

SELECTION OF DROUGHT TOLERANT AND HIGH WATER USE EFFICIENT RICE CULTIVARS THROUGH ^{13}C ISOTOPE DISCRIMINATION TECHNIQUE

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Abstract

Carbon isotope discrimination (Δ) has been suggested as an indirect tool for selecting plants having higher water use efficiency (WUE) and yield potential. Enhancing WUE is an important breeding objective as water scarcity is increasing with every passing day. This study was undertaken to assess the genotypic variation and relationship between leaf, straw, grain Δ , grain yield and WUE in eight aromatic rice cultivars grown in lysimeters under three water regimes, in absence of drainage and runoff. Highly significant positive correlations were found between above-ground biomass and WUE_B, and grain yield and WUE_G, due to the low variation in water consumed by different cultivars. Leaf, straw and grain Δ showed a consistent variation across treatments and cultivars. Under water stress conditions, both leaf and straw Δ were positively correlated to grain yield and WUE_G. In all the water treatments, WUE_G was positively correlated to harvest index and negatively to plant height. All the mutants from Basmati 385 had significantly higher Δ values as compared to the mutants from Basmati 370. It was concluded that the new cultivar, Basmati 385, represents a better genetic source for Δ improvement than the old cultivar, Basmati 370.

Introduction

Due to the increasing water scarcity for rice cultivation, enhancing yield under water-limited conditions is an important target of rice research. High transpiration efficiency (biomass/water transpired) is considered to be a useful trait, unless this trait has negative linkage with water uptake capacity and other traits for yield gain. Increasing agronomic water use efficiency (WUE) in rice is consequently a major goal for the next decades, considering that water resources are decreasing (Vörösmarty *et al.*, 2000). WUE of a crop is the ratio of total biomass or above-ground biomass or harvested yield, against total available water or evapotranspiration, or plant transpiration (Loomis & Connor 1992; Jones, 2004). Transpiration efficiency (TE), also referred as intrinsic water use efficiency (WUE_T) (Farquhar *et al.*, 1989), can be evaluated at the leaf level as the ratio of leaf CO₂ exchange rate to transpiration (Dingkuhn *et al.*, 1991; Morgan *et al.*, 1993) or as the ratio of biomass produced to transpiration. A term closely related to TE, CER/g_s, i.e., the leaf CO₂ exchange rate to leaf conductance has been proposed to evaluate transpiration efficiency when vapor pressure varies among measurements (Morgan & LeCain 1991). However, diurnal and seasonal variations in leaf gas exchange are such that thousands of measurements would be required to sufficiently integrate TE for wide genotypic comparison. An alternative is to assess gas exchange parameters and TE under controlled conditions (Condon *et al.*, 1993).

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Isotopic methods could represent another alternative to circumvent this difficulty. Carbon isotope discrimination (Δ) was shown to be negatively correlated with CER/g_s at the leaf level (Farquhar & Richards 1984; Akhter *et al.*, 2008). Significant negative correlation was also found between Δ and instantaneous measurements of TE (Condon *et al.*, 1993). Under drought conditions, carbon isotope discrimination (Δ) was shown to be negatively correlated to transpiration efficiency (TE) at the leaf level in wheat (Farquhar & Richards, 1984) and rice (Dingkuhn *et al.*, 1991; Scartazza *et al.*, 1998; Kondo *et al.*, 2004; Kuroda & Kumura 1990). Under well-watered conditions, the relationship between Δ and TE in C₃ cereals was found to be nil (Morgan *et al.*, 1993; Cabuslay *et al.*, 2002) or even positive (Monneveux *et al.*, 2004; FAO 2005).

The physiological basis for Δ variation in C₃ plants is related to the variation in the ratio of internal CO₂ (C_i) to ambient CO₂ concentrations (C_a). High Δ values resulting from high C_i/C_a reflect higher CO₂ assimilation rate to transpiration ratio (Farquhar *et al.*, 1989), *i.e.*, lower TE. A wide genetic variation has also been reported for Δ in *Oryza sativa* (Dingkuhn *et al.*, 1991; Samejima, 1985; Impa *et al.*, 2005) and *O. glaberrima* (Kondo *et al.*, 2004). Small genotype x environment interactions were found by Kondo *et al.*, (2004) and Impa *et al.*, (2005). Based on the relationship observed under drought between Δ and TE, several authors have proposed to use Δ as an indirect selection criterion in rice breeding programs for improving WUE (Keeling *et al.*, 1979; Dingkuhn *et al.*, 1991; Kondo *et al.*, 2004). The relationship between Δ and WUE in rice is however less clear. In pot experiments, Impa *et al.*, (2005) found a negative correlation between leaf Δ and WUE.

In contrast to other C₃ cereals, rice originated from a semi-aquatic ancestor and appears as a luxury water user (Lafitte & Courtois 2002). Upland rice requires much less water than lowland rice. Upland rice cultivars released so far have lower yield potential than lowland ones (Zhang & Yang 2003). Improving WUE of upland cultivars is therefore a priority for rice breeders. However, less is known about WUE of rice and how it relates to yield and transpiration efficiency under various water regimes. Relationships between Δ , TE and WUE have been mainly studied under controlled conditions and in a limited number of genotypes (SAS Institute 1987). Moreover, variation in Δ in different plant organs has not been very much studied in rice, despite the likelihood of improving the effectiveness of selection strategies. The research reported here was carried out with the objectives: 1) to investigate the relationship in grain yield, yield components, WUE and Δ of leaf straw and grain and 2) to find out any genotypic variation in these parameters, using eight aromatic rice genotypes grown in field lysimeters under three water regimes.

Materials and Methods

Plant material: Eight aromatic lowland rice cultivars were used in this study (Table 1). Among them, three were mutants of the cultivar Basmati 370 while others three were mutants of the cultivar Basmati 385. Basmati 370 and Basmati 385 were released in 1933 and 1985, respectively by the Kala Shah Kaku Rice Research Institute (KRRI) in Lahore, Pakistan. The mutants were evolved by γ irradiation at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. The two other cultivars were evolved by intraspecific hybridization. The first one is a cross between NIAB-6 (also called NIAB-IRRI-9), a salt tolerant mutant of IRRI-6 selected by NIAB, and DM-25, a mutant of Basmati 370, also developed by NIAB through cross between the salt tolerant variety Jhona 349 and Basmati 370.

Table 1. List and origin of the eight aromatic rice cultivars used in the study.

Rice cultivars	Origin
DM-3-89	Mutant of Basmati-370
DM-5-89	Mutant from Basmati-370
DM-38/88	Mutant of Basmati-370
DM-59418	Mutant from Basmati-385
NIAB-6 x DM-25	Cross between NIAB-6 x DM-25
Jhona-349 x Bas-370	Cross between Jhona-349 and Bas-370
DM-64198	Mutant of Basmati-385
DM-63275	Mutant from Basmati-385

Experimental conditions: The study was conducted at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad (31°2' N, 73°05' E), Pakistan. Sandy clay loam (45% sand, 33% silt and 22% clay) soil (fine-loamy, mixed, hyperthermic, Udic Halustepts, Inceptisols (FAO. 2005) from NIAB experimental field was used in these studies. Soil electrical conductivity (EC) was 0.5 dS m⁻¹, pH 7.6 and sodium adsorption ratio (SAR) 1.36. 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 (Fig. 1). The average annual rainfall, based on 30 years observations, is around 250 mm. Rainfall occurs mainly in March-April and July-August. The total rainfall recorded during the growth cycle was 296 and 206 mm in the 2004 (Fig. 1).

The eight genotypes were grown in cemented lysimeters (1m x 1m x 1m) laid out in a randomized block design with three treatments and three replicates. The soil used had an average bulk density of 1.4 g cm⁻³. Soil surface was carefully levelled to avoid run-off. In each lysimeter (thereafter designated as a plot), 5-week old seedlings were planted in 5 rows with 20 cm inter row and plant spacing. Four weeks after transplantation, plants were exposed to three water regimes, i.e., well-watered (WW), medium-watered (MW) and low-watered (LW) in which soil moisture was maintained at 100, 75 and 50% of the total available water (TAW), respectively. TAW was defined as the difference between soil moisture at field capacity and soil moisture at permanent wilting point. Field capacity and soil moisture at permanent wilting point were determined by placing the soil samples in plastic rings in a pressure membrane apparatus and measuring water retained by the soils at -0.03 MPa and -1.5 MPa, respectively. The amount of water required for each plot to maintain the respective soil water regime was estimated on the basis of readings taken with a neutron moisture meter (NMM, 503 Model CPN, USA) up to one meter depth. The NMM was calibrated in the same soil before the start of experiment according to Akhter et al. (1995). NMM readings were converted to water contents using the following calibration equation $\theta_v = 0.389n + 0.02$, where θ_v is volumetric water content, n is count rate ratio = observed counts/standard counts taken with NMM. The volume of water present in each lysimeter was estimated and the difference from required level was applied on alternate days except for few weeks of rainfall periods. The volume of water required for each plot was added through a locally fabricated irrigation system including a water pump, fixed pipes, water flowmeters and taps. EC of the irrigation water was 0.76 dS m⁻¹, pH 7.5 and SAR 2.0. The amount of water applied to each lysimeter was recorded and the cumulated amount was calculated at the end of the experiment. Rainfall was also recorded in mm and taken into account in the calculation of total water consumed during the growth cycle, referred as W_i . Fertilizer N (150 kg N ha⁻¹ as urea) and P (130 kg P₂O₅ ha⁻¹ as diammonium phosphate) were applied to all plots. Weeds were removed manually as and when required. Each year, transplanting was done during 1st week of July and the crop was harvested during 2nd week of November.

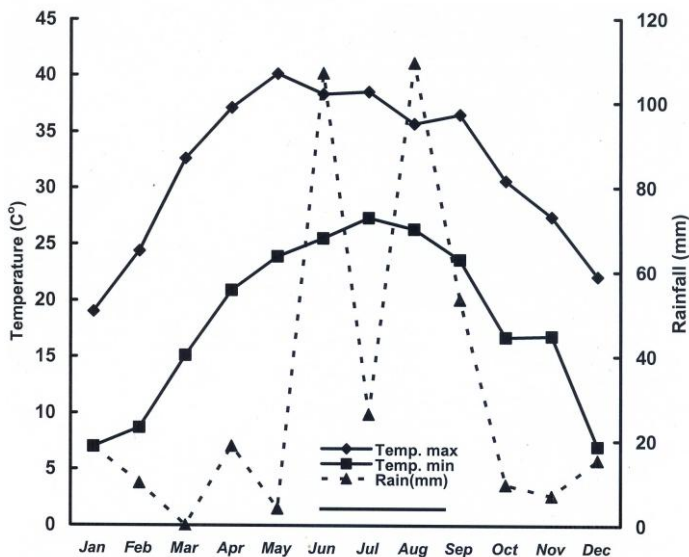


Fig. 1. Average monthly maximum and minimum air temperatures and rainfall during year 2004.

Measurements: The number of days to flowering was recorded when at least 50% of spikes of a given plot reached anthesis. Plant parameters including grain yield, above-ground biomass, plant height, number of tillers per plant, panicle length and number of grains per panicle were recorded at physiological maturity. Harvest index was determined as the ratio of grain yield to above-ground biomass. Water use efficiency on biomass basis (WUE_B) and water use efficiency on grain yield basis (WUE_G) were determined as the ratio of biomass and grain yield, respectively; to the quantity of water applied during the growth cycle (sum of rainfall and quantity of water applied through irrigation).

Carbon isotope discrimination (Δ) was determined by analysing the flag leaf, straw and grain. At anthesis, a total of 20 flag leaves were harvested per plot. At maturity, 10 g straw and 10 g grain samples were collected from each plot. Leaf, straw and grain samples were dried at 60 °C for 48 h 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 ml) using the relationship: □

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

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):

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

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. Carbon isotope discrimination in leaf, straw and grain have been thereafter referred to as ΔL , ΔS and ΔG , respectively.

Statistical analysis: The data were subjected to the analysis of variance (ANOVA), using SAS, version 8.1 (SAS Institute. 1987). The F test was used to identify treatment main effects and interactions followed by Duncan's Multiple Range Test for difference between treatment means at the 0.05 probability level.

Results

There were significant effects of genotype and year on grain yield, above-ground biomass, water use efficiency and Δ (Table 2). Treatment effect was also significant for all these traits, except WUE_G i.e. the agronomic water use efficiency on grain yield basis. The reduction in the amount of water applied by 25 and 50%, reduced grain yield by 22.4 and 41.7%, respectively. Above-ground biomass was reduced by 7.6 and 22.8%, in MW and LW, compared to WW conditions, while WUE_B increased by 18.7 and 38.0%, respectively.

Carbon isotope discrimination: Carbon isotope discrimination was 2.2, 3.0 and 2.6 ‰ lower in MW than in WW in leaf, straw and grain, respectively. There was non-significant effect of water regimes on leaf, straw and grain Δ . Leaf and straw Δ , averaged across treatments, were similar (20.70 and 20.84‰, respectively) and significantly higher than grain Δ (20.15‰). Grain yield in WW was positively correlated with grain yield in MW, but not with grain yield in LW (Fig. 2). Above-ground biomass at physiological maturity, averaged across years and water regimes ranged from 13.89 Mg ha⁻¹ (DM-64198) to 23.70 Mg ha⁻¹ (DM-3-89; Table 3). Conversely, the genotype DM-64198 showed the highest (5.90 Mg ha⁻¹) mean grain yield while DM-3-89 had the lowest yield (3.41 Mg ha⁻¹). The highest harvest index values were noted in the short-statured and early cultivars i.e., DM-63275, Jhona-349 x Bas-370 and DM-59418 (60.6, 54.6 and 51.5%, respectively).

Water use efficiency: Highly significant correlations were noted in all water regimes between WUE_B and above-ground biomass (Fig. 3a) and under WUE_G and grain yield (Fig. 3b). The slope of the regression, however, increased with water supply. In all water regimes, above-ground biomass and WUE_B showed highly significant negative correlations with harvest index and highly significant positive correlation with plant height (Table 4). Above-ground biomass and WUE_B were also negatively correlated to the number of grains per panicle in LW and positively to days to flowering in MW conditions. Grain yield and WUE_G were positively correlated to harvest index in all water regimes. They were also negatively correlated to plant height in WW and MW treatments. In the LW treatment, leaf and straw Δ were significantly positively correlated to grain yield (Table 5) and WUE_G (Fig. 4). In MW and WW treatments, these correlations were non-significant.

Discussion

Rice yield under limited water availability is related to potential yield, drought escape and drought tolerance *per se* (Fukai *et al.*, 1999). In the present study, potential yield (i.e., grain yield in WW) significantly explained the grain yield in MW, but not in LW conditions. Grain yield was significantly correlated with harvest index, as reported by Lanceras *et al.*, (2004) and in WW conditions with the number of grains per panicle.

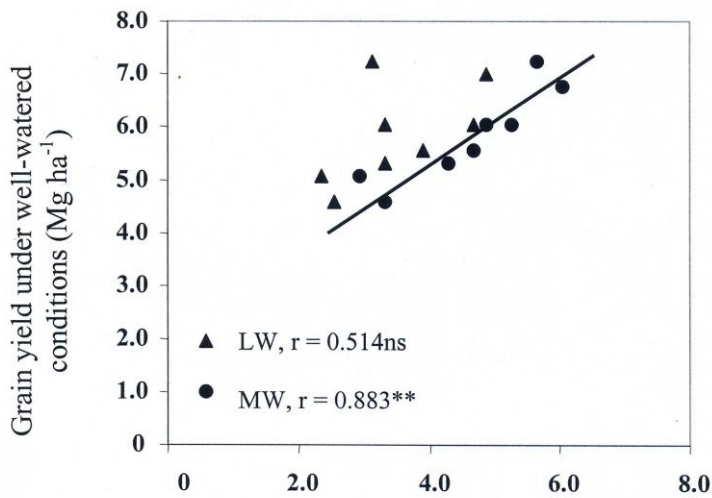


Fig. 2. Relationship between grain yield under well water levels (WW) and grain yield under water limited conditions (LW and MW).

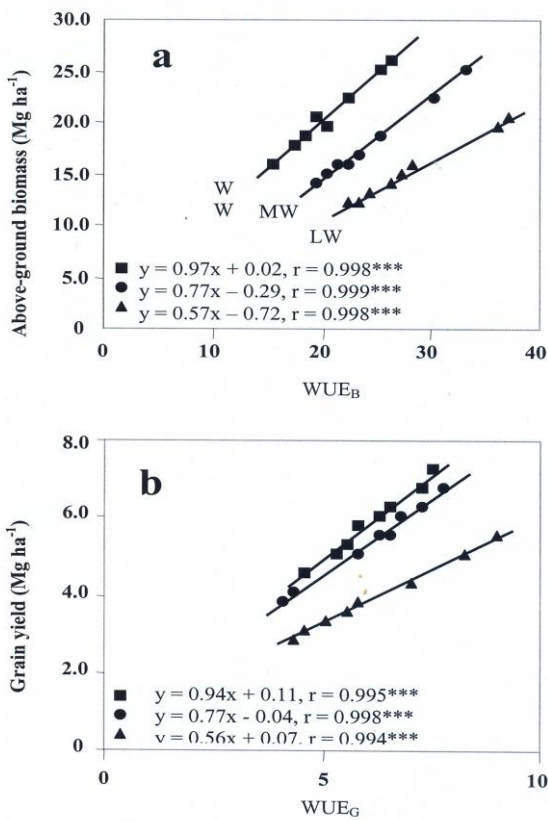


Fig. 3. Relationship of water use efficiency (WUE_G) with biomass yield (a) and grain yield (b) under three irrigation regimes.

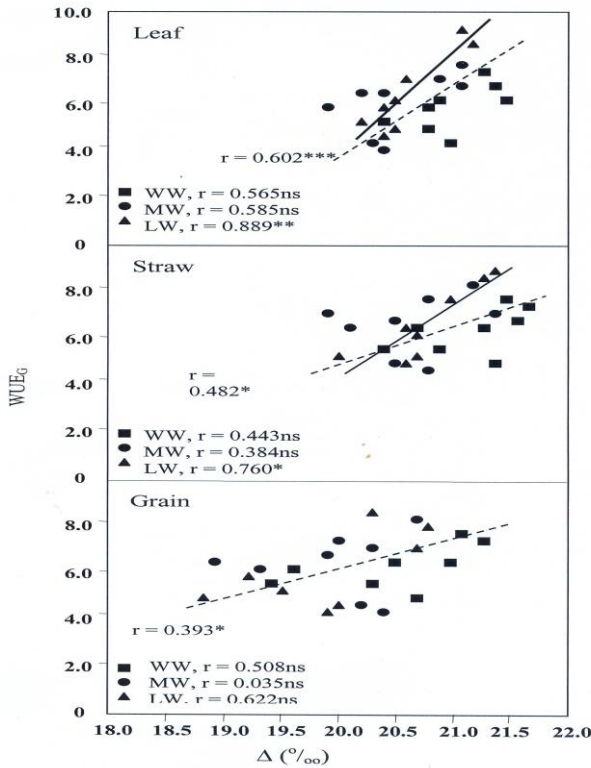


Fig. 4. Relationship between water use efficiency (WUE_G) and Δ of leaf, straw and grain at three irrigation regimes.

Table 4. Relationship among biomass (Biom), grain yield (GY), biomass water use efficiency (WUE_B) and grain based water use efficiency (WUE_G) with plant traits i.e. harvest index (HI), days to flowering (DF), plant height (PH), number of tillers per plant (NT), plant length (PL) and number of grains per panicle (NGPP) of eight rice genotypes grown at three irrigation regimes.

Water regimes / Parameters	HI	DF	PH	NT	PL	NGPP
Well watered (WW)						
Biom	-0.966***	0.602NS	0.831**	0.162NS	0.194NS	-0.548NS
GY	0.778*	-0.056NS	-0.720*	0.327NS	-0.472NS	0.724*
WUE_B	-0.968***	0.598NS	0.841**	0.145NS	0.192NS	-0.547NS
WUE_G	0.808*	-0.107NS	-0.715*	0.252NS	-0.479NS	0.731*
Medium watered (MW)						
Biom	-0.960***	0.711*	0.843**	-0.090NS	0.348NS	-0.062NS
GY	0.863**	-0.351NS	-0.755*	0.417NS	-0.285NS	0.275NS
WUE_B	-0.963***	0.704*	0.846**	-0.094NS	0.347NS	-0.071NS
WUE_G	0.870*	-0.371NS	-0.761*	0.419NS	-0.291NS	0.282NS
Low watered (LW)						
Biom	-0.949***	0.659NS	0.838**	0.054NS	0.320NS	-0.806**
GY	0.790*	-0.526NS	-0.543NS	-0.091NS	-0.071NS	0.481NS
WUE_B	-0.942***	0.631NS	0.840**	0.024NS	0.308NS	-0.812**
WUE_G	0.762*	-0.535NS	-0.467NS	-0.136NS	-0.046NS	0.423NS

*, **, Significant at 0.05 and 0.01 levels, respectively. NS, Non-significant.

Table 5. Relationship between grain yield (GY) and above-ground biomass (Biom) and leaf, straw and grain Δ under the three irrigation regimes.

Water regimes / Parameters	Leaf Δ	Straw Δ	Grain Δ
Well watered (WW)			
GY	0.530NS	0.402NS	0.469NS
Biom	-0.521NS	-0.349NS	-0.324NS
Medium watered (MW)			
GY	0.604NS	0.401NS	0.046NS
Biom	-0.352NS	-0.180NS	0.216NS
Low watered (LW)			
GY	0.870**	0.705*	0.553NS
Biom	-0.458NS	-0.217NS	-0.089NS

*, **, Significant at 0.05 and 0.01 levels, respectively. NS, Non-significant

Early flowering can play an important role to escape under terminal drought stress (Lafitte *et al.*, 2003). In the present study, however, water availability was reduced during the whole growth cycle and earliness did not affect yield and other plant characteristics, despite a large range in days to flowering among cultivars (almost 20 days). Consequently, grain yield under drought is expected to have depended more, particularly under the most severe drought treatment, on drought tolerance *per se* than on potential yield or drought escape.

Carbon isotope discrimination was reduced by water limitation, as already reported in rice by Pinheiro *et al.*, (2000) and Kondo *et al.*, (2004). The lower Δ values for grain, compared to that for leaf and straw are likely to be due to a high evaporative demand during grain filling, leading to lower stomatal conductance (Kuroda & Kumura, 1990). Carbon isotope discrimination exhibited a substantial genetic variation, however the variation was less as compared to earlier reports (Samejima, 1985; Dingkuhn *et al.*, 1991; Impa *et al.*, 2005) in which a larger range of *O. sativa* genetic resources were used.

While analyzing genotypic variation, it was noticed that significantly higher Δ values were found for all mutants from Basmati 385 compared to mutants from Basmati 370, indicating that the new cultivar Basmati 385 represents a better genetic source for Δ improvement than the old cultivar Basmati 370. Based on this observation it is also evident that selection for high yield in aromatic rice has resulted in an increase in its Δ value. Similar observation has also been documented for wheat where recently developed cultivars have higher Δ as compared to old cultivars (Fischer *et al.*, 1998).

In the present study, the correlation between leaf and straw Δ and grain yield was found significantly positive under LW conditions. An interrelationship between leaf or straw Δ and grain yield has also been reported in wheat under water limited environments (Merah *et al.*, 2001; Monneveux *et al.*, 2004). This correlation can be due to a combination of several factors in high Δ genotypes, such as higher stomatal conductance, faster crop growth rate, better ability to remobilize stored reserves, earlier flowering or better water extraction (Condon *et al.*, 2004; Monneveux *et al.*, 2005). It is interesting to note that if high leaf Δ values at anthesis or maturity show high values of Δ at early stages of growth, then high Δ and high yield may reflect faster growth rate (Condon *et al.*, 1993) which may contribute to a reduction in E_s and an increase in WUE_G . No relationship was found between Δ and WUE_G (or grain yield) under WW conditions. Under these conditions, stomatal conductance is likely to be high in all cultivars resulting in increased C_i/C_a and Δ values (Morgan *et al.*, 1993), while increased photosynthetic capacity potentially decreases C_i/C_a . The decrease in C_i/C_a associated with increased photosynthetic capacity is consequently offset by the C_i/C_a increase resulting from stomatal aperture, hence reducing the possibility of relationship between Δ and grain

yield (Monneveux *et al.*, 2005). As reported for wheat (Dingkuhn *et al.*, 1991; Monneveux *et al.*, 2005), Δ appears as a good predictor of grain yield in rice grown under water stress, but not under well irrigated conditions.

The positive correlation between leaf and straw Δ and WUE_G under water stress disagrees with results of Impa *et al.*, (2005) who found a negative correlation between leaf Δ and WUE in pot experiments. In both the experiments, runoff and drainage were negligible however, in the experiment carried out by Impa *et al.*, (2005), the soil surface was covered with a plastic film to minimize soil evaporation. The negative relationship between water use efficiency and Δ have probably reflected, in this case, a negative relationship between Δ and M/T , together with a low variation in harvest index. Conversely, the positive relationship between Δ and water use efficiency, in our study, could to be explained on the basis of higher harvest index and lower values of E_s/T for genotypes with high Δ . This is in good agreement with previous results for wheat showing that genotypes with high Δ have higher harvest index (Merah *et al.*, 2001) and transpiration (Morgan *et al.*, 1993) and can limit soil evaporation than to a faster growth rate (Condon *et al.*, 1993). It can be concluded from the present study that water use efficiency and grain yield in rice are mainly driven by harvest index, as already reported by Kondo *et al.*, (2004), Zhang & Yang (2003). Under water limited conditions, however, the use of Δ as indirect selection criterion may contribute considerably to increase grain yield and water use efficiency.

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