*Pak. J. Bot.*, 40(4): 1441-1454, 2008.

# RELATIONSHIPS BETWEEN CARBON ISOTOPE DISCRIMINATION AND GRAIN YIELD, WATER-USE EFFICIENCY AND GROWTH PARAMETERS IN WHEAT (*TRITICUM AESTIVUM* L.) UNDER DIFFERENT WATER REGIMES

**JAVED AKHTER*, S. ALI SABIR, ZAHID LATEEF[1] M. YASEEN ASHRAF AND M. AHSANUL HAQ**

*Nuclear Institute for Agriculture and Biology (NIAB), P.O. Box 128, Jhang Road, Faisalabad 38000, Pakistan. Email of corresponding Author (akhjniab@yahoo.com)*
*[1]Pakistan Institute of Nuclear Science and Technology, Islamabad 45650, Pakistan*

**Abstract**

The present studies were conducted to identify high yielding wheat genotypes for target environments and establish relationship between carbon isotope discrimination (Δ), grain yield (GY) and water use efficiency (WUE), and other parameters. A set of eight wheat genotypes screened previously for variation in Δ and higher GY were grown under four water regimes; well-watered (WW), medium-watered (MW), low-watered (LW) and stored soil moisture (SSM) conditions. Early leaf and grain samples collected at maturity were analysed for Δ. Plant parameters, such as number of tillers (NT), plant height (PH) heading days (HD), and maturity days (MD) were recorded. At harvesting spike length (SL), number of grains per spike (NGPS), thousand grains weight (TGW), biomass yield (BY), GY, harvest index (HI) and WUE on biomass basis ($WUE_B$) and grain basis ($WUE_G$) were determined. Significant effects of genotype and treatments on Δ of leaf (ΔL) and grain (ΔG), BY, GY, HI, $WUE_B$, $WUE_G$, HD, NT, PH, NGPS, TGW and SL were observed. Genotype x treatment interaction had a significant effect on HI, PH, SL, TGW, HD and MD, but the effect was non-significant on other traits. In all these genotypes ΔL and ΔG showed a variation of 1.3 and 0.91‰, respectively. All genotypes exhibited higher ΔL than ΔG under different water regimes. Water stress reduced both ΔL and ΔG and highly significant correlation (0.946**) was found between ΔL and ΔG. GY showed a wide variation among these genotypes and water stress resulted in a marked decrease in GY. Genotype Sitta produced highest mean GY (4.4 Mg $ha^{-1}$) with highest $WUE_G$ (16.99 kg $ha^{-1}$ $mm^{-1}$) averaged across the treatment. GY showed significant positive correlations with ΔL (r=0.779*) and ΔG (r=0.753*). GY was also strongly and positively correlated with HI (r=0.845**), SL (r=0.779**) and TGW (r=0.899**). GY had a significant negative correlation with NT (r=-0.884*) and HD (r=-0.708*). $WUE_G$ was positively correlated with ΔL (r=0.846*), ΔG (0.707*), HI (r=0.846**), SL (r= 0.784*), TGW (r=0.892**). $WUE_G$ was negatively correlated with NT (r=-0.814*) and HD (r= -0.743*). Sitta and FD-83 genotypes were found high yielder with greater increase in WUE under water stress and can be exploited to obtain high GY in rain-fed and water limited environments of the country. The results highlight significant positive correlations between Δ and GY or $WUE_G$ in bread wheat and carbon isotope discrimination as indirect selection criterion for grain yield in Pakistan.

## Introduction

Organic matter in plants is depleted in $^{13}C$ with lower $^{13}C/^{12}C$ ratio compared to atmospheric $CO_2$ (Craig, 1954; Bender, 1968). The magnitude of depletion depends upon

* Corresponding Author: javedakhterniab@yahoo.com

the photosynthetic pathways of $CO_2$ fixation (Smith & Epstein, 1971). Plants exhibiting $C_3$ pathways are the most depleted and plants endowed with $C_4$ pathways are the least depleted, while plants with crassulacean acid metabolism shows intermediate value (Bender *et al*., 1973). The difference in ratio ($^{13}C/^{12}C$) between $C_3$ and $C_4$ is correlated with isotopic fractionation present between the ribulose biphosphate carboxlase (RuBP) activity in $C_3$ plants and phosphoenolpyruvate (PEP) carboxylase activity in $C_4$ plants. RuBP discriminates more against $^{13}C$ than PEPEC (Christeller *et al*., 1976). Parameters used to characterize carbon isotope discrimination in plants are carbon isotope composition (δ) and carbon isotope discrimination (Δ). δ is calculated as $\delta^{13}C(‰)=[(Rsample/Rstandard -1) \times 1000]$, R is $^{13}C/^{12}C$ ratio and has negative values. Δ is calculated using formula (Farquhar *et al.* 1989): $\Delta(‰)=[(\delta a-\delta p)/(1+ \delta p)]x1000$. where δp is $\delta^{13}C$ of samples and δa, the $\delta^{13}C$ of atmospheric $CO_2$, -8‰. δ varies from -22 to -38‰ in $C_3$ plants and from -8 to -15‰ in $C_4$ plants (Yeh & Wang, 2001).

Transpiration efficiency (TE), referred as intrinsic WUE (Farquhar *et al*. 1989), can be evaluated at leaf level as the ratio of $CO_2$ exchange rate to transpiration (Morgan *et al*. 1993) or the ratio of biomass produced to transpiration. The physiological basis for Δ variation in $C_3$ plants is related to the variation in the internal $CO_2$ concentration ($C_i$) to ambient $CO_2$ concentration ($C_a$) ratio. High Δ values resulting from high $C_i/C_a$ reflect higher $CO_2$ assimilation rate to transpiration ratio (Farquhar *et al*., 1989), *i.e.*, lower TE. In wheat, grain Δ was found to be positively correlated with yield (Kirda *et al*., 1992; Condon & Richards, 1993; Araus *et al*. 1998). The positive correlation of GY with Δ in several $C_3$ species (Merah *et al*., 2001; Monneveux *et al*., 2006) lead to a positive correlation between Δ and WUE under same water availability for all genotypes. Wheat grain yield was found to be positively related to stem Δ under conditions of South Australia (Condon *et al*., 1987) and grain Δ in Syria (Araus *et al*. 1997), France (Merah *et al*. 2001), Greece (Tsialatas *et al*. 2001), Spain (Araus *et al*. 2003). In wheat, under post-anthesis water stress a positive correlation of GY with grain Δ in India (Misra *et al*. 2006) and strong positive correlation of GY with both leaf and grain Δ in China (Xu *et al*., 2007) have been observed.

Wheat is one of the major sources of food in many countries and being staple diet, it is the most important crop in Pakistan. It is grown in every part of the country in irrigated as well as in rainfed areas. Country has been divided into 10 agro-ecological zones : Indus Delta, southern irrigated plain, sandy desert, northern irrigated plains, Barani (rainfed) areas, wet mountains, northern dry mountains, western dry mountains, dry western plateau and the Sulaiman Piedmont (PARC 1980). The precipitation during the growing season is inadequate and varies greatly, both within the growing season and from year to year. Average yield per hectare of major crops including wheat is the lowest in the world (Hafizullah, 2002).

According to Passioura (1977, 1996) higher crop performance may be achieved through improvements in water use, water-use efficiency and harvest index. Water use is relevant when there is still soil water available at maturity or when deep-rooted genotypes access water deep in the soil profile that is not normally available. The other two factors become more significant when all available water is normally used up by the end of the crop cycle. The single most important attribute under water stress tackled so far has been phenology, matching crop development and seasonal rainfall patterns (Slafer & Araus, 1998; Villegas *et al*., 2000; Khan *et al*., 2007) which may affect either water use, or the efficiency of its utilization.

Selection of plants of high GY or WUE is desirable to improve crop production in water limited environments. Therefore, screening for high GY/ or water use efficient wheat genotypes is imperative to enhance wheat yields under different water environments. The information on screening of high GY or WUE is, however, limited due mainly to non-availability of fast screening techniques. The objective of the present study was to evaluate the potential of Δ as indirect selection criterion for bread wheat grain yield under different water regimes.

## Material and Methods

**Plant material:** Eight wheat (*Triticum aestivum L.*) genotypes used in this study were screened previously for variation in Δ and higher grain yield (Table 1). These genotypes were selected out of dozens obtained from local sources and CIMMYT at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. First, six (1-6 Table 1) were selected out of 71 and two (7-8) out of 45 in years 2004 and 2005, respectively by growing under well watered conditions. These were selected due to their high grain yield and large variation in leaf and grain Δ among these genotypes.

**Experimental conditions:** The study was conducted during the season 2006-7 at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad (31°2´ N, 73°05´ E), Pakistan. The climate in Faisalabad is semi-arid and characterized by large seasonal variations for both temperature and rainfall. The average monthly temperature ranges from 5-18 °C during winter and 20-47 °C during summer. The average annual rainfall, based on 30 years observations, is around 250 mm. Rainfall occurs mainly in March-April and July-August. Total rainfall recorded during the growth cycle 206 mm in year 2004. Plants were grown in cemented lysimeters (5m x 5m x 1m) in sandy clay loam (45% sand, 33% silt and 22% clay) soil (fine-loamy, mixed, hyperthermic, Udic Halustepts, Inceptisols; FAO) originated from the NIAB experimental field. The soil had been filled since long and had an average bulk density of 1.4 $gcm^{-3}$. Soil had an electrical conductivity (EC) 1.5$dSm^{-1}$, pH 7.6 and sodium adsorption ratio (SAR) of 1.36. In four lysimeters all genotypes were sown randomly in three replicate. Three live seeds per hill were sown in 5 rows with 20 cm row spacing and interplant space of 10cm adjusting seeding rate of 150 seeds $m^{-2}$. The sowing was done on 23 November 2006 and harvesting was carried out in 3rd week of April 2007.

Each lysimeter contained three PVC access tubes installed down to the bottom for soil water assessment using neutron moisture meter (NMM). The soil moisture before the start of experiment and after harvesting was estimated on the basis of readings recorded with NMM. The readings with NMM (503 Model CPN, USA) were taken at prefixed depths of 15, 25, 50 and 75 cm as and when required The readings were converted to volumetric water content using the equation $\theta_V$=0.389n+0.02, where $\theta_V$ is volumetric water content, n is count rate ratio = observed counts/standard counts taken with NMM. One pre-sowing irrigation was applied to each lysimeter. Lysimeters were randomly selected to impose pre-selected water treatments including well water (WW), medium water (MW), low water (LW) and no irrigation was applied in stored soil moisture (SSM) treatment. In WW, the soil was kept at 100% of total available water (TAW), under MW at 75% of TAW and in LW treatment at 50% of TAW. Required volume of water for each lysimeter was added through a locally fabricated irrigation system including a water pump, fixed pipes, water flow meters and taps, etc. Total water consumed was determined by adding water applied by irrigation and rainfall recorded during the course of study. The water used for irrigation had electrical conductivity of 0.76 $dSm^{-1}$, pH: 7.5 and SAR: 2. Fertilizer N urea was applied @ 120 kg $ha^{-1}$ to all treatments. Weeds were removed manually as and when required.

**Measurements:** The number of days to flowering was registered when at least 50% of spikes of a given plot reached anthesis counted from the day of sowing. Parameters including grain yield, above-ground biomass, plant height, number of tillers, spike length and number of grains per spike were recorded at physiological maturity. The physiological maturity was assumed when 90% of seed changed color from green to yellowish and stopped photosynthetic activity. Only fertile tillers were included in number of tillers. At maturity the central part of plot leaving the border rows was harvested by cutting the plants at ground level. The total dry matter included the oven dried stem, leaves, spike and grains at physiological maturity. The dry weight of the plot biomass was determined after drying a representative sample from each plot at 70°C to a constant weight and applying it to whole plot biomass. Number of grains and grain weight per spike were determined taking main spikes from replicated plants from each treatment. Average weight per grain was multiplied by 1000 to obtain thousand grain weight (TGW). Water use efficiency on biomass basis ($WUE_B$) and water-use efficiency on grain basis ($WUE_G$) were determined by dividing the biomass or grain yield by quantity of water applied (sum of rainfall and quantity of water added by irrigation) during the growth period by each genotype. Harvest index (%) was determined as the ratio of grain yield to above-ground biomass and multiplied by 100.

Carbon isotope discrimination (Δ) was analyzed on early leaf samples collected before water stress and grain samples collected at maturity. Leaf, samples were dried at 60°C for 48 hrs and ground to fine powder. The carbon isotopic ratio ($R={}^{13}C/{}^{12}C$) of the samples ($R_{sample}$) and standard ($R_{standard}$) was determined using an Isotope Ratio Mass Spectrometer (GD 150, MAT, Germany). R values were converted to $\delta^{13}C$ (in ‰ or per mil) using the relationship:

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

The standard is the $CO_2$ obtained from a limestone from Pee Dee Belmenite "PDB" formation in South Carolina, USA. The $\delta^{13}C$ values were converted to carbon isotope discrimination (Δ) values using the relationship established by Farquhar *et al.,* (1989):

$$\Delta\ (‰) = (\delta^{13}C_a - \delta^{13}C_p) / (1 - \delta^{13}C_p/1000)\ ,$$

where a and p represent air and plant, respectively. To convert $\delta^{13}C$ values to Δ values, -8.00 ‰ for air (Keeling *et al.*, 1979) was substituted in these calculations.

**Statistical analysis:** The data were subjected to the analysis of variance (ANOVA), using SAS, version 8.1. (SAS Institute 1987, Cary, NC, USA). The F test was used to identify treatment main effects and interactions followed by Duncan's Multiple range test at the 0.05 probability level.

## Results

There were significant effects of genotype and treatment on BY, GY, HI, WC and all agronomic traits. Treatment x genotype interaction effect was significant on HI, leaf and grain Δ, HD, MD, PH, SL and TGW and non significant on other traits (Table 2). Water stress resulted in marked decrease in BY. The extent of the decrease was dependent on genotype and degree of water stress. BY averaged across the treatments ranged from 13.4 $Mgha^{-1}$ (Bhittai) to 10.19 $Mgha^{-1}$ (Pfau) (Table 3). The results showed that the largest water stress-induced reduction in BY appeared in SSM. There was largest reduction in NT (62%) and PH (21%) under SSM compared to WW conditions. The genotype Sitta showed the highest (4.4 $Mgha^{-1}$) GY while Nesser exhibited the lowest GY (3.12 $Mgha^{-1}$).The highest GY was recorded under WW conditions (Table 2) and GY decreased with

**Table 1: Variation in grain yield (GY) and carbon isotope discrimination (Δ) among the eight wheat genotypes used in the study.**

| Genotypes | GY ($Mgha^{-1}$) | Leaf Δ (‰) | Grain Δ (‰) |
|---|---|---|---|
| **1. Sarsabz** | 4.644 | 21.20 | 19.46 |
| **2. NR-234** | 4.602 | 18.63 | 19.68 |
| **3. Nesser** | 4.206 | 19.38 | 19.45 |
| **4. Bhittai** | 4.100 | 22.19 | 20.28 |
| **5. NR-241** | 3.996 | 18.87 | 19.57 |
| **6. FD-83** | 3.983 | 21.42 | 18.84 |
| **7. Sitta** | 4.732 | 21.06 | 19.29 |
| **8. Pfau** | 4.759 | 21.90 | 19.60 |

**Table 2: Analysis of variance and mean values of agronomical traits of wheat genotypes under different water regimes, well watered (WW), medium- (MW), low- (LW) and stored soil moisture (SSM).**

| Parameters | T | G | TxG | WW | MW | LW | SSM |
|---|---|---|---|---|---|---|---|
| df | 3 | 7 | 21 | Mean values | | | |
| **Grain yield (Mg)** | ** | ** | ns | 4.73a | 4.43b | 3.67c | 2.91d |
| **Biomass yield (Mg)** | ** | ** | ns | 14.81a | 13.97b | 10.74c | 8.26d |
| **Harvest index (%)** | ** | ** | ** | 31.92b | 31.89b | 34.26a | 35.30a |
| **Water consumed (mm)** | ** | ** | ns | 405a | 329b | 252c | 138d |
| **$WUE_G$ ($kg\ ha^{-1}mm^{-1}$)** | ** | ** | ns | 11.68c | 13.47b | 14.54b | 21.08a |
| **$WUE_B$ ($kg\ ha^{-1}mm^{-1}$)** | ** | ** | ns | 36.59c | 42.52b | 42.76b | 59.85a |
| **Leaf Δ (‰)** | ** | ** | ** | 20.48a | 19.65b | 19.46c | 19.43d |
| **Grain Δ (‰)** | ** | ** | ** | 19.27c | 19.39b | 19.66a | 19.14d |
| **Heading days** | ** | ** | * | 96.56a | 92.19b | 92.75b | 92.18b |
| **Maturity days** | ** | ** | ** | 142.6a | 140.7b | 140.6b | 137.6c |
| **Plant Height (cm)** | ** | ** | ** | 102.8a | 100.1b | 83.63c | 81.81d |
| **Number of tillers** | ** | ** | ns | 11.25a | 8.063b | 5.125c | 4.313d |
| **Grain number/spike** | ** | ** | ns | 58.31a | 52.50b | 47.31c | 46.00c |
| **Spike length (cm)** | ** | ** | ** | 11.74a | 11.40b | 11.29b | 11.02c |
| **Thousand grain weight (g)** | ** | ** | ** | 41.13b | 37.74d | 45.78a | 38.41c |

Means followed by different letters in a row differ significantly at $P \leq 5\%$ level.
*, ** means significant at 0.05 and 0.01 level respectively. ns means non-significant.
T and G refer to treatment and genotype, respectively.

**Table 3: Mean values of agronomical traits of wheat genotypes averaged across the different water regimes.**

| | Sitta | FD-83 | NR-234 | Bhittai | Sarsabz | Pfau | NR-241 | Nesser |
|---|---|---|---|---|---|---|---|---|
| **GY ($Mgha^{-1}$)** | 4.40a | 4.37a | 4.30a | 3.89b | 3.86b | 3.80b | 3.71b | 3.12c |
| **BY ($Mgha^{-1}$)** | 11.74b | 11.44b | 1159b | 13.47a | 13.24a | 10.91b | 11.59b | 11.58b |
| **$WUE_B$(kg $ha^{-1}mm^{-1}$)** | 45.26b | 43.74b | 43.64b | 51.80a | 49.50a | 42.14b | 43.38b | 44.00b |
| **$WUE_G$(kg $ha^{-1}mm^{-1}$)** | 16.99a | 16.80b | 16.49bc | 14.93cd | 15.04cd | 14.69d | 14.09de | 12.51e |
| **H.I.%** | 37.53a | 38.46a | 37.28a | 28.86d | 29.98d | 34.66b | 32.30c | 27.70d |
| **PH (cm)** | 91.13e | 96.50c | 89.75e | 102.5a | 99.25b | 91.75d | 85.25f | 80.38g |
| **Leaf Δ (‰)** | 20.03d | 20.18a | 19.72e | 19.74e | 20.16a | 20.0c | 19.37f | 18.88g |
| **Grain Δ (‰)** | 19.28f | 19.71a | 19.50c | 19.64b | 19.40d | 19.26g | 19.33e | 18.80h |
| **HD** | 88.50d | 84.38e | 93.13c | 97.50a | 93.75bc | 98.75a | 96.13ab | 96.50a |
| **MD** | 139.5cd | 137.8e | 139.3d | 143.1a | 140.4bc | 140.5bc | 141.4b | 141.0b |
| **TGW (g)** | 43.08b | 46.06a | 46.98a | 38.44c | 39.09c | 34.59d | 38.49c | 32.93e |

Means followed by different letters in a row differ significantly at $P \leq 5\%$ level.

**Table 4: Mean values of carbon isotope discrimination (Δ) in leaf and grain in eight wheat genotypes grown under three water regimes.**

| ***Genotypes*** | ***Sitta*** | ***FD-83*** | ***NR-234*** | ***Bhittai*** | ***Sarsabz*** | ***Pfau*** | ***NR-241*** | ***Nesser*** |
|---|---|---|---|---|---|---|---|---|
| | | | | Well Watered | | | | |
| **Leaf Δ(‰)** | 20.56 | 20.93 | 20.75 | 20.87 | 20.52 | 20.53 | 20.21 | 19.53 |
| **Grain Δ(‰)** | 19.50 | 19.84 | 19.68 | 20.21 | 19.46 | 19.60 | 19.57 | 19.45 |
| | | | | Medium Watered | | | | |
| **Leaf Δ(‰)** | 19.75 | 20.70 | 19.52 | 18.68 | 19.97 | 19.86 | 20.04 | 18.68 |
| **Grain Δ(‰)** | 19.37 | 19.75 | 19.34 | 19.67 | 19.33 | 19.32 | 19.35 | 18.95 |
| | | | | Low Watered | | | | |
| **Leaf Δ(‰)** | 19.92 | 19.55 | 19.31 | 19.72 | 20.08 | 19.81 | 18.62 | 18.66 |
| **Grain Δ(‰)** | 19.25 | 19.59 | 19.56 | 19.34 | 19.45 | 19.11 | 19.27 | 18.56 |
| | | | | Stored Soil Moisture | | | | |
| **Leaf Δ(‰)** | 19.9 | 19.53 | 19.28 | 19.69 | 20.05 | 19.79 | 18.60 | 18.63 |
| **Grain Δ(‰)** | 18.98 | 19.64 | 19.43 | 19.32 | 19.35 | 19.02 | 19.11 | 18.23 |

**Table 5. Correlation coefficients among various parameters including biomass yield (BY), grain yield (GY), $WUE_B$ (biomass) and $WUE_G$ (grain), harvest index (HI), Head days (HD), maturity days (MD), number of tillers per plant (NT), Thousand grain weight (TGW) and carbon isotope discrimination (Δ) of leaf (ΔL) and grain (ΔG) of wheat genotypes under different water regimes.**

| | **H.I.** | **$WUE_B$** | **$WUE_G$** | **ΔL** | **ΔG** | **NT** | **SL** | **TGW** | **HD** | **MD** |
|---|---|---|---|---|---|---|---|---|---|---|
| **BY** | -0.557 | 0.984** | -0.036 | 0.157 | 0.319 | -0.224 | 0.533 | -0.016 | 0.152 | 0.532 |
| **GY** | 0.845** | 0.004 | 0.994** | 0.779* | 0.753* | -0.854** | 0.779* | 0.899** | -0.708* | -0.592 |
| **$WUE_B$** | -0.526 | - | 0.003 | 0.198 | 0.335 | -0.250 | 0.545 | -0.036 | 0.142 | 0.547 |
| **$WUE_G$** | 0.846** | -0.526 | - | 0.783* | 0.707* | -0.814* | 0.784* | 0.892** | -0.743* | -0.635 |
| **HI** | - | - | - | 0.572 | 0.451 | -0.571 | 0.373 | 0.766* | -0.694 | -0.809* |

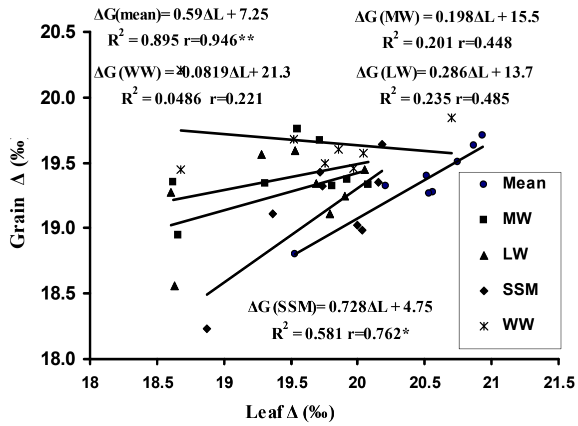


**Fig. 1. Relationship between Carbon Isotope Discrimination (Δ) of leaf and grain at different treatments and mean (averaged across the treatments).**

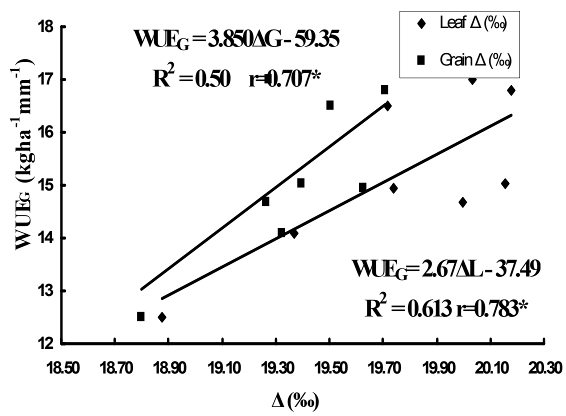


**Fig. 2. Relationship between average water-use efficiency ($WUE_G$) and Carbon Isotope Discrimination (Δ) of leaf and grain.**

increase in water stress. $WUE_B$ and $WUE_G$ also followed trends similar to BY and GY, respectively. There was a strong positive linear correlation between GY and HI. HI varied from 38.4-27.7 % and the highest HI was noted in early flowering and early maturing genotype FD-83. Water stress resulted significant decrease in PH, NT, GW and number of grains per spike.

Reduction in water applied by 25 and 50% reduced GY by 6% and 21% compared to WW treatment. Under SSM the GY reduction was 38% compared with WW treatment. WC was reduced 19% and 38% under MW and LW, respectively compared to WW conditions (Table 2).

There was a large range of variation for HD (15 days) and MD varied from 143 to 138 (5 days) averaged across the treatment (Table 3). BY showed non significant

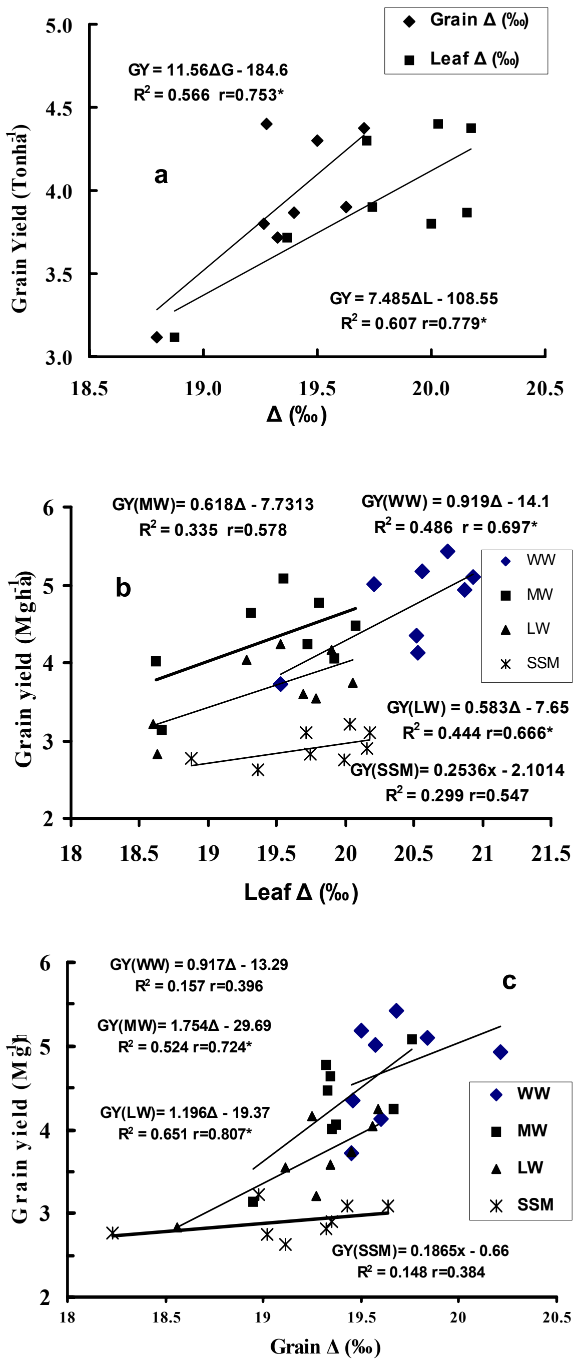


**Fig.3. Relationship between grain yield and Carbon Isotope Discrimination (Δ) a) leaf and grain, b) Leaf and c) grain at different treatments.**

correlations with most of the traits studied but had highly significant correlation (r=0.984**) with $WUE_B$. Grain yield showed highly significant positive correlations with HI, $WUE_G$, SL, TGW and highly significant negative correlation with NT and HD. (Table 5)

**Water-use efficiency and carbon isotope discrimination (Δ):** $WUE_G$ was strongly and linearly correlated with GY (Table 5) and showed poor correlation with BY. $WUE_B$ showed significant linear correlation with BY and inverse non-significant correlation with HI (Table 5). Both $WUE_G$ and $WUE_B$ increased with water stress and largest increase appeared in SSM under severe water stress. Genotype Sitta showed significantly higher $WUE_G$ (16.99 $kgha^{-1}mm^{-1}$) followed by FD-83 (16.8 $kgha^{-1}mm^{-1}$) due to their higher GY driven by relatively higher HI compared to other genotypes. Bhittai genotype exhibited significantly higher $WUE_B$ due to higher accumulation of BY and showed relatively lower GY/or $WUE_G$ because of lower HI compared to other genotypes.

Among these genotypes leaf and grain Δ showed a variation of 1.3 and 0.91‰, respectively. Both genotype and water treatment showed significant effects on **Δ** of leaf and grain (Table 2). All genotypes tended to have higher Δ in leaf than grain under different water regimes. In WW (0.82‰), MW (0.331‰), LW (0.191‰) and SSM (0.29‰) lower Δ was noted in grain than leaf averaged across the genotypes. Water stress reduced both ΔL and grain ΔG and difference between leaf and grain Δ decreased with water limitation and were genotype-dependent**.** The genotype FD-83 showed the highest ΔL (20.93‰) in WW and NR-241 the lowest ΔL (18.6‰) in SSM with highest water stress. The genotype, Bhittai exhibited the highest ΔG (20.21‰) in WW and Nesser the lowest mean ΔG (18.23‰) under SSM condition.

Leaf Δ showed significant positive linear correlation (0.946**) with ΔG averaged across the treatments (Fig 1). Under WW condition week correlation (r=0.221) between ΔL & ΔG was noted and slope of regression and r values increased with increase in water stress. Generally, GY was positively and linearly correlated with ΔL (r=0.779*) and ΔG (r=0.753*) (Fig. 3a) averaged across the treatment. GY showed significant positive linear correlation with ΔL under WW (r=0.697*) and LW (0.666*) but under MW and SSM condition the correlation was non-significant (Fig. 3b). GY and ΔG also showed positive linear relationship significant under MW and LW and non-significant under WW and SSM conditions Fig. 3c). $WUE_G$ was significantly correlated with leaf and grain Δ (Fig. 2). $WUE_G$ also showed highly significant positive correlations with HI, SL, TGW and highly significant inverse correlation with NT and HD (Table 5).

## Discussion

In this study, wheat genotypes grown were exposed to different water regimes by controlling soil available water with increasing water stress (WW, MW, LW and SSM under rain with no addition water supply) till maturity. The water stress decreased BY in all genotypes and was largely associated with decrease in NT and plant height. The different water treatments resulted in significant differences in GY among these genotypes. The reduction in GY with increase in water stress may be associated with decrease in soil AW adversely affecting NGPS, TGW and NT. The highest GY obtained by Sitta is likely due to relatively higher HI and highest TGW compared to other genotypes. The lowest GY obtained by genotype Nesser despite the relatively high BY with highest NT may be attributed to lowest HI, SL, and TGW.

In this study a week correlation between leaf and grain Δ was found under optimal conditions which, improved with increase in water stress (Fig. 1). Under drought conditions, significant correlations were reported between leaf and grain Δ by Araus *et al*. (1998), Hafsi *et al*. (2000) and Merah *et al*. (2001a). Under such conditions, stomatal conductance and Δ are reduced much earlier in the growth cycle and grain filling depends more on pre-anthesis reserves (Loss & Siddique 1994) accumulated during periods of reduced stress. As a consequence, leaf Δ isotopic imprint on final isotope composition of the grain is stronger than under more favourable conditions (Hannachi *et al*. 1996). The significant decrease in leaf Δ and less pronounced increase in grain Δ with water stress in the study is in agreement with previous reports on cowpea (Hall *et al*., 1990; Ismail & Hall, 1993), wheat (Farquhar & Richards, 1984), barley (Hubick & Farquhar, 1989), and Russian wild rye (Frank & Berdahl, 2001), indicating the consistent water stress effects on Δ. Scartazza *et al*., (1998) reported that Δ of structural material represents a long-term integration of Ci/Ca over the entire period when the contributing carbon was assimilated.

The genotype effect on Δ in leaf and grain under different treatments was found to be consistent. Lower Δ values in Nesser indicate that Nesser assimilated more $^{13}C$, while Sarsabz and Bhittai in WW discriminated against this heavier isotope to a greater degree. Condon *et al*. (1992) reported that genotypic variation in the response of stomata to soil water depletion may have caused the genotypic variation in Δ in wheat. Variation in photosynthetic capacity may also lead to cultivar variation in Δ (Condon *et al*., 1992). Greater photosynthetic capacity, lower stomatal conductance or both may result in lower values of Ci/Ca. This means that greater photosynthetic capacity should be reflected in lower values of Δ unless stomatal conductance also increases to balance the change in photosynthetic capacity and maintain constant Ci.

Grain yield was positively associated with both leaf and grain Δ (Fig 3) however the correlations were significant under moderate stress (MW and LW) but non-significant under optimal conditions (WW) and SSM (severe stress). Positive correlation between GY and grain Δ were found in a wide range of climatic conditions (Kirda *et al*.1992, Condon & Richards 1993, Sayre *et al*. 1995, Monneveux *et al*. 2004; 2005) while correlation between GY and leaf Δ was reported mainly under early drought conditions (Araus *et al*. 1998, Merah *et al*., 2001). Wheat grain yield was positively correlated to grain Δ under post anthesis water stress (Misra *et al*., 2006). Under Mediterranean conditions, a significant positive association was repeatedly found between grain yield and grain Δ (Bazza 1996; Merah *et al*. 2001a) and, under severe stress, between grain yield and flag leaf Δ (Merah *et al*., 1999; 2001b). Conversely, under residual soil moisture (out of season rainfall) conditions, genotypes with low Δ values in seedlings were found to be more productive (Condon *et al*. 2002), while the relationship between grain Δ and yield highly varied with total rainfall and its distribution (Condon & Hall, 1997; Condon *et al*. 2002; Misra *et al*. 2006; Waraich *et al*., 2007). A positive correlation between Δ and the production of grain and total biomass was reported in wheat under controlled conditions (Ansari *et al*. 1998). In China, Xu *et al*., 2007 found positive association of GY with ΔG and ΔL under terminal water stress at anthesis, strong correlation across the environment under residual moisture stress and no correlation under optimal irrigation indicating Δ as an indirect selection criterion for GY and a phenotyping tool under limited irrigation.

Results confirm strong and positive association between $WUE_G$ and both ΔL and ΔG. $WUE_G$ also showed significant correlation with HI and inverse correlations with NT, HD and MD. According to Passioura (1977), increase in $WUE_G$ can be obtained by enhancing either the quantity of water transpired by the crop, the transpiration efficiency, or the harvest index and the relative contribution of each component in $WUE_G$ variation is likely to depend upon their variation within the evaluated germ-plasm. Sitta and FD-83 genotypes were found high grain yielder with relatively greater increase in $WUE_G$ under increasing water stress. Results suggest that both Sitta and FD-83 with other desirable traits may be very useful genotypes for drought and water limited environments. The increase in $WUE_G$ or $WUE_B$ with water stress may be because of higher conductance reduction than assimilation reduction. Extended drought can increase WUE substantially (Li, 1999; Saranga *et al*., 1999; Sun *et al*., 1996; Akhter *et al*. 2003), although that effect may not always be found (Ehdaie, 1995; Walley *et al*., 1999). Between Δ and WUE, Akhter *et al*., (2005) found significant negative correlation in *Eucalyptus camaldulensis* and significant positive correlation in *Acacia ampliceps* subjected to different water regimes.

Increase in WUE appears to be an alternative strategy for improving performance where, additional water is not available to the crop (i.e. all the water available is exhausted during the crop cycle), Therefore, lines producing greater biomass and grain yield due to superior WUE will have lower $\Delta^{13}C$ and perform better in contrast to situations where lines perform better because of increased access to water (Slafer & Araus, 1998). The lowest leaf and grain Δ values noted in SSM, the most severe water stress corresponded to the lowest grain yield. Condon *et al*. (1992) reported that enrichment in $^{13}C$ in grain may be related to progressive soil drying and stomatal closure. $WUE_G$ was significantly positively correlated with leaf and grain Δ and HI and showed inverse correlation with heading and maturity days. In the present study water use efficiency and grain yield were mainly driven by harvest index, as already reported by Kondo *et al*. (2004) and Zhang & Yang (2003), due to the wide variation of this trait among the tested genotypes.

The present study confirms significant positive correlation between Δ (leaf and grain) and GY/ or $WUE_G$ in bread wheat genotypes indicating Δ, a reliable indirect selection tool for high grain yield and crop water-use efficiency under different (WW, MW, LW and SSM) water regimes. However, for selection/identification purposes early leaf Δ would be preferred over grain Δ as it is less time consuming. Genotypic variation in Δ and its relationship with water-use efficiency ($WUE_G$) or agronomic WUE can be exploited for increasing crop productivity by selecting water-use efficient genotypes for target environments.

**Acknowledgements:** This study was partly funded by the International Atomic Energy Agency (IAEA), Vienna, through Research Contract No 12656/RB.

**References**

Akhter, J., K. Mahmood, M.A. Tasneem, K.A. Malik, M.H. Naqvi, F. Hussain, and R. Serraj. 2005. Water-use efficiency and carbon isotope discrimination of *Acacia ampliceps* and *Eucalyptus* *camaldulensis* at different soil moisture regimes under semi-arid conditions. *Biologia Plantarum,* 49: 269-272

Akhter, J., K. Mahmood, M.A. Tasneem, M.H. Naqvi and K.A. Malik. 2003. Comparative water use-efficiency of *Sporobolus arabicus* and *Leptochloa fusca* and its relation with carbon-isotope discrimination under semi-arid conditions. *Plant and Soil,* 249: 263-269

Ansari, R., S.S.M. Naqvi, A.N. Khanzada and K.T. Hubick. 1998. Carbon isotope discrimination in wheat under saline conditions. ***Pak. J. Bot.,*** 30: 87-93.

Araus, J.L., D. Villegas, N. Aparicio, del. Garcia, L.F. Moral, S. El Hani, Y. Rharrabti, J.P. Ferrio and C. Royo. 2003. Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. *Crop Sci*., 43: 170-180.

Araus, J.L., T. Amaro, J. Casadesus, A. Asbati and M.M. Nachit. 1998. Relationships between ash content, carbon isotope discrimination and yield in durum wheat. *Aust. J. Plant Physiol*., 25: 835-842.

Araus, J.L., T. Amaro, Y. Zuhair and M.M. Nachit. 1997. Effect of leaf structure and water status on carbon isotope discrimination in field-grown durum wheat. *Plant Cell and Environment*, 20: 1484-1494.

Bazza, M. 1996. Carbon-13 discrimination as a criterion for identifying water use efficiency cultivars under water deficit conditions. In: Isotope Studies on Plant Productivity. *IAEA-TECDOC-889*., Vienna: pp. 123-130.

Bender, M.M. 1968. Mass spectrometric studies of carbon 13 variations in corn and other grasses. *Radiocarbon*., 10: 468-472.

Bender, M.M., I. Rouhani, H.M. Vines, C.C. Black. 1973. $^{13}C/^{12}C$ ratio changes in crassulacean acid metabolism plants. *Plant Physiol*., 52: 427-430.

Christeller, J.T., W.A. Laing, J.H. Troughton. 1976. Isotope discrimination by ribulose 1,5-diphosphate carboxylase. No effect of temperature or $HCO_3$- concentration. *Plant Physiol*., 57: 580-582.

Condon, A.G. and A.E. Hall, 1997. Adaptation to diverse environments: Genotypic variation in water-use efficiency within crop species. In: Jackson LE ed. Agricultural Ecology. *Academic Press, San Diego: pp.* 79-116.

Condon, A.G. and R.A. Richards. 1993. Exploiting genetic variation in transpiration efficiency in wheat : an agronomic view. In Ehleringer J. R. *et al*. (Eds.), stable isotopes and plant carbon-water relations. *Academic Press Inc., New York*., *USA: pp.* 435-450.

Condon, A.G., R.A. Richards and G.D. Farquhar. 1987. Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field grown wheat. *Crop Sci*., 27: 996-1001.

Condon, A.G., R.A. Richards and G.D. Farquhar. 1992. The effect of variation in soil water availability, vapour pressure deficit and nitrogen nutrition on carbon isotope discrimination in wheat. *Aust. J. Agric. Res*., 43: 935-947.

Condon, A.G., R.A. Richards and G.D. Farquhar. 1993. Relationships between carbon isotope discrimination, water use efficiency and transpiration efficiency for dry land wheat. *Aus. J. Agri. Res*., 44: 1693-1711.

Condon, A.G., R.A. Richards, G.J. Rebetzke and G.D. Farquhar. 2002. Improving water-use efficiency and crop yield. *Crop Sci*., 42: 122-132.

Craig, H. 1954. Carbon-13 in plants and the relationship between carbon-13 and carbon-14 variation in nature. *J. Geol*., 62: 115-149.

Ehdaie, B. 1995. Variation in water-use efficiency and its components in wheat: II. Pot and field experiment. *Crop Sci*., 35: 1617-1626.

Farquhar, G.D. and R.A. Richards. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Aust. J. Plant Physiol*., 11: 539-552.

Farquhar, G.D., J.R. Ehleringer and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Ann. Rev. Plant Physiol. *Mol. Biol*., 40: 503-537.

Frank, A.B. and J.D. Berdahl. 2001. Gas exchange and water relations in Diploid and Tetraploid Russian Wildrye. *Crop Sci*., 41: 87-92.

Hafizullah, M. 2002. Water resources management strategy. *Sci. Vision,* 7: 46-48.

Hafsi, M., W. Mechmeche, L. Bouamama, A. Djekoune, M. Zaharieva and P. Monneveux. 2000. Flag leaf senescence, as evaluated by numerical image analysis, and its relationship with yield under drought in durum wheat. *J Agron. and Crop Sci*., 185:275-280.

Hall. A.E, R.G. Mutters, K.T. Hubick and G.D. Farquhar. 1990. Genotypic differences in carbon isotope discrimination by cowpea under wet and dry field conditions. *Crop Sci*., 30: 300-305.

Hannachi, L., E. Deleens and P. Gate. 1996. Nitrogen and carbon isotope composition of wheat grain: alteration due to sink-source modifications at flowering. *Rapid Comm. Mass Spectrum*. 19: 979-986.

Hubick, K.T. and G.D. Farquhar. 1989. Carbon isotope discrimination and the ratio of carbon gained to water lost in barley cultivars. *Plant, Cell and Environ*., 12: 795-804.

Ismail, A.M. and A.E. Hall. 1993. Inheritance of carbon isotope discrimination and water-use efficiency in cowpea. *Crop Sci*., 33: 498-503.

Keeling, C.D., W.G. Mock and P.P. Tans. 1979. Recent trends in the $^{13}C/^{12}C$ ratio of atmospheric carbon dioxide. *Nature*., 277: 121-123.

Khan. A.J., F. Azam, A. Ali, M. Tariq, M. Amin and T. Muhammad. 2007. Wide and specific adaptation of bread wheat inbred lines for yield under rain-fed conditions. *Pak. J. Bot.*, 39: 67-71.

Kirda, C., A.R.A.G. Mohamed, K.S. Kumarasinghe, A. Montenegro and F. Zapata. 1992. Carbon isotope discrimination at vegetative stage as an indicator of yield and water use efficiency of spring wheat (*Triticum turgidum* L. var. *durum*). *Plant Soil*., 147: 217-223.

Kondo, M., P.P. Pablico, D.V. Aragones and R. Agbisit. 2004. Genotypic variations of $\delta^{13}C$ in rice (*Oryza sativa* L. and *Oryza glaberrima* Steud.) in relation to transipiration efficiency and biomass production as affected by soil water conditions and N. *Plant Soil*, 13:165-177.

Li, C. 1999. Carbon isotope composition, water-use efficiency and biomass productivity of *Eucalyptus microtheca* populations under different water supplies. *Plant and Soil*., 214: 165-171.

Loss, S.P. and K.H.M. Siddique. 1994. Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. *Advances in Agronomy*., 52: 229-276.

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

Merah, O., E. Deleens and P. Monneveux. 2001. Relationships between carbon isotope discrimination, dry matter production and harvest index in durum wheat. *Journal of Plant Physio*., 158: 723-729.

Merah, O., E. Deleens and P. Monneveux. 2001a. Relationships between flag leaf carbon isotope discrimination and several morphophysiological traits in durum wheat under Mediterranean conditions. *Environ. Exp. Bot*., 45: 63-71.

Merah, O., E. Deleens, I. Souyris, M. Nachit and P. Monneveux. 2001b. Stability of carbon isotope discrimination and grain yield in durum wheat. *Crop Sci*., 41: 677-681.

Misra, S.C., R. Randive, V. S. Rao, M.S. Sheshshayee, R. Serraj and P. Monneveux. 2006. Relationship between Carbon Isotope Discrimination, Ash Content and Grain Yield in Wheat in the Peninsular Zone of India. *J. Agron. & Crop Sci.*., 192: 352-362.

Monneveux, P., E. Rekika, D. Acevedo and O. Merah. 2006. Effect of drought on leaf gas exchange, carbon isotope discrimination, transpiration efficiency and productivity in field grown durum wheat genotypes. *Plant Sci*., 170: 867-872.

Monneveux, P., M. P. Reynolds, R. Trethowan, R. J. Pen˜ a. and F. Zapata. 2004: Carbon isotope discrimination, leaf ash content and grain yield in bread and durum wheat grown under full-irrigated conditions. *J. Agron. Crop Sci*., 190: 389-394.

Monneveux, P., M.P. Reynolds, R. Trethowan, H. R. Gonza lez-Santoyo, J. Pen and F. Zapata. 2005. Relationship between grain yield and carbon isotope discrimination in bread wheat under four water regimes. *European J. Agronomy*., 22: 231-242.

Morgan, J.A., D.R. LeCain, T.N. McCaig, J.S. Quick. 1993. Gas exchange, carbon isotope discrimination and productivity in winter wheat. *Crop Sci*., 33: 178-186.

PARC (Pakistan Agricultural Research Council). 1980. Agro-ecological Regions of Pakistan. *PARC, Islamabad*.

Passioura, J.B. 1977. Grain yield, harvest index and water use of wheat. *Journal of the Australian Institute of Agricultural Science*., 43: 117-120.

Passioura, J.B. 1996. Drought and drought tolerance. *Plant Growth Regulation*., 20: 79-83.

Saranga, Y., I. Flash, A.H. Paterson and D. Yakir. 1999. Carbon isotope ratio in cotton varies with growth stage and plant organ. *Plant Sci*., 142: 47-56.

SAS Institute. 1987. SAS/STAT User's Guide, version 6.SAS Inst., *Inc., Cary, NC*.

Sayre, K.D., E. Acevedo and R.B. Austin. 1995. Carbon isotope discrimination and grain yield for three bread wheat germplasm groups grown at different levels of water stress. *Field Crops Res*., 41, 45-54.

Scartazza, A., M. Lauteri, M.C. Guido and E. Brugnoli. 1998. Carbon isotope discrimination in leaf and stem sugars, water-use efficiency and mesophyll conductance during different developmental stages in rice subjected to drought. *Aust. J. Plant Physiol*., 25: 489-498.

Slafer, G.A. and J.L. Araus. 1998. Keynote address: Improving wheat responses to abiotic stresses. In: Slinkard AE, ed. Proceedings of the 9th international wheat genetics symposium vol. 1 (Section 7 Abiotic stresses - Keynote address). *Sasktchewan*., 201-213.

Smith, B.N. and S. Epstein. 19971. Two categories of $^{13}C/^{12}C$ ratios for higher plants. *Plant Physiol*., 47: 380-384.

Sun, Z.J., N.J. Livingston, R.D. Guy and G.J. Ethier. 1996. Stable carbon isotopes as indicators of increased water use efficiency and productivity in white spruce (*Picea glauca*) seedlings. *Plant Cell Environ*., 19: 887-894.

Tsialtas, J.T., I.S. Tokatlidis, E. Tamoutsidis and I.N. Xynias. 2001. Grain carbon isotope discrimination and ash content of cv Nestos bread wheat selected for high and low yield in absence of competition. *Cereal Res. Commun*., 29: 391-396.

Villegas, D., N. Aparicio, M.M. Nachit, J.L. Araus and C. Royo. 2000. Photosynthetic and developmental traits associated with genotypic differences in durum wheat yield across the Mediterranean basin. *Aust. J. Agri. Res.*., 51: 891-901.

Walley, F.L., G. P. Lafond, A. Matus and C. van. Kessel. 1999. Water-Use efficiency and carbon isotopic composition in reduced tillage systems. *Soil Sci. Soc. Am. J*., 63: 356-361.

Waraich, E.A. R. Ahmad, A. Ali and S. Ullah. 2007. Irrigation and nitrogen effects on development and yield of wheat (*Triticum aestivum* L.). *Pak. J. Bot.*, 39: 1663-1672.

Xu, X., H. Li. S. Yuan, R. Trethowan and P. Monneveux. 2007. Relationship between Carbon Isotope Discrimination and Grain Yield in Spring Wheat Cultivated under Different Water Regimes. *J. of Integr. Plant Bio.*, 49: 1497-1507.

Ye, H.W. and W.M. Wang. 2001. Factors affecting the isotopic composition of organic matter (1) Carbon isotopic composition of terrestrial plant materials. *Proc. Natl. Sci. Counc*., 25: 137-147.

Zang, J. and J. Yang. 2003. Cropy yield and water use efficieny: A case study of rice. In: M.A. Bacon. (Ed.) Water Use Efficiency in Plant Biology, Oxford and Carlton: Blackwell, Pp: 198-227.


(Received for publication 21 April, 2008)