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Comparative water-use efficiency of *Sporobolus arabicus* and *Leptochloa fusca* and its relation with carbon-isotope discrimination under semi-arid conditions

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Abstract

Water-use efficiency (WUE) of *Leptochloa fusca* (L.) Kunth (Kallar grass) and *Sporobolus arabicus* Boiss. was determined under different soil moisture regimes. Plants grown in lysimeters were subjected to three soil moisture regimes, viz. well-watered (100%), medium-watered (75%), and low-watered (50%) of total available water (TAW). The soil moisture was restored on alternate days by adding the required volume of water on the basis of neutron moisture meter readings taken from neutron access tubes installed in each lysimeter. The grasses were harvested after suitable intervals (~4 months) to obtain maximum biomass. Leaf samples collected at each harvest were analyzed for carbon-isotope discrimination (δ^{13}C) with an isotope ratio (^{13}C/^{12}C) mass spectrometer. Results indicated significant differences in WUE of both grasses subjected to different water regimes. *Sporobolus arabicus* showed higher WUE than Kallar grass. However, Kallar grass showed better value of yield response factor (k_y = 0.649) compared with *Sporobolus* (k_y = 1.06) over the entire season. The data confirm that these grasses can be grown successfully in water-limited environments by selecting an optimum soil moisture level for maximum biomass production. The mean carbon-isotope discrimination (δ^{13}C) of Kallar grass (−14.4‰) and *Sporobolus* (−12.8‰) confirm that both are C_4 plants. The carbon-isotope discrimination (Δ) was significantly and negatively correlated with WUE of the two species studied. The results of the present study confirm that δ^{13}C or Δ of leaves can be used as good predictor of WUE in some C_4 plants.

Introduction

Selection of plants of high water-use efficiency (WUE) is desirable to improve crop production in water-limited environments. The information on screening of water-use efficient plants is, however, limited due mainly to non-availability of fast screening techniques. Variation in ^{13}C/^{12}C ratio in C_3 and C_4 plants occurs during the fixation of CO_2 due to carbon isotope fractionation attendant on differential stomatal diffusivities of $^{13}CO_2$ and $^{12}CO_2$ (Farquhar et al., 1982). The variation in fractionation is due to many factors such as plant types, genotype, environment, etc., and has been used to study a variety of issues (Farquhar et al., 1989; O'Leary, 1981, 1988; Rundel et al., 1989; Vogel, 1980). Farquhar et al. (1982) have shown that δ^{13}C of plant material depends upon the weather and soil moisture conditions experienced by plant during growth. They reported a positive relationship between WUE and δ^{13}C of plant material and suggested a simple method for assessing WUE under field conditions.

Hubick et al. (1986) reported that plants grown under water stress exhibited higher WUE and δ^{13}C values compared to plants grown under adequate moisture conditions. Ehleringer and Cooper (1988) showed that plants growing along a soil moisture gradient (under water stress) had higher δ^{13}C values. The

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plant C isotope ratio varies due to moisture during growing season and $\delta^{13}C$ of the crop largely reflects changes in the available soil moisture (van Kessel et al., 1994). Richards and Condon (1993) summarized various studies indicating negative relationships between $\delta^{13}C$ and water-use efficiencies. A number of workers have related $\delta^{13}C$ of plants to water-use or transpiration efficiency and plant productivity (Farquhar et al., 1989; Virgona et al., 1990). Henderson et al. (1998) also found a relationship between transpiration efficiency and $\delta^{13}C$ in C_4 species, *Sorghum bicolor*. The variation in $\delta^{13}C$ has also been used as a tool to develop crops better adapted to water-limited environments (Hall et al., 1989; Hubick et al., 1988; White, 1993).

The use of isotope discrimination in screening of high WUE plants has advantages over the conventional techniques. The $\delta^{13}C$ is an integrated value over plant life, only small sample of dry matter is required, measurement is precise with coefficient of variation 2% and $\delta^{13}C$ can be measured at any stage of plant life. *Leptochloa fusca* (Kallar grass) and *Sporobolus arabicus* are included in the list of plants selected for extensive cultivation on salt-affected lands under biological approach (Malik et al., 1986). Shortage of irrigation water is considered one of the main factors responsible for low plant productivity in Pakistan. The scarcity of irrigation water demands that water should be applied according to the plant water requirements. The present studies were conducted to determine/compare the WUE of Kallar grass and *Sporobolus arabicus* at different water regimes and explore relationship between $\delta^{13}C$ and WUE of these two grasses under semi-arid conditions.

Materials and methods

The study was conducted at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad (31°2′N, 73°05′E), Pakistan. The area is semi-arid and characterized by large seasonal variations of both temperature and rainfall. During winter the temperature generally ranges from 5 to 18 °C and during summer it ranges from 20 to 47 °C. The average annual rainfall based on 30 years observations is 250 mm. The rainfall occurs mainly in March–April and July–August. The soil removed in bulk up to 1 m depth from a highly salt-affected area was air dried and passed through a 2-mm sieve. Soil texture was determined by sedimentation technique developed by Jennings et al. (1922) as described by Day (1965). Electrical conductivity (EC) and pH of irrigation water and saturated paste extracts of soil were determined by WTW conductivity meter LF-530 and Corning pH meter 130, respectively. Water and soil saturation extracts were analyzed for Na^+ and K^+ with a flame photometer (Model PFP7 Jenway). Ca^{2+}+Mg^{2+} and anions ($CO_3{}^{2-}$, $HCO_3{}^-$ and Cl^-) were determined by titration (U.S. Salinity Laboratory Staff, 1954) and $SO^{2-}{}_4$ by turbidimetry (Cunniff, 1995).

The water retained by the soil at different pressures (0.03 and 1.5 Mpa) was determined using pressure membrane apparatus by placing the soil in plastic rings. The water retained by the soil at field capacity was also confirmed *in situ* with neutron moisture meter readings after free drainage of 48 h. The soil was filled in 24 cemented lysimeters in equal weight. The lysimeters (1 × 1 × 1 m), called ‘plots’ here-after have been constructed deep in the soil with ridges 10 cm above the soil surface. The soil resumed natural conditions after 4 weeks with three irrigations and attained average bulk density ~1.4 Mg m^{-3}. In the center of each plot neutron moisture access tube was installed down to the bottom. The neutron moisture meter was calibrated in the same soil before the start of experiment by field method (Akhter et al., 1995). Two soil moisture tensiometers, one at 15 cm and other at 50 cm depth, were also installed in each plot.

Equal number of root stubbles of *Leptochloa fusca* (L.) Kunth (Kallar grass) and *Sporobolus arabicus* Boiss. were transplanted each in nine plots. Six plots were kept unplanted. During the first 3 months ~75 mm flood irrigation was applied to each plot as and when required on the basis of visual observation until the grasses established uniform cover.

Three replicate plots of each grass were randomly subjected to three water regimes (well-watered, medium-watered and low-watered). In well-watered treatment, the soil was kept at 100% of total available water (TAW), under medium-water treatment at 75% of TAW and in low-watered treatment at 50% of TAW. Total available water was determined by using the relation:

$$\begin{aligned}\text{TAW} &= \text{Soil moisture at 0.03 Mpa (FC)} - \\ &\quad \text{Soil moisture at 1.5 Mpa (PWP)},\end{aligned}$$

where, FC and PWP refer to field capacity and permanent wilting point, respectively. The amount of water required for each plot to maintain the respective soil water regime was estimated on the basis of readings from the neutron moisture meter (NMM). The tensiometer readings were used as an alternate check to confirm the soil moisture status. Generally the water regimes were restored on alternate days except for few weeks of rainfall periods during the year. The volume of water required for each plot was added through a locally prepared irrigation system including a water pump, a calibrated plastic water tank, fixed pipes and taps, etc. The rainfall during the experimental period was also recorded.

The grasses were harvested after suitable intervals (~4 months) to obtain maximum biomass. Fresh and dry biomasses of plants were determined. Composite samples of leaves collected at each harvest were dried and preserved for isotopic (δ^{13}C) analysis. The experiment was continued for 1 year. The total above-ground dry biomass and total water applied during 12 months were used to calculate the water-use efficiency (WUE) using the following relation:

$$\text{WUE} = \text{Total dry matter} / \text{Total water consumed}$$

Yield response factor k_y was calculated for each treatment over the whole growth period using the following relation:

$$1 - Y_a/Y_m = k_y(1 - \text{ET}_a/\text{ET}_m),$$

where Y_a is the actual yield, Y_m is maximum yield, ET_a is the actual evapotranspiration and ET_m is the maximum evapotranspiration (FAO, 1986). The isotopic ratios ($R = {}^{13}\text{C}/{}^{12}\text{C}$) of plant sample ($R_{\text{sample}}$) and of standard ($R_{\text{standard}}$) were determined using a ratio mass spectrometer. The R values were converted to δ^{13}C using the relation:

$$\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000‰$$

The standard is the carbon dioxide obtained from 'PDB' a limestone from Pee Dee Belmenite formation in South Carolina, USA and was provided by International Atomic Energy Agency (IAEA), Vienna, Austria. The δ^{13}C values were converted to Δ values using the relation:

$$\Delta = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_p)/(1 - \delta^{13}\text{C}_p/1000)‰,$$

where a and p represent air and plant, respectively. To convert δ^{13}C values to Δ values −8.00‰ for air (Keeling et al., 1979) was substituted in these studies. Data were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) for multiple comparison of paired means of treatments (Steel and Torrie, 1980). Simple linear regression analysis was also carried out. The regression coefficient (b) and correlation coefficient (r) were verified at the $p \leq 0.05$ levels.

Results

The soil was highly saline sodic (EC 1.25 Sm^{-1}; pH 8.5; SAR 60) non-gypsiferous sandy loam. Some selected soil properties and chemical composition of irrigation water used to grow grasses are presented in Table 1. Generally the highest biomass yield in both grasses was obtained under well-watered treatment (100% TAW) compared with medium- (75% TAW) and then low-watered plants (Table 2). The largest biomass reduction (38.2%) in *Sporobolus* was observed at low-watered treatment (50% TAW) followed by only 2.9% reduction at medium-water treatment compared with well-watered plants. Similar trend in biomass yield reduction of 31 and 4.6% in Kallar grass was observed for low- (50% TAW) and medium-watered (75% TAW) plants, respectively, compared with well-watered (100% TAW) plants. Total water consumed by both the grasses decreased with decrease in TAW of soil. Kallar grass used slightly more water compared to *Sporobolus* at well- and medium-watered conditions (Table 2).

Both the grasses exhibited significant differences in WUE at different water treatments (Table 3). *Sporobolus* showed significantly higher mean WUE under all water treatments compared with Kallar grass. *Sporobolus* indicated highest WUE (1.59 $\text{g m}^{-2}\ \text{mm}^{-1}$) under medium-water followed by well-watered and low-watered treatments. The WUE of Kallar grass increased with decrease in TAW (Table 3) and highest WUE was observed under low-water treatment.

In general, a decrease in total water used (TWU) decreased the yield of both the grasses linearly. The decrease in TWU showed a linear effect ($r = 0.96$) on Kallar grass yield by a decrease rate of 0.642 g mm^{-1} over the whole season (Yield = 0.642TWU+804.9). *Sporobolus* yield also decreased linearly ($r = 0.94$) with decrease in TWU at the rate of 1.783 g mm^{-1} (Yield = 1.783TWU − 786.5). The amount of water saved and corresponding yield reduction under low- and medium-water compared with well-water grasses are given in Table 4. Data indicated that by growing *Sporobolus* under medium-water, 13% water saving was achieved with 2.9% yield reduction.

Table 1. Some characteristics of soil and irrigation water used. FC refers to soil field capacity and PWP refers to permanent wilting point.

Parameter	Soil	Irrigation water
Sand (%)	68	-
Silt (%)	18	-
Clay (%)	14	-
Texture	Sandy loam	-
Moisture at FC ($cm^3\ cm^{-3}$)	29	
Moisture at PWP ($cm^3\ cm^{-3}$)	13	
Electrical conductivity (Sm^{-1})	1.25	0.12
pH	8.5	7.7
Sodium adsorption ratio	60	3.9
Ca^{2+}+Mg^{2+} (meq L^{-1})	8.8	5.4
Na^+ (meq L^{-1})	126	6.4
K^+ (meqL^{-1})	8.4	0.1
$CO_3{}^{-2+}$ (meq L^{-1})	0.1	Nil
$HCO_3{}^{2-}$ (meq L^{-1})	2.5	6.9
Cl^- (meq L^{-1})	50	1.8

Table 2. Dry biomass yield (g) and total water used (mm) by *Sporobolus arabicus* and Kallar grass grown at different soil moisture regimes: percent total available water (TAW). Values are means of three replicates.

TAW	*Sporobolus*		Kallar grass	
	Biomass	Water consumed	Biomass	Water consumed
100%	3922a	2976a	2635a	2752a
75%	3809a	2450b	2513a	2394b
50%	2420b	1660c	1817b	1873c

Means followed by different letters differ in column significantly at $p \leq 5\%$ level.

Table 3. Water-use efficiency of *Sporobolus arabicus* and Kallar grass as a function of total available water (TAW). Values are means of three replicates.

TAW (%)	Water-use efficiency ($g\ m^{-2}\ mm^{-1}$)		
	Sporobolus	Kallar grass	Mean (TAW)
100	1.425 b	0.885 b	1.155 b
75	1.591 a	1.028 a	1.309 a
50	1.292 c	1.095 a	1.193 b
Mean(species)	1.436A	1.002B	

Values followed by different letters in a column differ significantly at $p \leq 5\%$ level. Letters A and B following values in the last row indicate significant differences between overall mean WUE of the two species.

Table 4. Amount of water saved and corresponding reduction in biomass yield of *Sporobolus arabicus* and Kallar grass grown under medium- and low-water treatments.

Parameter	*Sporobolus*		Kallar grass	
TAW*	50%	75%	50%	75%
Water saved (%)	31.9	13	44.2	17.6
Yield reduction (%)	38.2	2.9	31	4.6

*TAW = total available water

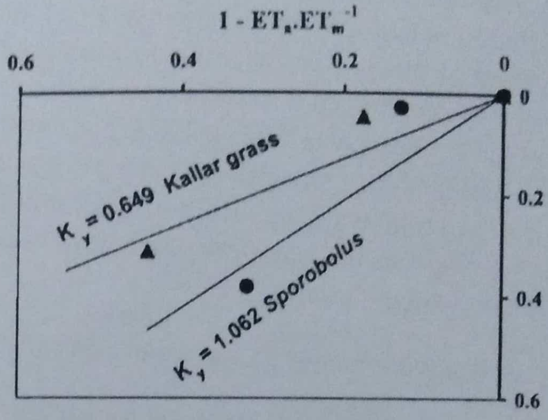

Figure 1. Relationship between relative yield decrease and relative decrease in water used by *Sporobolus arabicus* (●) and Kallar grass (▲).

In Kallar grass 17.6% water saving was noted with 4.6% yield reduction (Table 4). Kallar grass showed better value of yield response factor ($k_y = 0.649$) compared to *Sporobolus* ($k_y = 1.06$) over the entire season (Figure 1).

Carbon-isotope discrimination indicated that *Sporobolus*, with mean $\delta^{13}C$ value −12.37‰, and Kallar grass, with mean $\delta^{13}C$ value −14.38‰, are C_4 plant types (Table 5). In the present study, growing C_4 species at different soil moisture levels (TAW) caused a considerable variation in $\delta^{13}C$ of plant tissues (leaves). The $\delta^{13}C$ values of *Sporobolus* grown at 50% TAW varied by 4.16% and of Kallar grass by 3.53% compared to respective values for 100% TAW treatment (Table 5). Data revealed that carbon-isotope discrimination (Δ) in both the grasses was linearly and negatively correlated with water-use efficiency. The WUE of Kallar grass had a significant negative correlation with Δ (WUE = −0.189Δ + 2.219) with high value of correlation coefficient ($r = -0.917^{**}$). The WUE of *Sporobolus arabicus* increased with decrease in Δ (WUE = −0.305 Δ + 2.912) with high value of correlation coefficient ($r = -0.801^{**}$). The combined regression analyses between WUE and Δ of both the

Table 5. Carbon-isotope discrimination ($\delta^{13}C$; ‰) in leaves of *Sporobolus arabicus* and Kallar grass grown under different soil moisture regimes. Values are means of three replicates.

TAW* (%)	Carbon-isotope discrimination ($\delta^{13}C$/‰)	
	Sporobolus	Kallar grass
100%	−12.71	−14.71
75%	−12.88	−14.24
50%	−12.18	−14.19
Mean	−12.37	−14.38
Type	C-4	C-4

*TAW = total available water

$\delta^{13}C$ (‰) values for different plant types are: $C_4 = -9$ to -16; $C_3 = -22$ to -34 and CAM $= -16$ to -22 (Vogel, 1980).

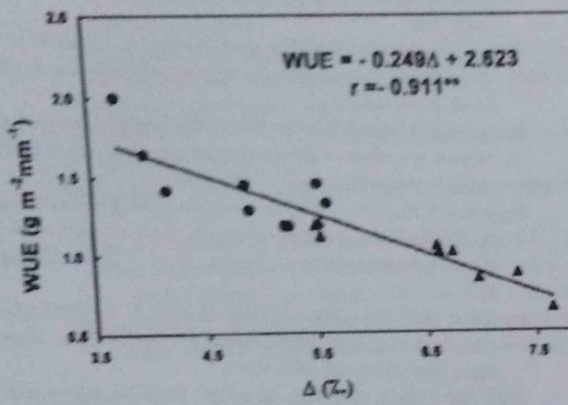

Figure 2. Relationship between water-use efficiency (WUE) and carbon-isotope discrimination (Δ (‰)) for combined data of *Sporobolus arabicus* (●) and Kallar grass (▲).

grasses (Figure 2) indicated that Δ decreased with increase in WUE with high *r* (−0.831**).

Discussion

The results of present study indicated that in both species (*Sporobolus* and Kallar grass) the biomass yield depends upon the soil TAW and yield is positively correlated with total water taken up by the plants at different soil moisture levels (Table 2). Soil moisture stress reduces the nutrient uptake in plants (Ashraf et al., 1992; Mbagwe and Osuigwe, 1985). Reduction in plant nutrient uptake occurs due to decrease in transpiration, impaired active transport and membrane permeability and decrease in diffusive flow of nutrients from soil matrix to root surface (Ashraf et al., 1998).

The concept of water-use and its importance in relation to plant productivity is now well recognized (Anonymous, 1993). In *Sporobolus* the highest WUE was observed in medium-watered plants while in Kallar grass highest WUE was recorded under low-water treatment. Both grasses responded differently to soil moisture deficit. It is well established now that plants adapt differently to low soil moisture conditions and several species develop tolerance to water deficit by osmotic adjustment in roots and leaves (Belhassen, 1995; Riga and Vartanian, 1999). Belhassen (1995) reported that to maintain high hydric potential under dry conditions or to resist the effects of low water potential plants involve physiological or phenotypical and morphological mechanisms.

Yield response factor is considered an important component of the evapotranspiration function that provides a useful means for defining plant requirements and thus better water management (IAEA, 1996). Kallar grass showed better adaptation with a yield response factor (K_y = 0.649) indicating least yield reduction compared with *Sporobolus* (K_y = 1.06) under water deficit conditions (Figure 1). Medium-water treatment (75% TAW) proved more suitable for obtaining optimum biomass production of the two grasses with water saving of 13% for *Sporobolus* and 17% for Kallar grass. However, under 50% TAW treatment Kallar grass performed better as more water was saved with a lower corresponding yield loss as compared with *Sporobolus* (Table 4).

It is generally accepted that C_3 plants grown under water stress become more water-use efficient and exhibit higher values of $\delta^{13}C$ than plants grown under adequate moisture conditions (Hubick et al., 1986). Farquhar et al. (1982) reported that water-use efficiency was positively correlated to $\delta^{13}C$ of the plant. The $\delta^{13}C$ values will tend to increase (less negative) and conversely, the Δ values will decrease (less positive) as the WUE of C_3 plants increases. Both grasses under study are C_4 plants and showed considerable variation in $\delta^{13}C$ values of leaves grown under different soil moisture levels. Significant negative correlations were observed between Δ and WUE of each plant species separately and in a combined (pooled) data (Figure 2). Van Kessel et al. (1994) reported that ^{13}C discrimination is species dependent and C isotope composition of the plant also varies as a function of plant-available water. The results of the present study are in agreement with the earlier findings (Ehleringer, 1993; Ehleringer et al., 1992; Hubick et al., 1986; Johnson and Tieszen 1993; White, 1993).

Many other studies on grasses (Johnson et al., 1990; Read et al., 1991), wheat (Ehdaei et al., 1991; Farquhar and Richards, 1984; Kirda et al., 1992), cowpea (Abdelbagi and Hall, 1993), cotton (Hubick and Farquhar, 1987), tomato (Martin and Thorstenson, 1988), and upland rice (Dingkuhn et al., 1991) have shown a negative correlation between ^{13}C discrimination and field water-use efficiency. Meinzer et al. (1990) suggested the use of Δ as a screening tool for annual crops of high water-use efficiency for high crop yield under drought conditions. C_3 plants which are more enriched in ^{13}C will have greater water-use efficiency under particular site conditions (Farquhar et al., 1982). The discrimination is a more complex phenomenon in C_4 species as the CO_2 concentrating mechanism is sensitive and leakiness of bundle sheath cells is apparently not stable under drought stress conditions (Williams et al., 2001). Henderson et al. (1998) have demonstrated the usefulness of ^{13}C discrimination as an indicator of water-use efficiency in C_4 species *Sorghum bicolor* under certain environmental conditions. The results of the present study confirm that $\delta^{13}C$ or Δ value of plants can be used to predict WUE of the plants from the equations developed from the pooled data within similar plant types. Screening of more C_4 species is desirable in this context.

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