of view, any trait has relevance only when it is associated with superior growth rates under limited water condition (Udaykumar and Prasad, 1994; Sheshshayee *et al*., 2003). Traits associated with water mining from deeper soil layers (roots), efficient use of water for biomass production (WUE - the amount of biomass produced per unit of water transpired) deserve greatest emphasis (Passioura, 1976 and 1986). Significant stimulation to selection for these traits was provided with the advent of high throughput stable isotope based approaches.

Theory linking the carbon isotope discrimination ($\Delta^{13}C$) with WUE is well established (Farquhar *et al*., 1989). It was validated in several species of crop plants (White *et al*., 1996; Ashok *et al*., 1999; Sheshshayee *et al*., 2003; Condon *et al*., 2004; Bindumadhava *et al*., 2005; Impa *et al*., 2005; Nadaradjan *et al*., 2005) including cotton (Saranga *et al*., 1998; Stiller *et al*., 2005).

However, progress in breeding for improved WUE was not encouraging as a reduction in biomass or yield was often noticed while selecting for high WUE (Rishards *et al*., 2002). A possible explanation for the reduction in biomass in high WUE types was provided by Udaykumar *et al*., (1998b) and Sheshshayee *et al*., (2003). More recently Blum (2009) proposed that effective use water (EUW) is more appropriate parameter than WUE. However, for a comprehensive improvement in growth rates under water limited conditions both water mining abilities as well as efficient use of water for biomass production are essential. This necessitates the assessment of genetic variability in both of these traits.

Carbon isotope discrimination ($\Delta^{13}C$) has been accepted as a time-averaged surrogate for WUE (Farquhar *et al*., 1989), it was shown that oxygen isotope enrichment is a good surrogate for transpiration rate (Sheshshayee *et al*., 2005 and 2010). In this work, the relevance of carbon and oxygen isotope ratios in identifying cotton lines with higher WUE despite relatively high transpiration is examined.

MATERIALS AND METHODS

Plant material:

This study was conducted during the summer of 2010 at the field facility of Department of Crop Physiology, University of Agricultural Sciences, Bangalore, India. Thirteen cotton lines (*Gossypium hirsutum* L.) in advanced stage of selections (F7) for yield (Table 1) were sown in carbonized rubber containers filled with approximately 30 kg rooting mixture of red loamy soil and farmyard manure in 3:1 proportion (v/v). The water held by the soil at field capacity was determined gravimetrically and it was 21% v/w. A single plant was maintained per container. The containers were arranged in Completely Randomized Design (CRD) with eight replications at the rate of two containers per replication.

Table 1. List of thirteen cotton lines (*G.hirsutum*).

Serial No.	Lines names
1	Br-04(a)1CHT 5401
2	Br-04(a)1CHT 5403
3	Br-04(a)1CHT 5404
4	Br-04(a)1CHT 5405
5	Br-04(a)1CHT 5407
6	Br-04(a)1CHT 5408
7	Br-04(a)1CHT 5409
8	Br-04(a)1CHT 5410
9	LRA-5166
10	DIS -22
11	CNH-32
12	CNH-29 I
13	DIS-380

Gravimetric approach to determine the WUE and associated physiological traits:

WUE and associated physiological traits were measured as per Udaykumar *et al*., (1998a) and Cernusak *et al.* (2008). Over a period of 30 days between 50 and 80 days after sowing (DAS). This period coincides with the peak vegetative growth in cotton. The method involved weighing the containers on a daily basis using a mobile weighing device. The soil surface of all containers was covered with pieces of plastic to minimize evaporation of water from the soil surface. The water status of the soil in containers was returned to field capacity every day during morning hours. At the start of the gravimetric experiment (50- DAS), the soil water status was brought to field capacity (FC) by adding the appropriate volume of water, predetermined for each container. The amount of water added over the experimental period of 30 days was summed up ($CWA_{planted}$) to arrive the total evapo-transpiration (ET). Simultaneously, a set of four containers filled with the same soil mixture were maintained without plants but with the soil surface covered with plastic pieces, to determine the direct soil evaporation. The water added to each of the containers without plant was also summed (CWA_{empty}) over the experimental period to measure the total evaporation (E). Containers were arranged randomly under a mobile rainout shelter (Chauhan *et al*., 1997) which was moved over the experimental area at night and during rain episodes (if any). Total water transpired by plants (i.e., cumulative water transpired or CWT) over the experimental period was calculated as the difference between ET ($CWA_{planted}$) and E (CWA_{empty}). At the start (50 DAS) and at the end (80 DAS) of the experiment, plants from a representative set of containers consisting of two replications (four containers) were carefully removed from soil and leaf, stem and root biomass was separately determined. The total leaf area was determined form each individual plant by using a leaf area meter (MK-2, Delta-T devices, England). The remaining 12 containers (six replications) were used to record the final data (on 80 DAS) as well as for determining total water transpired. WUE was calculated as the ratio of biomass produced during the experimental period to CWT and expressed as g biomass per liter water transpired.

The two physiological traits namely, Mean Transpiration Rate (MTR) and Net Assimilation Rate (NAR) were computed according to Hunt (1978) and Escalant and Kohashi (1993)

MTR = (CWT/LAD)

NAR = (TDM/LAD)

Where LAD, the leaf area duration during the experimental period, was determined using the equation $LAD = \{(LA_{50DAS} + LA_{80DAS})/2\} \times 30$ and expressed as m^2 days (Power, *et al*., 1967). Since the experimental period coincided with the peak vegetative phase, the increment in leaf area between 50 and 80 DAS was assumed to be linear.

While the net assimilation rate (NAR) is a time-averaged measurement of photosynthetic rate, MTR is a good measure of time-averaged measure of stomatal conductance (Udayakumar *et al*., 1998a; Impa *et al*., 2005).

Measurement of stable isotope ratios in leaf biomass:

Carbon isotope ratio: Carbon isotope ratio was determined with an isotope ratio mass spectrometer (IRMS; Delta-plus, ThermoFisher Scientific, Bremen, Germany) interfaced with an elemental analyzer

(NA1112, CarloErba, Italy) via a continuous flow device (Conflo-III, ThermoFisher Scientific, Bremen, Germany). A composite leaf sample, comprising of 10 mature leaves representing all positions of the plant canopy, were harvested and oven dried for 3 days at 70°C and homogenized to a fine powder with a ball mill. Six replications from each cotton line were analyzed for $\delta^{13}C_{lb}$ with an analytical uncertainty of < 0.1‰. Carbon isotope discrimination ($\Delta^{13}C$ expressed in ‰ per mil) was computed as follows, assuming the isotopic composition of atmospheric air ($\delta^{13}Ca$) to be -8‰ relative to Pee Dee Belemnite) (Farquhar *et al*., 1989):

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

Oxygen isotope ratio: The samples were pyrolysed at high temperatures using a temperature conversion elemental analyzer (TC/EA – Thermofisher Scientific, Brmen, Germany). The TC/EA was interfaced with the IRMS through a continuous flow device (Conflo-III). ^{18}O enrichment in leaf biomass ($\Delta^{18}O$) was computed (Sheshshayee *et al*., 2005) as:

$$\Delta^{18}O\ (‰) = \delta^{18}O_{lb} - \delta^{18}O_{iw}$$

Where, $\delta^{18}O$ is the isotopic composition in relation to VSMOW (Vienna Standard Mean Ocean Water) and subscripts lb and iw refer to leaf biomass and irrigation water, respectively. The $\delta^{18}O$ in water used to irrigate plants, was determined by a CO_2-H_2O equilibrating device (Gas Bench-III). The average oxygen isotope composition ($\delta^{18}O_{iw}$) was found to be -3.75‰ ±0.05.

All stable isotope measurements were made at the National Facility for Stable Isotope Studies, Department of Crop Physiology, University of Agricultural Sciences, Bangalore, India.

Statistical methods:

An Analysis of Variance (ANOVA) was performed for each parameter to determine whether there were differences among cotton lines. Line means were separated by the use of least Significant Difference (LSD) at $p \leq 0.05$ using MSTAT-C software (Anonymous 1998).

The relationship between parameters was analyzed via simple linear regression supported by Pearson’s correlation analysis. The analysis was conducted based on genotype mean.

To classify the genotypes based on variations in traits, the specific traits in question were transformed into their standardized normal distribution values as follows:

$$Z = (X - Xi)/\sigma$$

Where, X is the overall mean of a specific parameter and Xi is the average of the i^{th} individual; σ is the standard deviation of that parameter.

Weather parameters: The daily humidity and temperature data for the experimental period was obtained from the Agro meteorological station. Vapor pressure deficit (VPD) was computed based on the relative humidity and air temperatures measured twice a day at 7 AM and later at 2 PM as suggested by Tanner and Sinclair (1983)

$$VPD = VPs - VPh$$

Where, VPs is saturated vapor pressure of the air (mbar), was determined based on air temperature (t) using the equation (Allen *et al*., 1998) $VPs = (6.13753 \times (et(18.564 - (t/254.4))))/(t+255.57)$ and VPh is actual vapor pressure of the air (mbar), was determined based on relative humidity (RH) using the equation $VPh = (RH/100) \times VPs$

Early morning VPD did not show marked fluctuations while the mid day VPD varied marginally during the experimental period (Fig. 1).

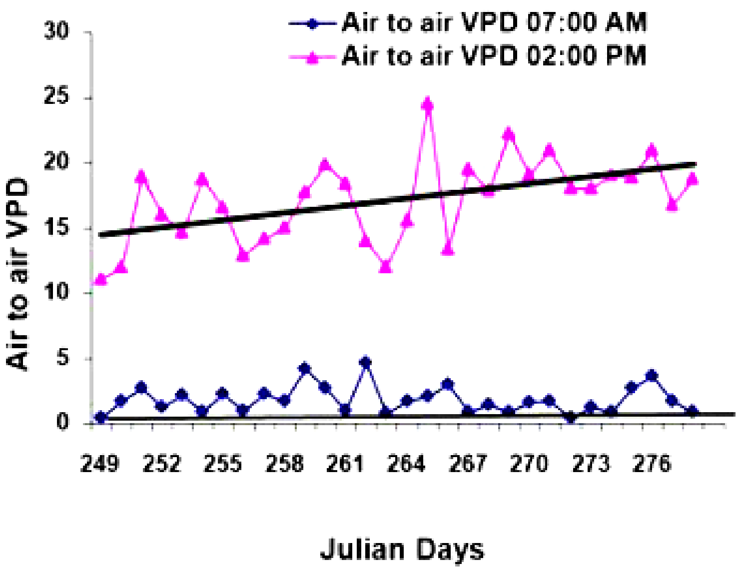

Fig.1 Regression of the air to air VPD during the experimental period (Julian days).

RESULTS:

Cotton lines differed significantly in the total amount of water transpired (CWT) which ranged from 11.40 to 16.90 L plant^{-1}. Total biomass accumulated over the same period varied between 41.31 to 62.25 g plant^{-1} (Table 2). Leaf area duration (LAD), a measure of canopy cover varied from 6.49 to 9.35 m^2 days representing a significant phenotypic variability (Table 2). TDM was strongly related with variations in CWT and

LAD (Fig. 2). At a given leaf area total transpiration depends on root traits. Accordingly a significant relationship between root weight and CWT was noticed (Fig. 3).

Table 2.
Mean of some physiological traits among thirteen cotton lines (*G.hirsutum*).

Cotton line	CWT	TDM	LAD	WUE	MTR	NAR	Root Wt.
	(LPt^{-1})	(g Pt^{-1})	(m^2 days)	(g L^{-1})	(L m^{-2}days)	(g m^{-2} days)	(g Pt^{-1})
Br-04(a)1CHT 5401	14.10	54.63	8.04	3.88	1.75	6.79	6.46
Br-04(a)1CHT 5403	16.41	62.25	9.35	3.41	1.95	6.65	8.91
Br-04(a)1CHT 5404	16.79	57.30	8.62	3.54	1.79	6.29	7.27
Br-04(a)1CHT 5405	16.13	56.81	9.03	3.84	1.77	6.73	8.03
Br-04(a)1CHT 5407	14.99	56.91	8.45	3.49	1.94	6.78	7.83
Br-04(a)1CHT 5408	11.40	41.31	6.93	4.02	1.77	7.06	6.27
Br-04(a)1CHT 5409	16.90	59.11	8.71	3.99	1.84	7.29	7.03
Br-04(a)1CHT 5410	13.50	53.71	7.62	3.45	1.97	6.77	6.11
LRA-5166	13.72	54.52	7.51	3.86	1.65	6.52	5.93
DIS -22	14.37	49.61	7.29	3.62	1.75	6.32	5.59
CNH-32	12.87	49.67	7.64	3.75	1.86	6.99	4.89
CNH-29 I	14.62	52.90	8.39	3.29	2.04	6.73	6.39
DIS-380	12.08	45.29	6.49	3.50	1.84	6.42	3.94
Mean	14.5	53.4	8.0	3.7	1.8	6.7	6.5
F test	**	**	**	**	**	NS	**
CV.%	9.19	9.23	7.67	7.35	8.30	8.00	10.03
LSD (5%)	1.68	6.16	0.77	0.34	0.19	NS	1.41

** Significant at the 0.01 probability level and NS: non-significant. Cumulative Water Transpired (CWT), Leaf Area Duration (LAD), Total Dry biomass (TDM), Water Use Efficiency (WUE), Net assimilation rate (NAR), mean transpiration rate (MTR), Root biomass (Root Wt.)

Water use efficiency (WUE), the amount of biomass produced per unit water used, was arrived at by computing the ratio of TDM to CWT and a significant variability was noticed (Table 2).

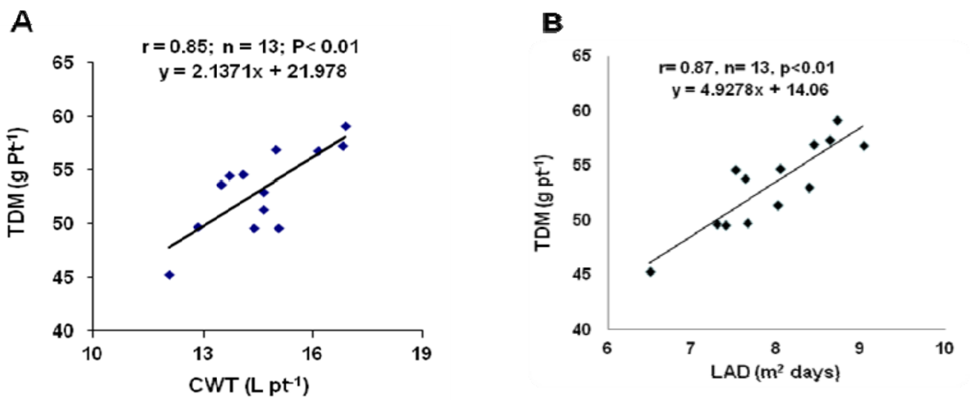

Fig.2. Relationship of total biomass (TDM) with (A) cumulative water transpiration (CWT) and (B) functional leaf area (LAD) amongst thirteen cotton lines (*G.hirsutum*).

Stable carbon isotope discrimination ($\Delta^{13}C$) routinely being used a surrogate for WUE, also differed significantly among the genotypes. As predicted by theory, a strong inverse relationship between $\Delta^{13}C$ and WUE was noticed (Fig. 4). Though WUE is one of the important determinants of biomass accumulation, no discernible relationship between WUE and TDM was observed (Fig. 5). This lack of relationship clearly emphasizes the need for understanding the physiological basis of the variation in WUE.

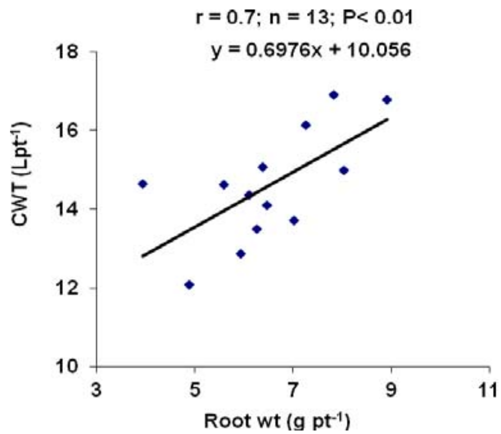

Fig.3. Relationship between CWT and Root biomass amongst thirteen cotton lines (*G.hirsutum*).

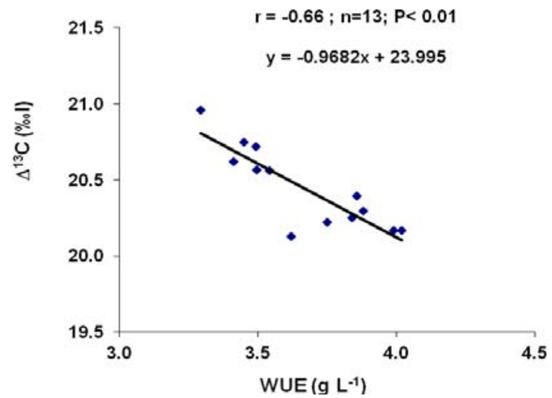

Fig.4. Relationship between water use efficiency (WUE) and carbon isotope discrimination ($\Delta^{13}C$) amongst thirteen cotton lines (*G.hirsutum*).

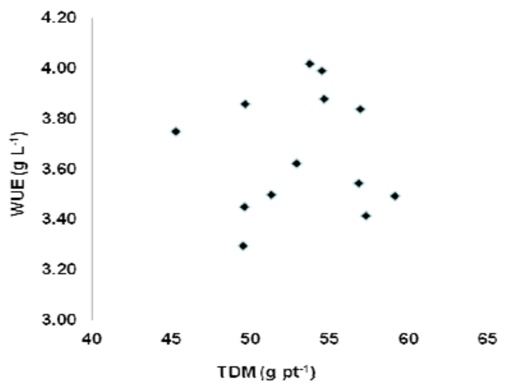

Fig.5. Relationship between water use efficiency (WUE) and total biomass (TDM) amongst thirteen cotton lines (*G.hirsutum*).

The two physiological processes associated with WUE, namely, net assimilation rate (NAR) and Mean transpiration rate (MTR) were determined. Significant phenotypic variability was noticed for MTR but not for NAR (Table 2).

Oxygen stable isotope enrichment ($\Delta^{18}O$) was determined as a time averaged surrogate for MTR, which ranged between 25.32 ‰ and 26.98 ‰ representing significant variation (Table 3). A strong relationship between MTR and $\Delta^{18}O$ illustrated in Fig. 6 reiterated the relevance of oxygen isotope enrichment as a convenient tool to rapidly assess variations in MTR.

Table 3.
Phenotypic variability in carbon isotope discrimination ($\Delta^{13}C$) and oxygen isotope enrichment ($\Delta^{18}O$) among thirteen cotton lines (*G.hirsutum*).

	$\Delta^{13}C$ (‰)	$\Delta^{18}O$ (‰)
Br-04(a)1CHT 5401	19.12	26.01
Br-04(a)1CHT 5403	20.62	26.31
Br-04(a)1CHT 5404	20.56	26.06
Br-04(a)1CHT 5405	20.25	26.56
Br-04(a)1CHT 5407	20.72	26.23
Br-04(a)1CHT 5408	20.17	25.79
Br-04(a)1CHT 5409	20.17	25.81
Br-04(a)1CHT 5410	20.75	26.78
LRA-5166	20.39	25.32
DIS -22	20.13	25.53
CNH-32	20.22	25.81
CNH-29 I	20.96	26.98
DIS-380	20.57	25.74
Mean	20.32	26.10
F test	**	**
CV.%	4.45	5.10
LSD (5%)	1.14	1.43

** Significant at the 0.01 probability level.

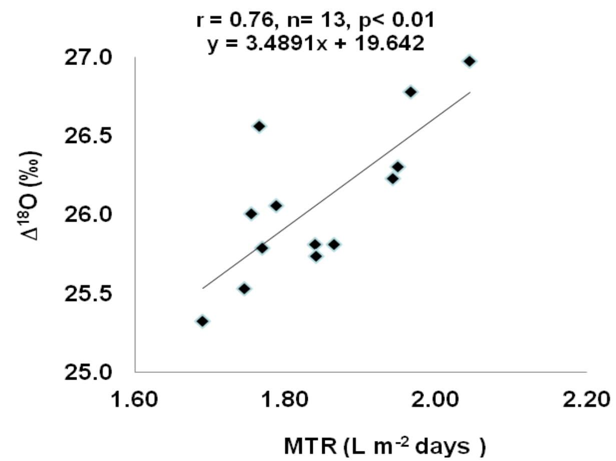

Fig.6. Relationship between mean transpiration rate (MTR) and oxygen isotope enrichment ($\Delta^{18}O$) amongst thirteen cotton lines (*G.hirsutum*).

A significant inverse relationship of WUE with MTR (Fig. 7) and an insignificant regression line between WUE and NAR suggest that stomatal factors control the differences in WUE in these cotton lines. Based on the variations in the two stable isotope ratios, the cotton lines were classified into four distinct categories (Table 4) and the total biomass of the lines in each of these groups was compared. Result presented in Table 4 revealed that at a given leaf area cover, differences in WUE and transpiration rate significantly contributed to total biomass. The lines categorized under low $\Delta^{13}C$ (high WUE) and high $\Delta^{18}O$ (high transpiration rate) recorded the highest biomass of 55.7 g $plant^{-1}$ while it was lowest among the category of high $\Delta^{13}C$ and low $\Delta^{18}O$ genotypes (50.48 g $plant^{-1}$) (Table 4). Root biomass closely followed the same trend.

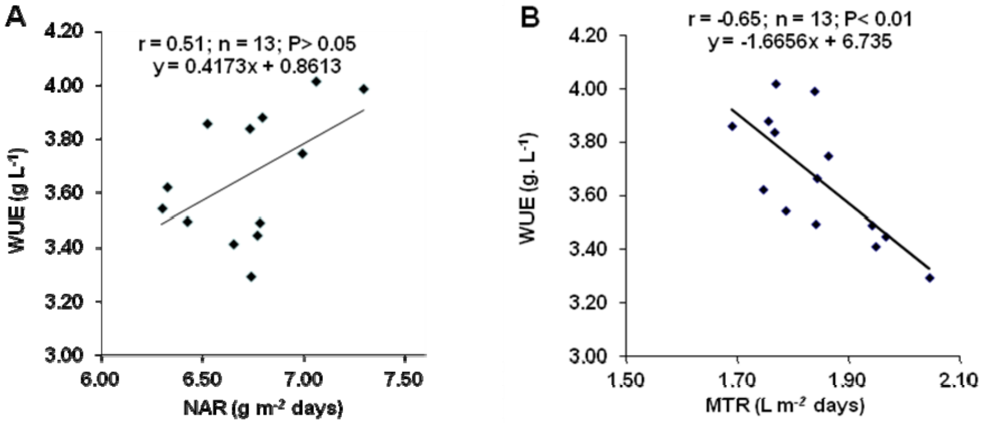

Fig. 7. Relationship of water use efficiency (WUE) with (A) net assimilation rate (NAR) and (B) mean transpiration rate (MTR) amongst thirteen cotton lines (*G.hirsutum*).

Table 4.
Mean of Some physiological traits of four categories

	n	CWT	LAD	TDM	WUE	NAR	MTR	Root wt	$\Delta^{13}C$	$\Delta^{18}O$
		(L Pt^{-1})	(m^2 days)	(g Pt^{-1})	(g L^{-1})	(g m^{-2}days)	(L m^{-2} days)	(g Pt^{-1})	(‰)	(‰)
High $\Delta^{18}O$ Low $\Delta^{13}C$	3	14.54b*	8.24a	55.77a	3.86a	6.76a	1.76b	7.25a	20.27c	26.34a
Low $\Delta^{18}O$ low $\Delta^{13}C$	3	13.48c	7.50b	51.60bc	3.85a	6.92a	1.80b	5.94b	20.17c	25.76b
Low $\Delta^{18}O$ high $\Delta^{13}C$	4	13.76c	7.83ab	50.48c	3.68b	6.47a	1.77b	4.93c	20.48b	25.61b
High $\Delta^{18}O$ high $\Delta^{13}C$	3	15.59a	8.08a	53.31b	3.43c	6.61a	1.94a	7.17a	20.72a	26.53a

Note: values in a column with the same letter are not significantly different at LSD (0.05). Cumulative Water Transpired (CWT), Leaf Area Duration (LAD), Total Dry biomass (TDM), Water Use Efficiency (WUE), Net assimilation rate (NAR), mean transpiration rate (MTR), Root biomass (Root Wt.), carbon isotope discrimination ($\Delta^{13}C$) and oxygen isotope enrichment ($\Delta^{18}O$) and number of cotton lines in each category (n).

DISCUSSION

Cotton production globally is severely constrained by water availability. With limited options for expanding area under irrigation, emphasis must be on improving drought tolerance of cotton lines to enhance productivity. A highly orchestrated “trait based” breeding strategy is needed to achieve improved productivity of crops (Richards, 1996; Condon *et al*., 2004; Reynolds and Tuberosa, 2008). Global research has therefore concentrated on identifying relevant traits for crop improvement (Richards *et al*., 2002; Sheshshayee *et al*., 2003; Blum, 2009).

Among several traits are those that help in maintaining tissue water relations and efficiency of water use for biomass production (Passioura, 1986; Sheshshayee *et al*., 2003; Reynolds and Tuberosa, 2008). Water uptake, a mass flow mechanism, is dependent on transpiration as well as the ability of roots to supply water to meet the transpirational demand of the canopy. Since stomata are the only pores that control the diffusion of both water vapor during transpiration and CO_2 during photosynthesis, a strong interdependence between transpiration and biomass accumulation is generally noticed (Fig. 2a). Evolutionarily, plants minimized transpiration as a water conservation strategy to survive water limited conditions. Because of a strong link between transpiration and photosynthesis, any reduction in transpiration is normally associated with reduced biomass. This results in a negative tradeoff between WUE and biomass production (Richard *et al.* 2002; Condon *et al.* 2004). Though evolutionarily significant, increase in WUE through a reduction in transpiration would be counterproductive (Sheshshayee *et al*., 2003; Reynolds and Tuberosa, 2008; Blum, 2009). This means that better uptake of water associated with roots appears to be a better strategy to improve productivity of dry land crops. Blum (2009), reviewing the ongoing crop improvement efforts opined that effective use of soil water is important for better growth of plants under water limited conditions.

A strong positive relationship between root biomass and transpiration (Fig. 3) and between transpiration and total biomass accumulated (Fig. 2a) within the cotton lines implied that improving root traits would be relevant. However, there are two problems associated with such attempts. The first is assessing the genetic variability in roots is a herculean task, while the second is enhancing water uptake under water limited rain fed conditions might become detrimental for productivity (Condon *et al*., 1993). Deep rooted plants are known to exhaust water early and would experience a definite end season stress.

Efforts have also been made to increase the rapidity of canopy cover as an approach towards decreasing the soil evaporation losses. A strong positive association between leaf area duration development and total biomass accumulated (Fig. 2b) within these cotton lines reiterates the possibility of achieving higher growth rates through canopy development. Canopy cover, besides increasing the surface area for photosynthesis also contributes to higher transpiration rate and hence may not help under water limited rain-fed conditions (Stastna *et al*., 2002; Impa *et al*., 2005). Hence a simultaneous improvement in the efficiency of water use (WUE) would be extremely relevant for achieving a comprehensive improvement of growth and productivity under water limited conditions (Sheshshayee *et al*., 2003; Reynolds and Tuberosa, 2008).

If all plants were provided with the same amount of water for transpiration, the genotypes with higher WUE would result in higher crop growth rate. This is in accordance with the Passioura yield model (Passioura, 1986) which has formed the framework of several breeding programs (Richards *et al*., 2002; Sheshshayee *et al*., 2003; Condon *et al*., 2004). A low genotype by environment interaction for WUE (Ismail and Hall 1992 and 1993; Hebbar *et al*., 1994; Ashok *et al*., 1999) and high broad sense heritability (Hubick *et al*., 1988; Merah *et al*., 1999; Ray *et al*., 1999; Zacharison *et al*., 1999; Rebetzke *et al*., 2002; Richards *et al*., 2002) have rendered WUE a very useful trait. Hence, besides assessing the genetic variability in CWT, LAD and root traits, it is equally important to generate information on the variability in WUE.

Methods for the assessment of variability in WUE range from the tedious though accurate, gravimetric approaches (Udayakumar *et al*., 1998a) to the time instantaneous gas exchange approaches (Caemerrer and Farquhar, 1981; Condon *et al*., 2002). However, a tremendous impetus to the efforts of assessing the phenotypic variability in WUE was provided with the discovery that plants discriminate against the heavy isotope of carbon (^{13}C) during photosynthesis (O'Leary, 1988) and the demonstration of its association with WUE in crop plants (Farquhar *et al*., 1989). Several workers have since validated the $\Delta^{13}C$ as a selectable parameter in breeding programs attempting to improve WUE (Udayakumar, 1998a; Saranga *et al*., 1998; Sheshshayee *et al*., 2003; Impa *et al*., 2005; Stiller *et al*., 2005).

A strong inverse relationship between $\Delta^{13}C$ and WUE measured gravimetrically (Fig. 4) reiterated the relevance of carbon isotope signature as a time averaged surrogate for WUE. Although variability was significant, the range in WUE was narrow (Table 2). Similar observation was also reported by Pettigrew and Meredith (1994) in cotton. This is may be due to the fact that modern cotton cultivars have been mainly selected for specific properties such as high yield, good fiber characteristics and adaptation to mechanical harvesting (Rosenow *et al*., 1983). Thus, the variability in several physiological parameters is expected to be narrow and efforts must be made to assess the actual variability in $\Delta^{13}C$ using diverse cotton accessions.

Despite the establishment of the relevance of WUE and development of a powerful high throughput screening option, many breeding attempts were unsuccessful. A reduction in total biomass or yield while selection for high WUE was one of the major reasons (Blum, 2005 and 2009). In the present investigation also, there was no relationship between WUE and total biomass (Fig. 5). Such lack of relationship between WUE and total biomass or yield has also been reported for several crop plants (Hubick *et al*., 1986; Richards and Condon, 1993; Richards, 1996; Condon *et al*., 2002 and 2004). On the contrary, depending upon the growing conditions, positive (Matus *et al*., 1995; Richards, 1996; Rebetzke *et al*., 2002; Richards *et al*., 2002) and negative relationships between $\Delta^{13}C$ and total biomass have also been reported (Richards and Condon, 1993; Condon and Hall, 1997; Condon *et al*., 2002). This high degree of inconsistency of relationship between WUE and biomass potentially dampened the enthusiasm of the plant breeders. To fully exploit the advantages of these traits like roots and WUE, it is essential to further dissect the traits and understand the physiological factors that regulate variations especially in WUE (Farquhar and Lloyd, 1993; Sheshshayee *et al*., 2003). The net assimilation rate (NAR) and mean transpiration rate (MTR) within the cotton lines were measured. The calculated genetic variation was significant (Table 2). Examining the relationship of WUE with these physiological traits is a simple approach to assess the physiological factor that determines the variability in WUE. The strong inverse relationship of WUE with MTR (Fig. 7b) and not with NAR suggests that WUE of these cotton lines is under stomatal control. In such lines called the “conductance” type, selection for high WUE would invariably result in reduction of biomass (Blum, 2009; Lu *et al*., 1996)

This necessitates the identification of genotypes where chloroplast capacity determines the variations in WUE. Farquhar and Lloyd, (1993) called such lines as “Capacity” types and several experiments reported that the negative tradeoff between WUE and biomass is weak among such capacity types that are characterized by high WUE despite a relatively high total transpiration (Ashok *et al*., 1999; Sheshshayee *et al*., 2006) Hence, one needs to assess the genetic variability in both WUE and transpiration.

While carbon isotope ratio is a powerful surrogate for WUE, experimental evidences for the relevance of oxygen isotope discrimination as a surrogate for transpiration rate was reported (Sheshshayee *et al*., 2005). In the present study, $\Delta^{18}O$ and MTR were significantly related (Fig. 6). Although the application of oxygen isotope ratio as a measure of stomatal conductance has been equivocal, there is considerable consensus to the fact that oxygen isotope ratio is a good surrogate for transpiration rate (Farquhar *et al*., 2007; Cernusak *et al*., 2008). Besides stomatal conductance vapor pressure deficit of air is an important determinant of transpiration. While a positive relationship between VPD and transpiration is generally recognized, stomatal conductance decreases as VPD increase (El-Sharkawy *et al*., 1985). Since all the genotypes of this study experienced the same VPD (Fig 1), the difference in MTR between the genotypes would arise predominantly due to differences in stomatal conductance. Hence the genetic difference in $\Delta^{18}O$ was primarily due to variations in stomatal conductance. A mathematical explanation has been reported earlier by Sheshshayee *et al*., (2010).

Genotypes with superior chloroplast capacity would typically display higher WUE despite a relatively high transpiration rate and are hence desirable (Blum, 2009). To identify such lines, the cotton lines were classified based on the difference in $\Delta^{13}C$ and $\Delta^{18}O$. The cotton lines with high $\Delta^{18}O$ and low $\Delta^{13}C$ (corresponding to high transpiration rate and high WUE) had the highest total biomass compared to genotypes with other combinations of the two stable isotope ratios (Table 4). This observation further proves that stable isotopes can be effectively used for identifying chloroplast capacity types.

The results from this study clearly suggest that WUE among cotton breeding lines is strongly controlled by stomatal factors. Promising “capacity” types can still be identified based on stable isotope ratios. The cotton lines that had higher WUE despite a relatively higher transpiration did so because of a high root biomass and significantly deeper roots. Thus, a stable isotope based approach would be extremely useful in assessing the physiological regulation of WUE. The identified lines can be used in hybridization programs and the dual stable isotope approach can be effectively adopted for identifying the trait put together lines from among the segregating populations.

REFERENCES

Allen, R.G., Pereira, L.S., Raes, D. and Smith M. 1998. Reference evapotranspiration (ETo) p. 15–86. In Crop evapotranspiration: Guidelines for computing crop water requirements. FAO, Irriga tion and Drainage Paper 56, Rome, Italy.

Anonymous, 1998. Compendium of environment statistics: Central statistical organization. Dept. of Statistics. Ministry of Planning and Programme Implementation, GOI, New Delhi

Ashok, I.S., Hussein, A., Prasad, T.G., UdayaKumar, M., Nageswara Rao, R.C. and Wright, G.C. 1999. Variation in transpiration efficiency and carbon isotope discrimination in cowpea (*Vigna unguiculata* L. Walp.) genotypes. Australian Journal of Plant Physiology. 21:507-516

Bindumadhava, H., Sheshshayee, M.S., Shashidhar, G., Prasad, T.G. and Udayakumar, M. 2005. Ratio of stable carbon and oxygen isotope discrimination ($\Delta^{13}C/\ \delta^{18}O$) reflects variability in leaf intrinsic carboxylation efficiency in plants. Current Science. 189(7):1256-1258

Blum, A. 2005. Drought resistance, water-use efficiency and yield potential – are they compatible, dissonant or mutually exclusive? Australian Journal of Agriculture Research. 56:1159-1168

Blum, A. 2009. Effective use of water (EUW) and not water use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crops Research. 112:119-123

Caemerrer, S.V. and Farquhar, G.D. 1981. Some relationship between the biochemistry of photosynthesis and the gas exchange of leaves. Planta. 153: 378- 383

Cernusak, L.A., Winter, K., Aranda, J. and Turner, B.L. 2008. Conifers, angiosperm trees, and lianas: growth, whole-plant water and nitrogen use efficiency, and stable isotope composition ($\Delta^{13}C$ and $\Delta^{18}O$) of seedlings grown in a tropical environment. Plant Physiology. 148:642–659

Chauhan, Y.S., Saxena, N.P., Nageswara Rao, R.C., Johansen, C. and Ravindranath, K. 1997. Portable rainout shelter, a useful tool in drought research. ACIAR Food Legume Newsletter. 25, 9

Collins, N.C., Tardieu, F. and Tuberosa, R. 2008. Quantitative trait loci and crop performance under abiotic stress: Where do we stand? Plant Physiology. 147: 469-486

Condon, A.G. and Hall, K.T. 1997. Adaptation to adverse environments; variation in WUE within crop species. In: Jackson, L.E (Ed.) Ecology in Agriculture, pp. 89-116. Academic Press, San Diego, USA

Condon, A.G., Richards, R.A. and Farquhar, G.D. 1993. Relationships between carbon isotope discrimination, water use efficiency and transpiration efficiency for dry land wheat. Australian Journal of Agriculture Research. 44:1693–1711

Condon, A.G., Richards, R.A., Rebetzke, G.J. and Farquhar, G.D. 2002. Improving intrinsic water use efficiency and crop yield. Crop Science. 42:122-131

Condon, A.G., Richards, R.A., Rebetzke, G.J. and Farquhar, G.D. 2004. Breeding for high Water Use Efficiency. Journal of Experimental Botany. 55:2447-2460

El-Sharkawy, M.A., Cock, J.H. and Hernández, A.D.P. 1985. Stomatal response to air humidity and its relation to stomatal density in a wide range of warm climate species. Photosynthesis Research. 7:137-149

Escalante, E. J. A and Kohashi, Y. J. S. 1993. The performance and growth of beans. Manual data collection. Graduate College. Montecillo. Municipality. Texcoco Mex. pp 84

Farquhar, J., Peters, M., Johnston, D.T., Strauss, H., Masterson, A., Wiechert, U. and Kaufman, A.J. 2007. Isotopic evidence for Mesoarchaean anoxia and changing atmospheric sulphur chemistry. Nature. 449:706-709

Farquhar, G.D. and Lloyd, J. 1993. Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. In: Ehleringer, J.R., Hall, A.E. and Farquhar, G.D.(Eds). Stable isotopes and plant carbon-water relations, pp. 47-70. Academic Press, San Diego, USA

Farquhar, G.D., Hubick, K.T., Condon, A.G. and Richards, R.A. 1989. Carbon isotope fractionation and plant water use efficiency. In: Rundel, P.W., Ehleringer, J.R. and Nagy, K.A. (Eds.). Stable Isotope in Ecological Research, pp. 21-40.Springer-Verlag: New York, USA

Hebbar, K.B., Sashidhar, V.R., UdayaKumar, M., Devendra, R. and Rao, R.C.N. 1994. A comparative assessment of water use efficiency in groundnut (*Arachis hypogea*) grown in containers and in field under water – limited conditions. Journal of Agriculture Science. 122: 249- 434

Hubick, K.T., Farquhar, G.D. and Shorter, R. 1986. Correlation between water use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. Australian Journal of Plant Physiology. 13:803–816

Hubick, K.T., R. Shorter, and G.D. Farquhar. 1988. Heritability and genotype × Environment interaction of carbon isotope discrimination and transpiration efficiency in peanut. Aust. J. Plant Physiol. 15:799 –813.

Hunt, R. 1978. Plant growth analysis. Studies in biology no.96.Edward Arnold (publishers).London.67 p.

Impa, S.M., Nadaradjan, S., Boominathan, P., Shashidhar, G., Bindhumadhava, H. and Sheshshayee, M. S. 2005. Carbon Isotope Discrimination accurately reflects variability in WUE Measured at a whole plant level in Rice. Crop Science. 45:2517– 2522

Ismail, A.M. and Hall, A.E. 1992. Correlation between water use efficiency and carbon isotope discrimination in diverse cowpea genotypes and isogenic lines cowpea. Crop Science. 32:7 -12

Ismail, A.M. and Hall, A.E. 1993. Inheritance of Carbon Isotope Discrimination and water use efficiency in cowpea. Crop Science. 33:498-503

Lu, C., Richards, G.P., Sharti, M.R. and Zeiger, E. 1996. Genetic variation in carbon isotope discrimination and its relation to gs in pima cotton. Australian Journal of Plant Physiology. 23:127-132

Matus, A., Slinkard, A. and Kessel, C.V. 1995. Crop breeding, genetic and cytology, CID: potential for indirect selection for yield in canola. Crop Science. 35:1267-1271

Merah, O., Deleens, E. and Monneveux, P. 1999. Grain yield, carbon isotope discrimination, mineral and silicon content in durum wheat under different precipitation regimes. Physiology Planta. 107: 387-394

Miflin, B. 2000. Crop improvement in the 21st century. Journal of Experimental Botany. 51(342):1-8

Nadaradjan, S., Impa, S.M., Shashidhar, G. P., Sheshshayee, M.S., Udayakumar, M. and Prasad T.G. 2005. Overlapping QTLs for WUE and Carbon isotope discrimination in DHLs of rice. Journal of Plant Biology. 32:1-8

O'Leary, M.H. 1988. Carbon isotope in photosynthesis. Bio Science. 38:325-336

Passioura, J.B. 1976. Physiology of grain yield in wheat growing on stored water. Australian Journal of Plant Physiology. 3:559-565

Passioura, J.B. 1986. Resistance to drought and salinity: Avenues for improvement. Australian Journal of Plant Physiology. 13:191-201

Pettigrew, W.T., and W.R., Meredith. 1994. Leaf gas exchange parameters vary among cotton genotypes. Crop Science. 34:700- 705

Power, J.F., Willis, W.O., Grunes, D.L. and Reichman, G.A. 1967. Effect of soil temperature, phosphorus, and plant age on growth analysis of barley. Agron. J. 59:231-234.

Ray, I.M., Townsend, M.S., Muncy, C.M. and Henning J.A. 1999. Heritabilities of WUE traits and correlation with agronomic traits in water stressed Alfalfa. Crop Science. 39:494-498

Rebetzke, G.J., Condon, A.G., Richards, R.A. and Farquhar, G.D. 2002. Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. Crop Science. 42:739-745

Reynolds, M., and Tuberosa, R. 2008. Translational research impacting on crop productivity in drought-prone environments. Current Opinion Plant Biology. 11:171-179

Richards, R. A. 1996. Defining selection criteria to improve yield under drought. Plant growth regulation. 20:157-166

Richards, R.A., Rebetzke, G.J., Condon, A.G. and Van Herwaarden, A. F. 2002. Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. Crop Science. 42:111-121

Richards, R.A. and Condon, A.G. 1993. Challenges ahead in using carbon isotope discrimination in plant breeding programs. p. 451–462. In: Ehleringer, J.R., Hall, A.E. and Farquhar, G.D.(Eds). Stable isotopes and Plant carbon/water relations, . pp. 451-462. Academic Press, San Diego, USA

Rosenow, D.T., Quisenberry, J.E., Wendt, C.W. and Clark, L.E. 1983. Drought tolerant sorghum and cotton germplasm. Agriculture Water Management. 7:207-222

Saranga, Y., Flash, I. and Yakir, D. 1998. Variation in water use efficiency and its relation to carbon isotope ratio in cotton. Crop Science. 38:782-787

Sheshshayee, M. S., Bindumadhava, H., Shankar, A.G., Prasad, T.G. and Udayakumar, M. 2003. Breeding strategies to exploit water use efficiency for crop improvement. Journal of Plant Biology. 30(2):253-268

Sheshshayee, M.S., Bindumadhava, H., Ramesh, R., Prasad, T.G., Lakshminarayana, M.R. and Udayakumar, M. 2005. Oxygen isotope enrichment ($\Delta^{18}O$) as a measure of time averaged transpiration rate. Journal of Experimental Botany. 422(56):3033-3039

Sheshshayee, M.S., Bindumadhava, H., Rao, N. R., Prasad, T.G., Udayakumar, M., Wright, G.C. and Nigam, S.N., 2006. Leaf cholorophyll concentration relates to transpiration efficiency in Peanut. Annual of Applied Biology. 148: 7-12

Sheshshsayee, M.S., Bindumadhava, H., Ramesh, R., Prasad, T.G. and Udayakumar, M. 2010. Relationship between oxygen isotope enrichment ($\Delta^{18}O$) in leaf water, biomass stomatal conductance. Isotopes in Environment and Health Studies. 46(1): 122-129

Štastná, M., Eitzinger, Z., Žalud, J. and Dubrovský, M. 2002. A comparison of water stress in particular wheat growth stages under the present (1x CO_2) and modified (2xCO_2) climate. (Eds. J. Rožnovský, and T. Litschmann). XIV.

Czech-Slovak Conference Bioclimatology, Lednice in Moravia, 2 to 4 September

Stiller, W.N., Read, J.J., Constable, G.A. and Reid, P.E. 2005. Selection for water use efficiency traits in cotton breeding program cultivar difference. Crop Science. 45:1107-1113.

Tanner, C.B. and T.R. Sinclair. 1983. Efficient water use in crop production: Research or re-research? p.1–27. In H.M. Taylor et al. (Ed.) Limitations to efficient water use in crop production. ASSA, CSSA, SSSA, Madison, WI.

Udayakumar, M. and T.G. Prasad. 1994. ^{13}C isotope discrimination in plants- A potential technique to determine WUE. In: Rao, R.C.N. and Wright G.C. (Eds.), Selection for WUE in Grain Legumes, pp. 42–45. Report of Workshop held at ICRISAT. Andhra Pradesh, India 5-7 May

Udayakumar, M., Rao, R.C.N., Wright, G.C., Ramaswamy, G.C., Gangadhar, G.C. and Hussain, A. 1998a. Measurement of transpiration efficiency in field condition. Journal of Plant Physiology and Biochemistry. 1:69-75

Udayakumar, M., Sheshshayee, M.S., Nataraj, K.N., Bindumadhava, H., Devendra, R., Hussain, A. and Prasad, T.G. 1998b. Why breeding for water use efficiency has not been successful. An analysis and alternate approach to exploit this trait for crop improvement. Current Science. 74: 994-1000

White, D.S., Bell, M.J. and Wright, G.C. 1996. The potential to use carbon isotope discrimination as a selection tool to improve water use efficiency in soybean. In: Michalk, D.L. and Pratley, J.E. (Eds.) "Agronomy - Science with its sleeves rolled up". Proc. of the 8th Australian agronomy conference, 30 January – 2 February. The University of Southern Queensland, Toowoomba, Queensland

Yu, Z.H. and Kohel, R.J. 1999. Cotton genome mapping and applications. In Proceedings from the Plant and Animal Genome Conference VII, pp. 60. San Diego, CA

Zacharison, M.H., Brick, A.G., Fisher, J.B., Ogg, J.B. and Ehleringer, J.R. 1999. Relationships between productivity and carbon isotope discrimination among dry bean lines and F2 progeny. Euphytica. 105:239-250

تقييم التباين المظهري لاستخدام الماء وكفاءة استخدام الماء اعتمادا على المحددات الفيزيولوجية في القطن (*Gossypium hirsutum* L.)

إيهاب أبو خير

قسم التقانات الحيوية، مركز بحوث السويداء، الهيئة العامة للبحوث العلمية الزراعية

السويداء، سوريا

الملخص:

تقع مساحات واسعة مزروعة بالقطن تحت ظروف الهطول المطري المحدود. مما فرض دخول هذا المحصول ذي الأهمية الاقتصادية الكبيرة في العديد من البرامج التربوية لتحسين قدرته على تحمل الإجهاد الجفافي. وذلك بالبحث وتقييم الاختلافات للعديد من الصفات خاصة الجذور، كفاءة استخدام الماء وغيرها من الصفات الفيزيولوجية التي لها أهمية في تحسين التحمل للإجهاد المائي. يهدف هذا البحث إلى تقييم الاختلافات الوراثية لبعض صفات تحمل الإجهاد الجفافي وتأسيس استخدام نظائر الكربون والأوكسجين المستقرة كبدائل لكفاءة استخدام الماء والنتح على الترتيب.

زرعت ثلاث عشرة سلالة محسنة من القطن (F7) في أصص سعتها 30 كغ تربة. وبعد ترسيخ البادرات (50 يوم بعد الزراعة) قيمت الصفات الفيزيولوجية التي تؤثر على كفاءة استخدام الماء (WUE) وتراكم الكتلة الحيوية خلال المرحلة الخضرية من النمو (من 50 إلى 80 يوم من الزراعة) في مزرعة قسم فيزيولوجيا المحاصيل - جامعة العلوم الزراعية، بانغلور، الهند. حيث لوحظ تباين وراثي معنوي في صفات المساحة الورقية الفعالة في عملية التمثيل الضوئي (LAD) وكمية الكتلة الحيوية الجافة وكمية الماء المفقود بالنتح (CWT).

أبدت الكتلة الحيوية الجافة ارتباطا معنويا مع كل من المساحة الورقية وكمية النتح وليس مع كفاءة استخدام الماء. اعتمادا على العلاقات ما بين كفاءة استخدام الماء وكل من المعدل الوسطي للنتح (MTR) والمعدل الصافي للتمثيل الضوئي (NAR) وجدنا أن كفاءة استخدام الماء في السلالات المدروسة تخضع لتحكم مسامي قوي. كما كشفت نتائج النظائر المستقر لكل من الكربون والأوكسجين عن تأثير ضعيف للتمثيل الضوئي على كفاءة استخدام الماء.

أثبتت النتائج بأن هناك إمكانية لانتقاء بعض السلالات ذات كفاءة تمثيل ضوئي عالية للصانعات الخضراء من بين هذه السلالات المدروسة والتي تنتمي للنمط ذي التحكم المسامي. وعليه يمكن استخدام نهج النظائر المزدوجة (كربون وأوكسجين) بوصفها استراتيجية مفيدة للغاية في تحسين محصول القطن.

الكلمات المفتاحية: القطن، كفاءة استخدام الماء، نسبة التميز النظيري للكربون ^{13}C المستقر ، نسبة الوفرة النظيرية للأوكسجين ^{18}O المستقر.