Theor Appl Genet (2009) 118:1065–1081
DOI 10.1007/s00122-009-0963-z

ORIGINAL PAPER

Leaf-level water use efficiency determined by carbon isotope discrimination in rice seedlings: genetic variation associated with population structure and QTL mapping

Yunbi Xu · Dominique This · Roman C. Pausch · Wendy M. Vonhof · Jason R. Coburn · Jonathan P. Comstock · Susan R. McCouch

Received: 27 February 2008 / Accepted: 8 January 2009 / Published online: 18 February 2009

Abstract Increasing the water use efficiency (WUE) of our major crop species is an important target of agricultural research. Rice is a major water consumer in agriculture and it is also an attractive genetic model. We evaluated leaf-level WUE in young rice seedlings using carbon isotope discrimination ($\Delta^{13}C$) as an indicator of the trait. A survey of $\Delta^{13}C$ was undertaken in 116 diverse germplasm accessions representing *O. sativa*, *O. glaberrima* and four wild *Oryza* species. *O. sativa* cultivars were classified into sub-populations based on SSR markers, and significant differences in $\Delta^{13}C$ were observed among the five genetically defined groups. While individual accessions explained a greater proportion of the variation than did sub-population, *indica* rice varieties had the lowest $\Delta^{13}C$ values overall, indicating superior WUE, while *temperate japonica* had the highest $\Delta^{13}C$. *O sativa* accessions had a similar or greater range of $\Delta^{13}C$ values than wild *Oryza* species, while domesticated *O. glaberrima* had a narrower range. Correlation analysis identified leaf morphological and physiological traits that were significantly associated with $\Delta^{13}C$, including longer leaves, more drooping leaves, higher tillering ability, and lower leaf nitrogen content. These trait associations were investigated by quantitative trait locus (QTL) mapping using backcross inbred lines derived from a cross between Nipponbare (*temperate japonica*) and Kasalath (*aus*). Seven QTL for $\Delta^{13}C$ were identified using composite interval analysis, located in five chromosomal regions. The QTL with the largest additive effect came from Kasalath and co-localized with QTL for leaf length, tiller number and nitrogen content.

Communicated by F. van Eeuwijk.

Y. Xu · D. This · J. R. Coburn · J. P. Comstock · S. R. McCouch (✉)
Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY 14853-1901, USA
e-mail: srm4@cornell.edu

R. C. Pausch · W. M. Vonhof · J. P. Comstock
Boyce Thompson Institute for Plant Research at Cornell University, Ithaca, NY 14853, USA

Present Address:
Y. Xu
CIMMYT, Apdo. Postal 6-641, 06600 Mexico, DF, Mexico

Present Address:
D. This
UMR DAP, Montpellier-SupAgro-CIRAD, Avenue Agropolis, 34398 Montpellier Cedex 5, France

Introduction

The global utilization of fresh water by human beings has exceeded the rate at which water resources can be replenished by hydrologic cycles of rain and snow-melt. As a result, ground-water resources in many regions are being unsustainably depleted. On a worldwide basis, 70–85% of water withdrawals are used in irrigated agriculture (Gleick 2003; Rosegrant 1998). In Asia, more than 50% of irrigation water is used to irrigate rice (Barker et al. 1999). The most environmentally friendly and durable solution to the problem of water shortage is to complement more efficient irrigation approaches with crops that have improved water use efficiency (WUE).

Direct measurement of WUE relies either on extensive leaf gas-exchange data or long-term measures of plant water consumption and biomass production. These approaches are logistically difficult in large-scale individual plant screening efforts. It has been demonstrated,

however, that carbon isotope discrimination (Δ) during growth can be an excellent surrogate for direct measurement of WUE (Farquhar and Richards 1984). During photosynthesis, plants naturally discriminate against the heavier stable isotope of carbon, ^{13}C, which makes up approximately 1.14% of total carbon in the earth's atmosphere. The ratio of $^{13}C/^{12}C$ in plant tissues shows subtle but systematic variations among different plant genotypes and/or plants grown under different conditions, and one of the principle mechanisms underlying this variation in $\Delta^{13}C$ acts through variation in the intercellular CO_2 concentration (c_i) maintained in leaves (Farquhar and Richards 1984). The linkage between $\Delta^{13}C$ and WUE is predicated on the concept that both are functionally dependent on c_i (Farquhar et al. 1989).

In this study, WUE is investigated at the level of the photosynthesizing leaf and is defined as the ratio of photosynthesis (A) to the amount of water lost in transpiration (E)

$$\mathrm{WUE} \equiv \frac{A}{E} \approx \frac{(c_a - c_i)}{1.6(w_i - w_a)} \quad (1)$$

where c and w refer to CO_2 and water vapor, respectively, and subscripts i and a refer to intercellular and external atmospheric concentrations, respectively (Condon et al. 2002; Farquhar and Richards 1984).

More than 90% of the water that a plant needs in its lifetime is lost indirectly by transpiration during the photosynthetic process. Plants therefore have opposing needs to capture carbon dioxide and to avoid excessive water loss.

These opposing needs must be balanced through the coordinated regulation of carboxylation capacity and stomatal control of leaf diffusive conductance (g_s), which together determine the value of c_i

$$c_i = c_a - {}^{A}/_{g_{CO_2}} \quad (2)$$

where g_{CO_2} is equal to g/1.6 (accounting for differences in diffusivity between water vapor and CO_2). Control of c_i is the principle means by which plants may alter the WUE as expressed by Eq. 1. When the stomatal conductance is high relative to carboxylation capacity, the plant can capture carbon dioxide at a high rate, but WUE is low. The trade-off between plant growth rate and high WUE is influenced by the integration of stomatal behavior, leaf photosynthetic capacity, root uptake capacity, canopy construction costs, and the transport functions within each plant, and is further subjected to the genetic control of all these functions. Greater WUE represents a strategy by which plants can increase the production of biomass when growing in water-limiting conditions (Cregg and Zhang 2000; Passioura 1977).

Recent reports in wheat have demonstrated the potential for selecting high WUE (low $\Delta^{13}C$) lines that have improved yields under drought conditions but equivalent yields under high water availability (Condon et al. 2002, 2004). Nonetheless, wheat and peanut breeders have noted that low WUE (higher $\Delta^{13}C$) may be associated with higher yields under well-watered conditions in some crops (Araus et al. 2003; Condon et al. 1987; Wright et al. 1993). An association of $\Delta^{13}C$ with leaf characters and other physiological traits has been reported for several plant species, including wheat, forage, knotweed and cotton (Condon et al. 1990; Geber and Dawson 1997; Johnson 1993; Saranga et al. 1999). However, the physiological and genetic basis for such associations is poorly understood.

Quantitative variation for $\Delta^{13}C$ has been reported for several plants, with higher WUE identified in some wild species compared to their cultivated counterparts (Franks and Farquhar 2001; Larcher 1995). McKay et al. (2003) reported a 3.0‰ total range of $\Delta^{13}C$ values among 39 diverse climatic ecotype accessions of *Arabidopsis*, which is equivalent to that reported here for 116 wild and cultivated accessions of *Oryza* and consistent with other rice reports (Dingkuhn et al. 1991; Kondo et al. 2004; Peng et al. 1998). Quantitative trait loci (QTL) for $\Delta^{13}C$ have been reported in *Arabidopsis* (Juenger et al. 2005; Masle et al. 2005), rice (Ishimaru et al. 2001b; Price et al. 2002; Laza et al. 2006; Takai et al. 2006), soybean (Specht et al. 2001), cotton (Saranga et al. 2004), and barley (Handley et al. 1994; Teulat et al. 2002).

In the study reported here, genetic diversity for $\Delta^{13}C$ was investigated using 116 diverse accessions representing six AA genome species of *Oryza*. Population sub-structure, influenced by constrasting demographic and breeding histories, was assessed by neutral SSR makers (Garris et al. 2005). We used QTL analysis to identify regions of the rice genome associated with variation for $\Delta^{13}C$ as well as leaf morphological and physiological characters. Specifically, we were interested in the following questions: (1) what is the range of $\Delta^{13}C$ available in a diverse collection of AA genome species of *Oryza*? (2) is there a significant difference in $\Delta^{13}C$ between Asian cultivated rice (*O. sativa*) and African cultivated rice (*O. glaberrima*) and other AA genome species? (3) is genetic variation for $\Delta^{13}C$ associated with the genetically identifiable subpopulations of *O. sativa* in any predictable way? (4) what is the relationship between $\Delta^{13}C$ and leaf morphological and physiological characters? and (5) how is $\Delta^{13}C$ genetically controlled? By addressing these questions, we aim to provide new insights into the genetics and evolution of $\Delta^{13}C$ in rice and to enhance the efficiency of breeding efforts aiming to improve the photosynthetic WUE of this important crop.

Materials and methods

Plant material

A total of 116 rice accessions were used to evaluate genetic diversity of $\Delta^{13}C$ representing six AA genome species of *Oryza*: *O. sativa* (66 accessions; Table 1), *O. glaberrima* (22), *O. barthii* (7), *O. glumaepatula* (4), *O. nivara*/*O. rufipogon complex* (7), *O. longistaminata* (10) (Table 2). The 66 *O. sativa* accessions are from 21 countries and the 50 other accessions are from 30 different countries. The rice accessions were grown in two experiments. The first experiment (E-1) contained 57 *O. sativa* accessions listed in Table 1 and 48 accessions of other species as listed in Table 2 (21 from *O. glaberrima* and 27 from four wild species). The second experiment (E-2) contained 72 accessions including 60 *O. sativa* accessions (Table 1), and four accessions each from *O. glaberrima*, *O. longistaminata*, and *O. nivara*/*O. rufipogon* complex (Table 2). Among the four *O. glaberrima* accessions used in E-2, three were also included in E-1, which results in a total of 22 *O. glaberrima* accessions used in the two experiments.

A total of 98 BC1F5 lines (hereafter referred to as backcross inbred lines, or BILs), derived via single-seed descent from the backcross (Nipponbare/Kasalath//Nipponbare) (Ishimaru et al. 2001b; Lin et al. 1998), were used for QTL mapping. Seeds and molecular segregation data from the BILs were kindly provided by the National Institute of Agrobiological Sciences, Japan (http://www.rgrc.dna.affrc.go.jp/ineNKBIL98.html). Two independent QTL mapping experiments (E-3 and E-4) were conducted using these 98 BILs and a fifth experiment (E-5) was conducted on a subset of 32 BILs to explore the relationship between $\Delta^{13}C$ and gas exchange.

Each of the first four experiments, two for genetic diversity analysis (June–July 2002 for E-1; May–June, 2003 for E-2) and two for QTL mapping (Dec 2002 for E-3; March 2003 for E-4), was planted in a randomized block design; there were three replications in the diversity analysis and two replications for the QTL study, and each block contained one plant per genotype. E-5 (July 2004) consisted of 32 BILs representing eight genotypic categories selected to confirm QTL results. All plants were grown in glasshouse environments with controlled temperature, humidity, and supplemental HID lighting (a bank of alternating 1,000 W Na-vapor and Me-Halide lamps) as described by Comstock et al. (2005). The greenhouses were located at the Boyce Thompson Institute for Plant Research on the Cornell University campus in Ithaca, New York, and monitored during growth for mean photosynthetically active radiation (400–700 nm; PAR), temperature, humidity, and ambient [CO_2] within each greenhouse bay.

All plants were phenotyped at the early vegetative stage, 3–4 weeks from germination. Plants were grown in 0.15 m diameter pots with 2.5 l soil volume and watered every 2 days. The potting soil was a mixture of 6:3:3:1:1 vermiculite:peat:fritted clay:sand:topsoil with dolomitic lime, gypsum, superphosphate, and two micronutrient supplements (unimix III and micro-max) added as amendments at rates of 3.9, 1.5, 0.38, 0.71 and 0.28 kg m^{-3}, respectively. Plants were fertilized once every other day beginning 1 week after germination with Peters 20:10:20 and an iron chelate (Sprint 330) applied at a concentration giving 100 ppm available nitrogen and 0.45 g l^{-1} chelate.

DNA extraction and SSR genotyping

For DNA extraction, leaf tissue was harvested after 3–4 weeks of growth in the greenhouse and stored frozen at −80°C. DNA extractions were performed as described by Tai and Tanksley (1990), except that frozen tissues were ground in liquid nitrogen with a mortar and pestle before extraction.

For the genetic diversity analysis, genotypic information for 60 *O. sativa* accessions (Table 1, used in E-2) was downloaded from the supplemental tables provided by Garris et al. (2005) and Lu et al. (2005). The genotypic data consisted of 169 SSR markers distributed along the 12 chromosomes with an average distance between markers of approximately 9 cM and an average of 14 markers per chromosome. All other *O. sativa* and *Oryza* germplasm (56 accessions) was genotyped as part of this study using SSR marker amplification, allele detection and allele calling protocols as described in Garris et al. (2005) and Lu et al. (2005).

Isotopic analysis

$\Delta^{13}C$ was evaluated as described in Comstock et al. (2005) using a Finnigan Matt Delta Plus isotope ratio mass spectrometer (IRMS) at the Cornell Stable Isotope Laboratory (COIL). Isotope ratio data were provided by COIL relative to the IAEA standard PDB, as:

$$\delta^{13}C = \left(\frac{\frac{^{13}C}{^{12}C}\text{sample}}{\frac{^{13}C}{^{12}C}\text{standard}} - 1 \right) \times 1,000‰ \quad (3)$$

$\delta^{13}C$ was measured for plant samples from each experiment, and $\Delta^{13}C$ was calculated as:

$$\Delta^{13}C = \frac{\delta^{13}C_{air} - \delta^{13}C_{plant}}{1 + \frac{\delta^{13}C_{plant}}{1,000}}‰ \quad (4)$$

The evaluation of $\delta^{13}C$ of atmospheric CO_2 was measured directly only at the beginning of the project to establish a relationship between $\delta^{13}C_{air}$ and 1/[CO_2] (Keeling 1958) in

Table 1 66 *Oryza sativa* accessions analyzed in this study

Accession name	RA#[a]	IRGC#[b]	GRIN #[c]	Country of origin	Sub-population	Experiment
Arang	RA4949	43,322		Indonesia	*indica*	E-1, E-2
ARC 10352	RA4981	12,440		India	*aromatic*	E-1
Asominori	RA1971			Japan	*temperate japonica*	E-2
Azucena	RA1295			Philippines	*tropical japonica*	E-2
Baber	RA4941	33,984		India	*temperate japonica*	E-1, E-2
Badkalamkati	RA4991	45,011		India	*indica*	E-1, E-2
Basmati	RA5325		PI 385418	Pakistan	*aromatic*	E-1, E-2
Basmati 1	RA4933	27,798		Pakistan	*aus*	E-1, E-2
Bico Branco	RA4950	38,994		Brazil	*aromatic*	E-1, E-2
Calrose	RA5118		CIor 12027	USA (California)	*temperate japonica*	E-1, E-2
Carolina Gold	RA4876	1,723		Madagascar	*tropical japonica*	E-1
Champa Tong 54	RA4936	30,238		Thailand	*aus*	E-1, E-2
Chhote Dhan	RA4978	58,930		Nepal	*admixture*	E-1, E-2
Cica8	RA5134		PI 439199	Colombia	*indica*	E-1, E-2
Cuba 65	RA5015	10,658		Cuba	*aromatic*	E-1, E-2
Cypress	RA5322		PI561736	USA (Louisiana)	*tropical japonica*	E-1, E-2
Dom Sofid	RA4929	12,880	PI 584607	Iran	*aromatic*	E-1, E-2
Dular	RA4985	32,561		India	*admixture*	E-1, E-2
DV85	RA5323	8,839		Bangladesh	*aus*	E-1, E-2
Early Wataribune	RA5319		CIor 9738	Japan	*temperate japonica*	E-1, E-2
Firooz	RA4952		PI 584569	Iran	*aromatic*	E-1, E-2
Fortuna	RA5045		CIor 1344	USA (Louisiana)	*tropical japonica*	E-1, E-2
Gharib	RA4937	32,303		Iran	*admixture*	E-1, E-2
Gogo Lempuk	RA4957	43,394		Indonesia	*tropical japonica*	E-1, E-2
Gotak Gatik	RA4959	43,397	PI 584572	Indonesia (C. Java)	*tropical japonica*	E-1, E-2
Hill Long Grain	RA5047		CIor 9052	USA (Texas)	*tropical japonica*	E-1, E-2
Honduras	RA5129		CIor 1643	Honduras	*tropical japonica*	E-1, E-2
IAC 25	RA4982	19,642		Brazil	*tropical japonica*	E-1
IR64	RA472			Philippines	*indica*	E-2
IRAT 177	RA5348			French Guiana	*tropical japonica*	E-1, E-2
IRGA 409	RA5279	77,483		Brazil	*indica*	E-1, E-2
Jefferson	RA5150			USA (Texas)	*tropical japonica*	E-2
Kalamkati	RA4963	45,975		India	*aus*	E-1, E-2
Kaniranga	RA5014	9,145		Indonesia	*admixture*	E-1, E-2
Kasalath	RA5339			India	*aus*	E-2
Kashmir Basmati	RA5358			Japan	*aromatic*	E-1, E-2
Khao Gaew	RA5033	24,224		Thailand	*aus*	E-1, E-2
Koshihikari	RA5337	10,907		Japan	*temperate japonica*	E-2
Kotobuki Mochi	RA4882	2,545		Japan	*tropical japonica*	E-1, E-2
Kun-min-tsieh-hunan	RA5011		PI 584544	China	*indica*	E-1
L-202	RA5001	66,292		USA (California)	*tropical japonica*	E-1, E-2
LAC 23	RA5035	14,957		Liberia	*tropical japonica*	E-1, E-2
Lady-Wright	RA5058		CIor 5451	USA (Louisiana)	*tropical japonica*	E-1, E-2
Lemont	RA4998	66,756		USA (Texas)	*tropical japonica*	E-2
Moroberekan	RA5342		PI 434632	Guinea	*tropical japonica*	E-1, E-2
N 22	RA4999	6,264		India	*tropical japonica*	E-1, E-2
Nep Hoa Vang	RA4945	40,748		Vietnam	*temperate japonica*	E-1, E-2
Nipponbare	RA1411			Japan	*temperate japonica*	E-2

Table 1 continued

Accession name	RA#[a]	IRGC#[b]	GRIN #[c]	Country of origin	Sub-population	Experiment
Oiran	RA4903	8,257		Japan	*admixture*	E-1
Oryzica Llanos	RA5298		PI 584668	Colombia	*indica*	E-1, E-2
OS-6	RA5364		PI 458474	Nigeria	*tropical japonica*	E-1, E-2
Pagaiyahan	RA5013			China (Taiwan)	*admixture*	E-1
Pankhari 203	RA4888	5,999		India	*aromatic*	E-1, E-2
Pappaku	RA4908	8,268		China (Taiwan)	*indica*	E-1, E-2
Peh-Ni-Nuo	RA4907	8,266		China	*admixture*	E-1, E-2
Pin Tawng	RA4943	40,673		Thailand	*indica*	E-1, E-2
Rathuwee	RA4911	8,952	PI 584605	Sri Lanka	*indica*	E-1, E-2
Rexoro	RA4875	1,715		USA	*tropical japonica*	E-1, E-2
Shinriki	RA5327		CIor 1642	Japan	*temperate japonica*	E-1, E-2
Shoemed	RA5123		PI 392539	USA	*temperate japonica*	E-1, E-2
Short Grain	RA5019	5,075		Thailand	*admixture*	E-1, E-2
Shuang-Chiang	RA4900	8,242		China (Taiwan)	*indica*	E-1, E-2
Taipei 309	RA5397		PI 366153	China (Taiwan)	*temperate japonica*	E-1, E-2
Teqing	RA5130		PI 536047	China	*indica*	E-2
Texas Patna	RA5137		Clor 8321	USA (Texas)	*tropical japonica*	E-1, E-2
TKM6	RA5381	237		India	*indica*	E-1, E-2

[a] In-house Cornell University Rice germplasm collection. http://ricelab.plbr.cornell.edu/

[b] International Rice Germplasm Collection at IRRI in the Philippines. http://www.irri.org/GRC/requests/requests.htm

[c] GRIN (Germplasm Resources Information Network) run by the NGRP (National Genetic Resources Program). http://www.ars-grin.gov/npgs/orders.html

the growth facility. In each experiment reported here, atmospheric [CO_2] was measured continuously in each greenhouse bay throughout the growth interval, and mean [CO_2] from the week preceding sampling was converted to an estimate of $\delta^{13}C_{air}$ using

$$\delta^{13}C_{air} = 4,429 \times \frac{1}{[CO_2]} - 21.45‰ \quad (5)$$

Leaves for isotopic analyses were chosen from the youngest cohort of leaves that had completed the phase of rapid expansion. These represented the largest leaves on the young vegetative plants, and occupied upper canopy positions experiencing maximal illumination. Two full leaf blades from each of two or more tillers per 3–4 week old rice plant were sampled. After drying 48 h at 60°C, leaf samples were ground into a homogeneous powder and 2 mg subsamples were weighed for isotopic analysis. In addition to $\delta^{13}C$, COIL analyses provided elemental composition in %N, with measurement precisions of ± 0.1‰, and ± 0.1%, respectively.

Morphology

Each plant in E-2 of the germplasm survey and one of the QTL analysis trials (E-4) was evaluated for leaf width, leaf length, tiller number, leaf blade flatness and erectness indices. Flatness index was scored as 1 for flat leaf, 2 for the intermediate type and 3 for a leaf with a semi-circular, channeled shape in cross-section. Leaf erectness index was scored as 1 for erect leaf, 2 for the intermediate type and 3 for a leaf which curved from base to tip such that the tip pointed back towards the ground.

Gas exchange

Leaf photosynthetic gas-exchange was measured on 32 of the BIL using a LICOR6400 portable photosynthesis system. Leaves were measured on plants in the greenhouse. To ensure that all plants were uniformly exposed to high light intensities [similar to bright midday conditions in their normal growth positions (ca 1,000 μmol (PAR) m^{-2} s^{-1})], gas exchange measurements were taken at a station that provided extra HID lighting to plants situated under a heat-shield. The heat shield consisted of circulating water in a suspended 1 × 2 × 0.1 m plexiglass tray. This arrangement allowed for rapid measurement of light-saturated rates of photosynthesis on target leaves while maintaining normal whole-plant activity (temperature and relative humidity). Each measurement consisted of two stages, the first was done on a leaf clamped in an ambient cuvette [CO_2] of 370 μL L^{-1} (for 7 min), and the second at 340 μL L^{-1} (for 4 min). Reported values of leaf diffusive

Table 2 50 *Oryza glaberrima* and wild species accessions used in this study

Population	Source[a]	Accession no.[b]	RA no.[c] (Cornell)	Variety name	Country of origin	Experiment
O. barthii	IRRI	100933	RA3235	NA	Sudan	E-1
O. barthii	IRRI	100936	RA3236	NA	Niger	E-1
O. barthii	IRRI	103895	RA3241	NA	Senegal	E-1
O. barthii	IRRI	103909	RA3242	NA	Tanzania	E-1
O. barthii	IRRI	104066	RA3244	NA	Chad	E-1
O. barthii	IRRI	106208	RA3248	NA	Mali	E-1
O. barthii	IRRI	106291	RA3251	NA	Mauritania	E-1
O. glaberrima	WARDA	01139	RA3039	5324 TOG	Nigeria	E-1
O. glaberrima	WARDA	01160	RA3077	5397 TOG	Nigeria	E-1
O. glaberrima	WARDA	01221	RA3062	5486 TOG	Nigeria	E-1
O. glaberrima	WARDA	01400	RA3117	6154 TOG	Nigeria	E-1
O. glaberrima	WARDA	01413	RA3126	6203 TOG	Guinea Bissau	E-1
O. glaberrima	WARDA	01431	RA3135	6222 TOG	Burkina Faso	E-1, E-2
O. glaberrima	WARDA	01529	RA3168	6324 TOG	Liberia	E-1
O. glaberrima	WARDA	01653	RA3187	6461 TOG	Mali	E-1
O. glaberrima	WARDA	02029	RA3192	7103 TOG	Mali	E-1
O. glaberrima	WARDA	02085	RA3199	7173 TOG	Senegal	E-1
O. glaberrima	WARDA	02097	RA3201	7192 TOG	Ivory Coast	E-1, E-2
O. glaberrima	WARDA	02171	RA3203	7274 TOG	Cameroon	E-1
O. glaberrima	WARDA	02235	RA3208	7417 TOG	Mali	E-1
O. glaberrima	WARDA	04325	RA3211	CG14	Ivory Coast	E-2
O. glaberrima	WARDA	Unknown	RA3218	DC Kono	Sierra Leone	E-1
O. glaberrima	WARDA	Unknown	RA3219	PA DC Kono	Sierra Leone	E-1
O. glaberrima	WARDA	Unknown	RA3224	Sali Foreh	Sierra Leone	E-1
O. glaberrima	WARDA	0695	RA3215	T2	Ivory Coast	E-1, E-2
O. glaberrima	WARDA	0665	RA3220	YS 168	Guinea Conakry	E-1
O. glaberrima	WARDA	0602	RA3223	YS 179	Guinea Conakry	E-1
O. glaberrima	WARDA	0628	RA3221	YS 230	Guinea Conakry	E-1
O. glaberrima	WARDA	0556	RA3222	YS 351	Guinea Conakry	E-1
O. glumaepatula	IRRI	100968	RA3387	NA	Suriname	E-1
O. glumaepatula	IRRI	103812	RA3392	NA	Venezuela	E-1
O. glumaepatula	IRRI	105465	RA3399	NA	French Guiana	E-1
O. glumaepatula	IRRI	105670	RA3386	NA	Brazil	E-1
O. longistaminata	IRRI	101202	RA3388	NA	Nigeria	E-1
O. longistaminata	IRRI	101210	RA3389	NA	Ivory Coast	E-1
O. longistaminata	IRRI	101222	RA3390	NA	Mali	E-1
O. longistaminata	IRRI	101223	RA3391	NA	Mali	E-1
O. longistaminata	IRRI	103886	RA3393	NA	Tanzania	E-1
O. longistaminata	IRRI	104302	RA3394	NA	Zambia	E-1
O. longistaminata	IRRI	104637	RA3395	NA	Ethiopia	E-1, E-2
O. longistaminata	IRRI	105078	RA3396	NA	Africa	E-1, E-2
O. longistaminata	IRRI	105183	RA3397	NA	Ghana	E-1, E-2
O. longistaminata	IRRI	105205	RA3398	NA	Ethiopia	E-1, E-2
O. nivara	IRRI	105343	RA2744	NA	India	E-1
O. nivara	IRRI	105428	RA3381	NA	Sri Lanka	E-1
O. nivara	IRRI	105706	RA3379	NA	Nepal	E-1, E-2
O. rufipogon	IRRI	105491	RA2747	NA	Malaysia	E-2
O. rufipogon	IRRI	105567	RA3382	NA	Indonesia	E-1

Table 2 continued

Population	Source[a]	Accession no.[b]	RA no.[c] (Cornell)	Variety name	Country of origin	Experiment
O. rufipogon	IRRI	105868	RA3385	NA	Bangladesh	E-1, E-2
O. rufipogon/O. nivara	IRRI	100596	RA3383	NA	Taiwan, China	E-1, E-2

[a] Source = Germplasm repository from which accessions can be requested: IRRI (http://www.irri.org/GRC/requests/InterimMTA.htm); WARDA (http://www.sgrp.cgiar.org/CGIARSystem/AfricaRiceCenter.htm)

[b] Accession no. = accession number to be used for ordering seeds

[c] RA no. = rice accession (RA) number indexed in the Cornell collection

conductance (g) and c_i were taken from the 370 ambient [CO_2] point, while the CO_2 step was used to interpolate linearly a photosynthetic rate at a common c_i value [$A_{(\text{mean } c_i)}$] for all plants, assuming that the A-c_i relation is linear over this small range of CO_2 levels, to allow an estimate of photosynthetic capacity independent of stomatal influence.

Statistical analysis

Analysis of variance for genetic diversity was implemented using the statistical software package JMP V5.0 (SAS institute Inc.). For evaluation of subpopulation effects in *O. sativa,* genetic subpopulation was entered as the primary model effect with individual accessions nested within subpopulations as random effects. Comparison of multiple means was performed using the Tukey–Kramer HSD test.

Population structure analysis was undertaken using the program STRUCTURE 2.0 (Falush et al. 2003; Pritchard et al. 2000). This method uses multilocus data to infer the fraction of an accession's genetic ancestry that belongs to a particular sub-population, given a specified number of populations (K). Population structure was inferred for 60 *O. sativa* accessions (in E-2) that had previously been genotyped with 169 SSR markers (a subset of those analyzed by Garris et al. (2005)) using a burn-in period of 100,000 with 100,000 of MCMC replications, where $K = 6$ and the model allowed for admixture and correlated allele frequencies. Five independent runs yielded consistent results.

QTL mapping

Segregation data for 245 RFLP markers distributed along the 12 chromosomes of rice on 98 Nipponbare/Kasalath BILs was kindly provided by the National Institute of Agrobiological Sciences, Japan (http://rgp.dna.affrc.go.jp/publicdata/genotypedataBILs/genotypedata.html). In this study, 11 SSR markers were added to the existing RFLP map to increase the mapping resolution across putative QTL regions. QTL analysis was performed using composite interval mapping implemented in the software Windows QTL Cartographer 2.5 (Wang et al. 2006). A critical LOD (log of odds) for declaring the presence of putative QTL additive effects was determined by permutation tests based on 10,000 iterations.

Results

Species level comparisons in the genus *Oryza*

By screening a diverse collection of 105 rice germplasm accessions (in E-1) including 27 accessions from four wild species of *Oryza,* 21 accessions of the African cultivated

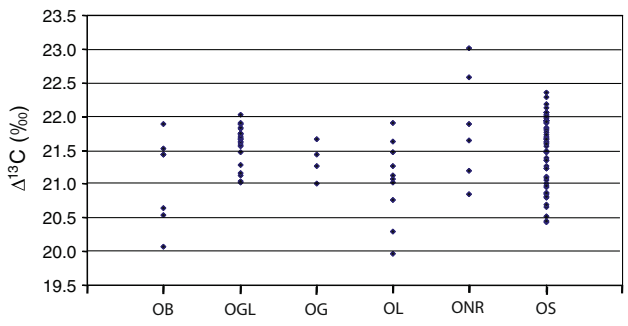

Fig. 1 Distribution of $\Delta^{13}C$ (‰) in six AA genome species of *Oryza*: *OB O. barthii*, *OGL O. glaberrima*, *OG O. glumaepatula*, *OL O. longistaminata*, *ONR O. nivara/O.rufipogon* complex, *OS O. sativa*

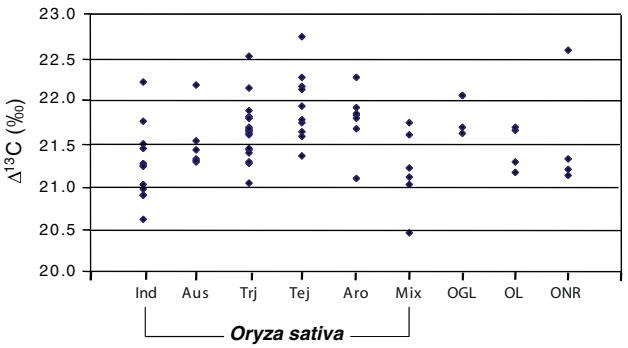

Fig. 2 Distribution of $\Delta^{13}C$ (‰) in subpopulations of *O. sativa*: (*Ind indica*, *Aus aus*, *Trj tropical japonica*, *Tej temperate japonica*, *Aro aromatic*, *Mix admixture*); *OG O. glaberrima*, *OL O. longistaminata*; *ONR nivara/O. rufipogon* complex

species *O. glaberrima* (Table 2), and 57 cultivars of *O. sativa* (Table 1) under favorable conditions, we found considerable genetic variation in $\Delta^{13}C$ (Fig. 1; Table S1). An ANOVA explaining $\Delta^{13}C$ based on accession (genotype) alone had an adjusted R^2 (coefficient of determination) of 0.70 and 0.62 in E-1 and E-2, respectively, and a correlation of 0.73 for accession mean values across the two experiments. Species level effects were formally tested in E-1 where a sufficient number of accessions were included for species other than *O. sativa,* while associations between $\Delta^{13}C$ and other plant traits were evaluated in E-2.

The 21 accessions of the African cultivated species *O. glaberrima,* showed significantly lower variance in $\Delta^{13}C$ than other species (Fig. 1; Table S1). These accessions included representatives from four of the cryptic subpopulations identified by Semon et al. (2005), including eight corresponding to Group 1, the sub-population that was most widely distributed throughout West Africa, 12 that were classified as admixtures (Groups 2 and 3), and six corresponding to a population sharing ancestry with a *japonica*-like *O. sativa* subpopulation (Group 5). When the data was analyzed using Welch ANOVA, which allowed for unequal variances, *O. glaberrima* was found to have significantly higher average $\Delta^{13}C$ (lower WUE) than either of the African wild species, *O. longistaminata*, or *O. barthii* ($P = 0.03$).

O. sativa and *O. glaberrima* had similar mean values of $\Delta^{13}C$, but *O. sativa* had a significantly larger standard deviation for $\Delta^{13}C$ values among contrasting cultivars, with variation equivalent to that observed among the 27 wild species accessions. *S*ome *O. sativa* cultivars had $\Delta^{13}C$ values that were similar to the best wild accessions, suggesting that they would be good sources of WUE. In E-2, the wide range of behaviors among contrasting *O. sativa* cultivars was very apparent (Fig. 2; Table S2). The ranking of means for the species *O. glaberrima*, *O. longistaminata*, and *O. sativa* was similar in E-1 and E-2, but the variation of *O. glaberrima* and *O. longistaminata* fell within the range of the *O. sativa* distribution in E-2. We therefore undertook an evaluation to determine how variation in $\Delta^{13}C$ was distributed within the sub-populations of *O. sativa*, and also investigated the inheritance of $\Delta^{13}C$ based on QTL analysis using a mapping population derived from two contrasting *O. sativa* cultivars.

Population structure in *Oryza sativa* accessions

The 60 *O. sativa* cultivars used in E-2 are a subset of those evaluated by Garris et al. (2005) where five significant subpopulations were identified within the species. We ran a parallel population structure analysis (Pritchard et al. 2000) and confirmed the previous results using this subset of 60 accessions (Table 1). Based on the 169 SSR markers, 54 of the 60 cultivars shared ≥70% ancestry with one of the five previously identified groups (Garris et al. 2005): *indica* (12 accessions), *tropical japonica* (19), temperate *japonica* (10), *aromatic* (7) and *aus* (6) (Table 1; Fig. 2; Table S2). Six (10%) of the accessions clustered separately and were classified as admixtures, with varying levels (40.5–68.1%) of ancestry shared among the five groups. Of these six varieties, Chhote Dhan (from Nepal) and Kaniranga (Indonesia) shared 68 and 55% ancestry, respectively, with *indica*, Short Grain (Thailand) and Dular (India) both shared 51% ancestry with *tropical japonica*, and Peh-Ni-Nuo (China) and Gharib (Iran) shared 64 and 41% ancestry, respectively, with *temperate japonica*.

Genetic variation in $\Delta^{13}C$ and other traits within *O. sativa*

The distribution of $\Delta^{13}C$ among the 60 *O. sativa* accessions evaluated in E-2 is shown in Fig. 2; (Table S2). Genotypes were planted in a randomized block design with three replications and $\Delta^{13}C$ showed an approximately normal distribution (skewness = 0.0668; Kurtosis = 0.2941) with an average of 21.57 ± 0.44‰ and a range of 20.45‰ (Chhote Dhan) to 22.73‰ (Taipei 309).

Seven other traits were measured in addition to $\Delta^{13}C$. These included four measures of leaf morphology including leaf width and length, and additional leaf indices describing the tendency of the blade to be either flat or 'channeled' on its upper surface, and whether the leaf blade was held 'erect' in a single steeply inclined line from base to tip, or had an arcing shape with recurved leaf tips pointing back towards the ground. Leaf nitrogen content as a percentage of total mass (%N) was measured from the same leaf blade samples as isotopic ratio, and incipient tiller number was counted. R^2 values (coefficient of determination) for respective single level ANOVAs based on accession were highly significant for all traits measured (Table 3). Several of the leaf morphological traits were correlated with each other. 'Erect' leaves tended to be shorter, and more deeply 'channeled'. Higher nitrogen contents were weakly associated with shorter leaves and low propensity for early tillering. $\Delta^{13}C$ was also significantly correlated with several of these traits, with the strongest correlations being with leaf length and %N.

All of the above mentioned traits were further evaluated for dependency on genetic subpopulation within *O. sativa,* as discussed above. Only the 54 accessions clearly associated with a single subpopulation (i.e., admixtures were ignored) were included in these analyses. All of the traits examined showed some degree of dependency on subpopulation, but in some cases it was a relatively weak effect. Comparing sums of squares (SS) distributed between subpopulation and accession (genotype) (nested as random

Table 3 Correlations among carbon isotope discrimination ($\Delta^{13}C$), leaf morphological traits, tiller number and leaf nitrogen content (%N) based on means of three replicates each for 60 *O. sativa* accessions

	$\Delta^{13}C$	%N	Leaf width	Leaf length	Leaf flatness	Leaf erectness	Tiller number
% nitrogen	0.44**[a]						
Leaf width	−0.23	−0.08					
Leaf length	−0.51**	−0.52**	0.31*				
Leaf flatness	0.27**	0.37**	−0.40**	−0.30*			
Leaf erectness	−0.37**	−0.36**	0.27*	0.54**	−0.71**		
Tiller number	−0.32*	−0.52**	−0.12	0.08	−0.30*	0.37**	
R^2	(0.72)[b]	(0.43)	(0.87)	(0.79)	(0.53)	(0.72)	(0.69)

[a] R^2 values calculated from single level ANOVAs where * $P < 0.05$ and ** $P < 0.01$

[b] Numbers in parentheses represent the total amount of variation associated with 'accession' for each trait, based on ANOVAs

effects within subpopulation in ANOVA) gives perspective on traits that are strongly divergent among subpopulations (Table 4). Only for leaf width did sub-population have a greater SS than accession (genotype). In this case the most divergent subpopulations were the *temperate japonicas* (narrower leaves) versus *tropical japonicas* (wider leaves), which had non-overlapping ranges for the trait. For leaf length, erectness and %N, sub-population contributed about 30–40% of total explained SS, and for leaf channeling, tiller number, and $\Delta^{13}C$, sub-population contributed only about 25–30%. The detailed distribution of values is graphed by subpopulation for $\Delta^{13}C$ in Fig. 2. Throughout all of these analyses, the *temperate japonica* sub-population tended to be the most phenotypically extreme. Individuals in this group had the narrowest, shortest, most erect, and most strongly channeled leaves of any of the sub-populations. They also had low tiller number, high %N, and high $\Delta^{13}C$, indicating low WUE. *Indica* was the most phenotypically divergent from *temperate japonica*, as seen specifically for $\Delta^{13}C$, %N, tiller number, leaf erectness, and leaf channeling. This association of trait values among contrasting subpopulations is also consistent with the correlations among traits seen at the level of all accessions (Table 4). The so-called "admixture" group showed the lowest $\Delta^{13}C$ values (Fig. 2).

QTL analysis of $\Delta^{13}C$ and other traits in a cross of two *O. sativa* cultivars

To gain insight into the genetic control of variation in $\Delta^{13}C$, QTL analyses were performed using a mapping population of 98 backcross inbred lines (BILs) derived from contrasting *temperate japonica* (cv. Nipponbare = N) and *aus* (cv. Kasalath = K) parents. Two screenings of the

Table 4 Association of trait values with individual accession and genetic sub-population in *O. sativa*

Leaf width		Leaf length		Leaf flatness		Leaf erectness		Tiller number		%Nitrogen		$\Delta^{13}C$	
Sub-P	Mean[a]	Sub-P	Mean	Sub-P	Mean	Sub-P	Mean	Sub-P	Mean	Sub-P	Mean	Sub-P	Mean
TrJ	11.9a	*Aus*	70.7a	*TeJ*	2.3a	*Ind*	2.4a	*Ind*	13.0a	*TeJ*	4.8a	*TeJ*	21.9a
Aus	10.9ab	*Aro*	66.6a	*Aro*	1.9ab	*TrJ*	2.2a	*Aus*	10.9ab	*trJ*	4.7ab	*Aro*	21.8a
Ind	9.6bc	*Ind*	66.3a	*TrJ*	1.7b	*Aus*	2.2a	*TrJ*	9.5b	*Aro*	4.5abc	*TrJ*	21.6a
Aro	9.0bc	*TrJ*	63.4a	*Ind*	1.7b	*Aro*	2.0ab	*TeJ*	9.4b	*Aus*	4.5bc	*Aus*	21.5a
TeJ	8.5c	*TeJ*	53.2b	*Aus*	1.4b	*TeJ*	1.2b	*Aro*	9.1b	*Ind*	4.5c	*Ind*	21.3ab

	Leaf width		Leaf Length		Leaf flatness		Leaf erectness		Tiller number		%N		$\Delta^{13}C$	
	SS[b]	*P* val	SS	*P* val	SS	*P* val	SS	*P* val	SS	*P* val	SS	*P* val	SS	*P* val
Sub-Pop	290	***	4606	***	12	***	26.4	***	357	*	3.18	***	8.4	*
Accession	266	***	6758	***	34.4	***	40.4	***	1092	***	7.33	***	20.5	***
Error	50		1625		20		14		370		6.79		6.5	
Total	606		12989		66.4		80.8		1819		17.3		35.5	

Sub-P subpopulation, *Aro aromatic*, *Aus aus*, *Ind indica*, *TeJ temperate japonica*, *TrJ tropical japonica*

* $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$

[a] Tukey HSD comparison of means for all sub-populations. Different letters are significantly different from each other at $P < 0.05$

[b] SS sum of squares from ANOVA with 'accession' nested as a random variable within 'sub-population'

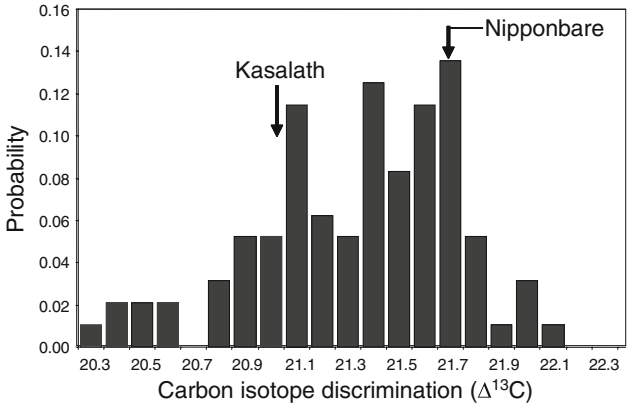

Fig. 3 Frequency distribution of $\Delta^{13}C$, showing genetic variation observed in 98 backcross inbred lines (BILs) derived from two *O. sativa* accessions, Nipponbare (*temperate japonica*) and Kasalath (*aus*)

full mapping population were performed, with a smaller experiment (E-5) conducted on 32 selected lines to confirm QTL effects. In E-3, only $\Delta^{13}C$ and the analytically associated %N traits were measured, in E-4, all of the morphological traits discussed above were added to examine the associations, and E-5 was undertaken to evaluate gas exchange. The frequency distribution of $\Delta^{13}C$ values among the BILs, averaged for the two experiments (M), was approximately normal (Fig. 3; skewness = −0.4694; kurtosis = 0.0393), with an average of 20.75‰ and a range of 19.66–21.59‰.

A total of seven QTL were identified for $\Delta^{13}C$, which were co-localized in five genomic intervals on chromosomes 1, 4, 7, 8 and 11 (LOD > 3.23) (Table 5; Fig. 4). The QTL on chromosomes 1, 4, and 8 had low $\Delta^{13}C$ (putatively high WUE) alleles coming from Kasalath, while the QTL on chromosomes 7 and 11 had low $\Delta^{13}C$ alleles derived from Nipponbare. The percent of the phenotypic variation explained by the largest QTL was 22.2% ($\Delta^{13}C1.1\_E4$), while the QTL with the smallest effect explained 7.6% ($\Delta^{13}C11.1\_M$), as detected using the mean values from E-3 and E-4. $\Delta^{13}C1.1\_E4$ (and $\Delta^{13}C1.1\_M$) co-localized with a QTL for leaf length that explained 35.7% of the phenotypic variation for the trait as well as with QTL for tiller number and %N (Fig. 4). In this genomic region, the Kasalath alleles were associated with lower $\Delta^{13}C$ (suggesting higher WUE), longer leaves, fewer tillers and lower %N.

QTL analysis also identified three QTL for leaf width (two on chromosome 1 and one on chromosome 12), four for leaf length (one each on chromosomes 1, 3, 4 and 12), one for leaf flatness (chromosome 7), four for tiller number

Table 5 QTL associated with six morphological and physiological traits in rice and the relative magnitude of QTL effects

Trait	Chr[a]	QTL	Peak marker	Peak LOD score	Additive allele effect[b]	R^{2c}
Leaf width_E4[d]	1	*LW1.1*	C955	3.43	−0.27	0.11
	1	*LW1.2*	C808	3.44	0.22	0.10
	12	*LW12.1*	C1069	4.39	0.30	0.14
Leaf length_E4	1	*LL1.1*	RM3520	13.41	−2.84	0.36
	3	*LL3.1*	C1488	3.34	−1.18	0.08
	4	*LL4.1*	R514	3.30	1.55	0.08
	12	*LL12.1*	C1069	4.29	1.65	0.10
Leaf flatness_E4	7	*LF7.1*	C596	5.20	0.26	0.17
Tiller number_E4	1	*TN1.1*	C742	5.28	1.25	0.15
	2	*TN2.1*	G132	5.07	−1.48	0.25
	2	*TN2.2*	R1826	5.72	−1.17	0.17
	3	*TN3.1*	C1677	3.45	0.99	0.10
%Nitrogen_E3	1	*%N 1.1*	RM3520	3.97	0.11	0.12
	9	*%N 9.1*	C570	3.89	0.11	0.13
%Nitrogen_M	1	*%N 1.1*	RM3520	4.45	0.11	0.12
	6	*%N 6.1*	R1954	3.43	−0.11	0.14
	9	*%N 9.1*	C506	3.65	0.10	0.10
$\Delta^{13}C\_E3$	8	*Δ^{13}C8.1*	C166	4.25	0.25	0.12
$\Delta^{13}C\_E4$	1	*Δ^{13}C1.1*	R2414	8.75	0.23	0.22
	4	*Δ^{13}C4.1*	R288	5.34	0.17	0.14
$\Delta^{13}C\_M$	1	*Δ^{13}C1.1*	R2414	5.49	0.18	0.14
	4	*Δ^{13}C4.1*	R288	5.07	0.16	0.14
	7	*Δ^{13}C7.1*	C1057	4.08	−0.17	0.11
	11	*Δ^{13}C11.1*	C794A	3.37	−0.13	0.08

[a] *Chr* chromosome

[b] Additive-allele effect is positive when the Nipponbare allele increases the trait value; allele effect is negative when Nipponbare allele decreases the trait value

[c] R^2 phenotypic variation explained by the QTL effect

[d] Suffix following trait name or abbreviation indicates the experiment in which the QTL was identified: E3 experiment 3, E4 experiment 4, M mean from E3 and E4

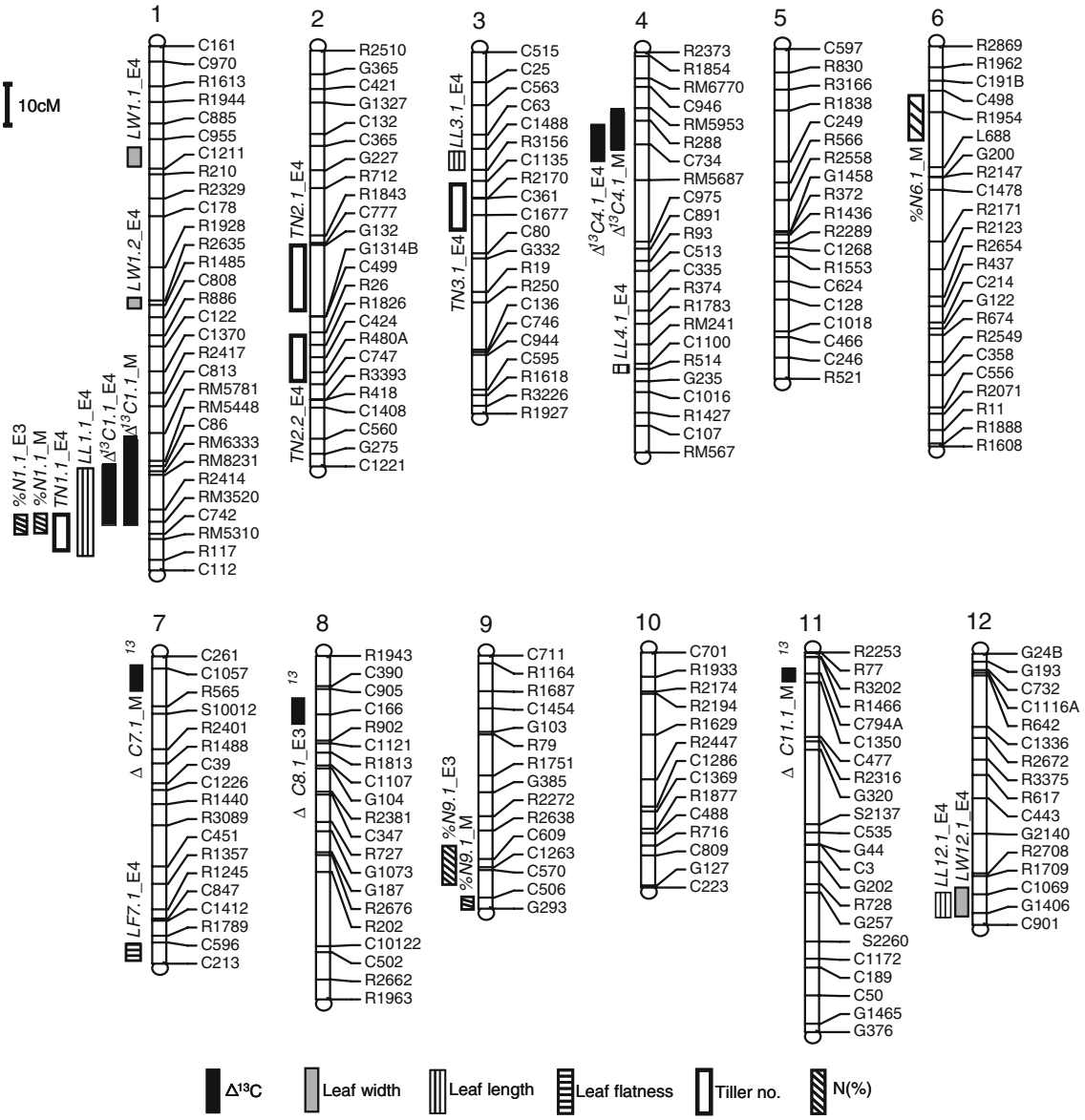

Fig. 4 Co-localization of QTL for $\Delta^{13}C$, leaf width, leaf length, leaf flatness, tiller number and leaf nitrogen content (N%) identified using 98 backcross inbred lines (BILs) derived from two *O. sativa* accessions, Nipponbare (*temperate japonica*) and Kasalath (*aus*). Empirical thresholds were determined based on 10,000 permutations where the LOD score for a significant QTL is 3.23 ($P < 0.05$)

(two on chromosome 2, and one each on chromosomes 1 and 3), and four for %N (two on chromosome 9 and one each on chromosomes 1 and 6). For all the traits with more than one QTL identified, each parent contributed positive alleles at some loci and negative alleles at others (Table 5).

$\Delta^{13}C$ and gas-exchange measurements among Nipponbare X Kasalath BILs

Targeting the three most significant $\Delta^{13}C$ QTL, residing on chromosomes 1, 4, and 8, BIL lines were categorized based on all possible combinations of Nipponbare and Kasalath alleles at markers located within these QTL regions. Eight categories were so defined, and four BILs were chosen to represent each category. These 32 BILs were evaluated for gas-exchange in E-5. Gas exchange measurements were taken to assess net photosynthetic rate at a standardized c_i [$A_{(\text{mean } c_i)}$); see "Materials and methods"], leaf conductance to diffusive exchange (modulated via stomatal aperture g), and c_i directly. This experiment reconfirmed the association between an isotopic signal and marker alleles at both $\Delta^{13}C$*1.1* and $\Delta^{13}C$*4.1*, and also indicated that the isotopic signal was associated with the expected shifts in c_i in both of these cases ($P = 0.008$ and 0.02, respectively). Markers across the $\Delta^{13}C$*8.1* QTL showed a $\Delta^{13}C$ effect as expected but only a non-significant trend ($P = 0.07$) in the predicted

direction for c_i. Since c_i is directly related to WUE (Eq. 1) while $\Delta^{13}C$ is an indirect proxy, this provided important confirmation from an independent measurement technique that these putative QTL, identified via $\Delta^{13}C$ analysis, were in fact associated with WUE differences.

It should be noted that under our cuvette measurement conditions of constant c_a and humidity gradient, c_i and instantaneous leaf-level WUE are virtually perfectly correlated in the 32 BILS ($R^2 = -0.98$). This is expected from the measurement conditions in which ambient CO_2 and the leaf-to-air water vapor gradient were held constant for all leaves in the measurement cuvette. Since WUE $= (c_a - c_i)/1.6(w_i - w_a)$ and c_i was the only variable, they should be perfectly correlated by definition. The minor deviation from the theoretical correlation of 1.0 is due to a small amount of noise in the control functions of the LICOR 6400 instrument. Instantaneous water-use efficiency measured under the cuvette conditions was therefore significantly correlated ($P < 0.01$) with the longer-term, integrated measure of WUE during growth reflected in Δ.

Additional insight into WUE mechanisms operating in this rice mapping population could be found in the patterns of the correlation matrix of $A_{(\text{mean } c_i)}$, g, c_i, $\Delta^{13}C$, and %N for the overall dataset based on mean values of the 32 BILs (Table 6). $A_{(\text{mean } c_i)}$ was strongly, positively correlated with stomatal opening. This indicates that plants with high g also had higher biochemical capacity for photosynthetic carbon uptake as seen in a wide range of wild and domesticated plants (Wong et al. 1979). If the relative variation in $A_{(\text{mean } c_i)}$ and g were always in perfect proportion, there would be no variation in c_i (Eq. 2), but in fact there was a substantially larger range of variation in g such that variation in c_i was strongly, positively correlated with g. c_i was also positively associated with $A_{(\text{mean } c_i)}$. Consistent with theoretical relationships, $\Delta^{13}C$ was then significantly correlated with c_i and g, but not with $A_{(\text{mean } c_i)}$. %N was positively correlated with $A_{(\text{mean } c_i)}$ as is generally expected due to the high nitrogen requirements of photosynthetic capacity in carboxylating enzymes, pigments, and electron transport components.

Table 6 Correlation matrix of gas-exchange characters, % nitrogen (%N), and carbon isotope discrimination ($\Delta^{13}C$) in the 32 BILs

	$A_{(\text{mean } c_i)}$ (μmol m^{-2} s^{-1})	c_i (μL L^{-1})	g (mol m^{-2} s^{-1})	%N (g g^{-1} 100)
c_i (μL L^{-1})	0.41*[a]			
g (mol m^{-2} s^{-1})	0.75**	0.89**		
%N	0.35*	0.00	0.16	
$\Delta^{13}C$	0.19	0.49**	0.42*	0.30

[a] * $P < 0.05$; ** $= P < 0.01$ where correlations are based on mean values of 32 backcross introgression lines (BILs). The means were based on 4-plant reps per BIL, and gas exchange characters were measured twice on each plant. Critical value of pairwise correlation coefficients = 0.34 ($P < 0.05$) and 0.44 ($P < 0.01$). Under our Cuvette measurement conditions of constant c_a and humidity gradient, c_i and instantaneous, leaf-level WUE are almost perfectly correlated ($r = -0.98$)

Discussion

Five questions motivated this work and provide a framework for interpreting the significance of these diversity studies.

(1) *What is the range of $\Delta^{13}C$ to be found in AA genome species of Oryza?* Use of a randomized block design and statistical comparisons of group means helped reduce the environmental error and increase the statistical power of our experiments. This was in contrast to our pre-trials where we had no replications and did not take population structure into account. Environmental factors (temperature, irradiance, and cultural conditions) were well controlled in this study, following the recommendations from a comprehensive multi-factor experiment reported previously (Comstock et al. 2005). The data presented from a total of 116 rice accessions representing seven different rice species is the most thorough survey published on rice to date and allows comparison with other well studied crops such as barley and wheat.

The mean value of $\Delta^{13}C$ for the rice accessions was 21.51‰, a fairly high discrimination value indicating moderately low WUE, but comparable to values reported for many other crops evaluated in the vegetative stage under water replete conditions, including mustard, canola and pea (Knight et al. 1994), sunflower (Lambrides et al. 2004), potato (Minhas et al. 2003), breadwheat (Monneveux et al. 2005), and cotton (Stiller et al. 2005). A substantial range was found among the accessions in our study, spanning 19.97–23.02‰ in E-1 and 20.45–22.73‰ in E-2. These values are equal to or greater than previously reported values for rice. Dingkuhn et al. (1991) reported a range of $\Delta^{13}C$ values between 19.8 and 21.5‰ for 28 upland rice cultivars grown under mild water stress, Kondo et al. (2004) reported a range of 20.3–21.9‰ obtained in shoots of 11 cultivars and Cabuslay et al. (2002) reported a range of 20.1–21.8‰ obtained for 27 cultivars grown under water deficit for 6 days. This is substantial variation, but still less than seen in some other crops and wild species.

(2) *What are the species level patterns for $\Delta^{13}C$ variation within the genus Oryza?* Distinct differences were found among species, but there were no general patterns between cultivated and wild species. *O. glaberrima* ($n = 22$) and perhaps *O. glumaepatula* ($n = 4$) had very narrow ranges of variation and high mean $\Delta^{13}C$, indicating low WUE. *O. sativa* and the other wild species all had much larger ranges of variation in $\Delta^{13}C$, which were

similar in E-1 and largest in *O. sativa* in E-2. Two of the African wild species, *O. barthii* and *O. longistaminata,* had lower overall mean values of $\Delta^{13}C$ than the cultivated species, *O. sativa* and *O. glaberrima* (suggesting higher WUE) but the Asian wild species most closely related to *O. sativa,* the *O. nivara*/*O. rufipogon* complex, did not. The broad variation observed in *O. sativa* is particularly interesting in view of common expectations that wild species tend to have higher WUE than their domesticated relatives (Franks and Farquhar 1999; Larcher 1995). This expectation is due, in part, to breeding practices that emphasize maximal yield, which will often select for very high diffusive conductance. *O. sativa* appears to have similar, high $\Delta^{13}C$ values as compared to its Asian wild ancestors, along with a large fraction of low $\Delta^{13}C$ lines (high WUE). This could be related to the initial wet-land ancestry of *O. sativa*, where very open stomata and high $\Delta^{13}C$ is likely to represent the ancestral state. The broad range of ecological conditions to which rice cultivars have become adapted during the long history of crop development, coupled with the diversity of cultural practices in different ecological and geographic regions, have certainly influenced crop evolution, and may be responsible for the selection of some lower $\Delta^{13}C$ (higher WUE) accessions within *O. sativa*.

(3) *Is genetic variation for* $\Delta^{13}C$ *partitioned within O. sativa in any predictable way?* We found significant variation in all traits examined as a function of genetic subpopulation identity within *O. sativa* (Table 4). At our sample sizes, averaging 11 accessions per subpopulation, this variation was most often statistically significant only in contrasts of the most extreme groups for each trait, and substantial overlap of trait values between subpopulations was the rule in all cases. The trait showing the greatest divergence among subpopulations was leaf width. Interestingly, the two subpopulations forming the extreme ends of the distribution were the *temperate* versus *tropical japonicas*, with narrow and wide leaf blades, respectively. These two groups are much more closely related to each other than to the other subpopulations (Garris et al. 2005; Glaszmann 1987).

These observations suggest that ecological and agronomic factors associated with the development of individual cultivars may be as or more important in generating the variation in these particular trait values than were measures of genetic association per se. We did not have sufficient information on the conditions of use and/or development of all the accessions to make any explicit test of this idea. The need for explicit information about each accession before forming expectations of trait values is particularly true for $\Delta^{13}C$, which showed a wide range of states in all subpopulations. Knowledge of subpopulation alone provided little certainty of $\Delta^{13}C$ value, but accessions with the most promising (low) values for $\Delta^{13}C$ clearly belonged to the *indica* group. In this context, the four accessions consistently having the very lowest $\Delta^{13}C$ among all *O. sativa* varieties independently ranked in both trials were: Pappaku, Rathuwee, and IRGA 409 (all *indicas*), and Chhote Dhan (an admixture sharing 68% ancestry with *indica*). In contrast, the two *indicas* with the highest $\Delta^{13}C$ (lowest WUE), Teqing (22.2‰) and Oryzica Llanos (21.8‰), were distinctly higher than the average for all *O. sativa* (21.6‰).

Our results contrast with some previous studies that have reported a trend of lower $\Delta^{13}C$ for *japonica* rather than *indica* varieties (Dingkuhn et al. 1991; Peng et al. 1998; Kondo et al. 2004). Our study screened a substantially larger number of accessions than the previous work and was supported by population structure analysis using genome-wide SSR markers to establish genetically distinct groups. Results were also undoubtedly influenced by growth conditions; our study was conducted on potted material in a greenhouse situation while previous work was conducted on field plantings. In our case, extensive prior attention was paid to the effects of planting conditions and soil volumes to avoid pot-bound effects on $\Delta^{13}C$ (Comstock et al. 2005). Growth conditions and water status are likely to have been more consistently favorable in the greenhouse regime, and our results should therefore reflect fully unstressed potentials. Our sampling also targeted an earlier vegetative stage than the other studies, minimizing the effect of uncontrolled environmental factors.

(4) *What is the relationship between* $\Delta^{13}C$ *and leaf morphological and physiological characters?* One of the most basic questions in regard to $\Delta^{13}C$ is whether variation can be attributed primarily to variation in just A, or just g (affecting Eq. 2), or if simultaneous variation in both variables is important. Since A and g form a ratio in Eq. 2, a proportional change in both at once might have no effect on c_i, while a comparable change in either A or g, achieved while the other variable stayed constant, would cause a substantial shift in c_i. In this case, both $A_{(\text{mean } c_i)}$ and g varied, but with a strong positive correlation. The variation in g was proportionally greater, however, and g, not $A_{(\text{mean } c_i)}$, drove the variation in c_i and $\Delta^{13}C$. This pattern holds for effects associated with the two QTL, $\Delta^{13}C1.1$ and $\Delta^{13}C4.1$, as well as with overall correlations among BILs. This is also consistent with previous reports in rice where variation in stomatal aperture and leaf conductance was identified as driving variation in WUE (Dingkuhn et al. 1991; Kondo et al. 2004). The principle effect of the positive correlation between A and g is to greatly reduce the variation that would otherwise occur in c_i from the observed range in g if A were constant.

Other traits may be correlated with $\Delta^{13}C$ indirectly through influences on g or $A_{(\text{mean } c_i)}$. %N is often related to

$A_{(\text{mean } c_i)}$ due to the high nitrogen requirements of photosynthetic enzymes and electron transport (Mae 1997; Makino 2003), which in some cases results in a negative correlation with $\Delta^{13}C$ [reviewed by Hausmann et al. (2005); Sparks and Ehleringer (1997); Turner (1993)]. In this study, %N was positively correlated with $A_{(\text{mean } c_i)}$ (Table 6) but, consistent with the effects of the interactions between $A_{(\text{mean } c_i)}$ and g discussed above, %N had a weak positive (non-significant) correlation with c_i and $\Delta^{13}C$. When evaluated on the entire mapping population with a larger sample size, %N was significantly, positively correlated with $\Delta^{13}C$.

Leaf length was negatively correlated with $\Delta^{13}C$ in rice. This was also true for the limited sample of other *Oryza* species accessions surveyed in E-2 ($r = -0.69$; $P = 0.01$). This correlation could be due to many factors related to leaf morphology and development impacting photosynthetic performance, but it is noteworthy that it is consistent with a hydraulic limitation concept in which the longer pathways for water transport ultimately result in more conservative water use patterns to avoid depression of leaf water potential.

In a sister study (D. This, in preparation) based on a QTL mapping population from a cross of *O. sativa* cultivars, IR64 (*indica*) and Azucena (*tropical japonica*), a very similar suite of characters relating to $\Delta^{13}C$ was observed. Trait values for g and $A_{(\text{mean } c_i)}$ were positively correlated, and the greater variation in g also dominated correlations with c_i and $\Delta^{13}C$. In that study, the positive correlations of $\Delta^{13}C$ with $A_{(\text{mean } c_i)}$ and %N were significant. Morphological correlations were similar with a significant negative correlation between $\Delta^{13}C$ and plant height.

(5) *How are $\Delta^{13}C$ and related traits genetically controlled?* As would be expected from the complex nature of a trait representing the ratio of two major processes, carbon assimilation and transpiration, the control of $\Delta^{13}C$ is strongly quantitative in rice. Moreover, only limited overlap has been found among the set of $\Delta^{13}C$ QTL studies reported to date in rice. No single, large-effect QTL have been identified in rice, and QTL breaking the strong correlation between A and g have not yet been discovered.

Since the pioneering work in QTL mapping of $\Delta^{13}C$ in tomato (Martin et al. 1989), there have been reports in other plant species including *Arabidopsis* (Hausmann et al. 2005; Masle et al. 2005), soybean (Specht et al. 2001), *Stylosanthes scabra* (Thumma et al. 2001), maritime pine (Brendel et al. 2002), barley (Diab et al. 2004; Teulat et al. 2002), *Castanea sativa* (Casasoli et al. 2004), and cotton (Saranga et al. 2004). In the first QTL study of $\Delta^{13}C$ undertaken on rice (Price et al. 2002), nine genomic regions were found to be associated with $\Delta^{13}C$. In that study, an F6 RIL population from a Bala × Azucena cross was grown under irrigated conditions during the dry season at IRRI (Los Baños, Philippines) in 1996 and WARDA (Bouake, Ivory Coast) in 1997 and $\Delta^{13}C$ was measured on fully expanded young leaves of rice plants (Price et al. 2002). None of the QTL detected at IRRI coincided with those detected at WARDA. Recently, Laza et al. (2006) and Takai et al. (2006) identified 13 and seven QTL, respectively, for carbon isotope discrimination evaluated at different growth stages under field conditions. Except for a chromosomal region on chromosome 2, no overlapping QTL were identified in the two studies, and none overlapped with QTL identified in our study. Nonetheless, the QTL *$\Delta^{13}C4.1$* identified in this study overlapped with a QTL identified by D. This (personal communication). The small size of the BIL population used in the current study may have limited our ability to identify *$\Delta^{13}C$* QTL with small effect. This, along with the sensitivity of $\Delta^{13}C$ measurements to environment, may help explain why so few QTL overlapped among studies.

Despite the small size of the BIL population, five QTL associated with *$\Delta^{13}C$* were identified, and together, they presented contrasting alleles between the two parents. Kasalath had favorable alleles for WUE at three loci on chromosomes 1, 4 and 8 but unfavorable alleles at the QTL on chromosomes 7 and 11, and vice versa for Nipponbare. The dispersal of favorable QTL alleles between the parents provides an explanation for the transgressive segregation observed in the Kasalath × Nipponbare BIL population for $\Delta^{13}C$ (Fig. 3).

The fact that different QTL were identified for $\Delta^{13}C$ and %N in E-3 and E-4 suggests that QTL × environment interaction influences the expression of both traits. Documented differences in environmental conditions were present in the greenhouses for E-3 and E-4, particularly in terms of light intensity. Further studies are needed to understand precisely how light intensity, humidity and other environmental factors contribute to the expression of water use efficiency at different stages in plant development, and to confirm whether the QTL identified here are stably expressed in other environments and during later growth stages in rice.

Correlations (Tables 3, 6) between $\Delta^{13}C$ and leaf morphological and physiological traits including leaf length, tiller number and leaf nitrogen content, were partially explained at the level of co-localized QTL (Fig. 4). On chromosome 1 at *$\Delta^{13}C$ 1.1*, near to the *sd1* locus, Kasalath alleles favored lower $\Delta^{13}C$, diminished tiller number and N%, and increased leaf length. Using a DH population derived from IR64 × Azucena (Yan et al. 1999), a QTL for flag leaf length was found in the same region as *$\Delta^{13}C$ 1.1*. Using two different mapping populations, QTL for $\delta^{13}C$ ($\Delta^{13}C$) were identified on barley chromosome 3H near the semi-dwarf gene *sdw1* (Ellis et al. 2002; Teulat et al. 2002) in a region that is homoeologous to rice chromosome 1 where *$\Delta^{13}C$ 1.1* is located.

It is known that both leaf %N and Rubisco content strongly affect photosynthesis (Mae 1997; Makino 2003). About 50% of total soluble protein and 25% of total N are associated with Rubisco protein in rice leaves (Makino 2003). Consistent with the expectations associated with increased %N, a slightly depressed $A_{(\text{mean } c_i)}$ was seen in association with Kasalath alleles at *Δ13C1.1*, but, as in the overall correlations, this was also associated with a proportionally larger decrease in *g* so that the $\Delta^{13}C$ effect was opposite to what would be predicted from $A_{(\text{mean } c_i)}$ alone (Eq. 2). Stomatal frequency, a trait that is of interest because it would be expected to correlate with leaf conductance and have possible effects on c_i and $\Delta^{13}C$, was mapped by Ishimaru et al. (2001a). Interestingly, one of four QTL identified for adaxial and abaxial stomatal frequencies was colocated with *Δ13C 1.1* in our study.

A study in *Arabidopsis* showed that *ERECTA*, a putative leucine-rich repeat receptor-like kinase (LRR-RLK), known for its effects on inflorescence development, was a major contributor to a locus for $\Delta^{13}C$ on *Arabidopsis* chromosome 2 (Masle et al. 2005). Blasting the sequence of this gene identified homologs near the top of the short arm of rice chromosome 6. No $\Delta^{13}C$ QTL was identified at that location in this study. One of the QTL identified for grain $\Delta^{13}C$ by Ishimaru et al. (2001b) was located in the middle of chromosome 6, while the QTL identified for leaf $\Delta^{13}C$ on chromosome 6 by Price et al. (2002) was near the telomere of the long arm. Therefore, alignment of the *ERECTA* homologs with the $\Delta^{13}C$ QTL that have been reported in rice so far revealed no co-localization. We therefore conclude that variation in the ERECTA gene may not be associated with variation of $\Delta^{13}C$ in rice. It is notable that in the Masle study, pleiotropic effects of ERECTA promoted simultaneous decreases in *g* and increases in *A*, and this resulted in a substantially larger shift in c_i and $\Delta^{13}C$ than any of the QTL yet investigated in rice, where the correlation of *A* and *g* has been consistently positive and c_i effects thereby muted.

Implications for crop improvement in WUE

Rice has considerable genetic potential for improvement of WUE. From initial QTL studies, this appears to be strongly quantitative and controlled by many genetic factors rather than a few loci of large effect. For genetic improvement of *O. sativa*, the full range of phenotypic variation is found among contrasting cultivars, suggesting no immediate need to tap the genetic resources available in related wild species. Nonetheless, the strongly multigenic nature of the trait and the transgressive variation observed in our mapping population suggests that even lines that do not, themselves, have high WUE might still contribute favorable alleles, and related wild species might similarly have unique alleles that would be of value for improvement of WUE. The case is different with respect to *O. glaberrima,* which had low overall water-use efficiency and much less variation among accessions than was seen in either *O. sativa* or some of the African wild species relatives. Improvement of WUE in *O. glaberrima* would, therefore, benefit more dramatically from tapping the genetic resources of related taxa. It is also of interest to determine whether the genetic control of $\Delta^{13}C$ in rice, a plant with recent aquatic origins, differs from that in cereals such as wheat and barley, which have been exposed to water-limited conditions for a great portion of their evolutionary history. Comparative analysis across different cereals will shed light on the similarities and differences related to the genetic control of WUE, providing useful insights about how to leverage information from one species to another.

Acknowledgments We wish to thank Christine F. Fleet for her dedicated data collection and analysis, Brian E. Gollands for data management, Paul S. King for coordination and mentoring of high school and undergraduate interns, Rebecca Rudicell, who collected data for the Keeling plots relating $\delta^{13}C$ of greenhouse air to atmospheric [CO_2], Anna Nowogrodski and Laura Vineyard who assisted with gas-exchange measurements, and to Masahiro Yano, from the National Institute of Agrobiological Science in Japan for sharing the populations of Nipponbare × Kasalath BILs and associated RFLP dataset. This work was supported by the National Science Foundation (Plant Genome Research Project Grant DBI-0110069, *Genomic Analysis of Plant Water Use Efficiency*).

References

Araus JL, Villegas D, Aparicio N, Garcia del Moral LF, El Hani S, Rharrabti Y, Ferrio JP, Royo C (2003) Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. Crop Sci 43:170–180

Barker R, Dawe D, Tuong TP, Bhuiyan SI, Guerra LC (1999) The outlook for water resources in the year 2020: challenges for research on water management in rice production. 19th session of the International Rice Commission. Food and Agriculture Organization, Cairo, pp 99–109

Brendel O, Pot D, Plomion C, Rosenberg P, Guehl JM (2002) Genetic parameters and QTL analysis of delta C-13 and ring width in maritime pine. Plant Cell Environ 25:1248–1257

Cabuslay GS, Ito O, Alejar AA (2002) Physiological evaluation of responses of rice (*Oryza sativa* L.) to water deficit. Pl Sci 163:815–827

Casasoli M, Pot D, Plomion C, Monteverdi MC, Barreneche T, Lauteri M, Villani F (2004) Identification of QTLs affecting adaptive traits in Castanea sativa Mill. Plant Cell Environ 27:1088–1101

Comstock JP, McCouch SR, Martin BC, Tauer CG, Vision TJ, Xu Y, Pausch R (2005) The effects of resource availability and environmental conditions on genetic rankings for carbon isotope discrimination during growth in tomato and rice. Funct Plant Biol 32:1089–1105

Condon AG, Richards RA, Farquhar GD (1987) Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat. Crop Sci 27:996–1001

Condon AG, Farquhar GD, Richards RA (1990) Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat leaf gas exchange and whole plant studies. Aust J Plant Physiol 17:9–22

Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2002) Improving intrinsic water-use efficiency and crop yield. Crop Sci 42:122–131

Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2004) Breeding for high water-use efficiency. J Exp Bot 55:2447–2460

Cregg B, Zhang J (2000) Carbon isotope discrimination as a tool to screen for improved drought tolerance. In: 11th METRIA conference, Gresham, Oregon

Diab AA, Teulat-Merah B, This D, Ozturk NZ, Benscher D, Sorrells ME (2004) Identification of drought-inducible genes and differentially expressed sequence tags in barley. Theor Appl Genet 109:1417–1425

Dingkuhn M, Farquhar GD, De Datta SK, O'Toole JC (1991) Discrimination of 13C among upland rices having different water use efficiencies. Australian J Ag Res 42:1123–1131

Ellis RP, Forster BP, Gordon DC, Handley LL, Keith RP, Lawrence P, Meyer R, Powell W, Robinson D, Scrimgeour CM, Young G, Thomas WT (2002) Phenotype/genotype associations for yield and salt tolerance in a barley mapping population segregating for two dwarfing genes. J Exp Bot 53:1163–1176

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587

Farquhar GD, Richards RA (1984) Isotopic composition of plant carbon correlates with water use efficiency of wheat genotypes. Aust J Ag Res 11:539–552

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. In: Briggs WR (ed) Annual review of plant physiology and plant molecular biology, vol 40. Annual Reviews Inc., Palo Alto, pp 503–538

Franks PJ, Farquhar GD (1999) A relationship between humidity response, growth form and photosynthetic operating point in C3 plants. Plant Cell Environ 22:1337–1349

Franks PJ, Farquhar GD (2001) The effect of exogenous abscisic acid on stomatal development, stomatal mechanics, and leaf gas exchange in *Tradescantia virginiana*. Plant Physiol 125:935–942

Garris AJ, Tai TH, Coburn JR, Kresovich S, McCouch S (2005) Genetic structure and diversity in *Oryza sativa* L. Genetics 169:1631–1638

Geber MA, Dawson TE (1997) Genetic variation in stomatal and biochemical limitations to photosynthesis in the annual plant, *Polygonum arenastrum*. Oecologia 109:535–546

Glaszmann JC (1987) Isozymes and classification of asian rice varieties. Theor Appl Genet 74:21–30

Gleick PH (2003) Water use. Annu Rev Environ Resour 28:275–314

Handley LL, Nevo E, Raven JA, MartInez-Carrasco R, Scrimgeour CM, Pakniyat H, Forster BP (1994) Chromosome 4 controls potential water use efficiency (delta13C) in barley. J Exp Bot 45:1661–1663

Hausmann NJ, Juenger TE, Sen S, Stowe KA, Dawson TE, Simms EL (2005) Quantitative trait loci affecting delta13C and response to differential water availability in *Arabidopsis thaliana*. Evolution Int J Org Evol 59:81–96

Ishimaru K, Shirota K, Higa M, Kawamitsu Y (2001a) Identification of quantitative trait loci for adaxial and abaxial stomatal frequencies in *Oryza sativa*. Plant Physiol Biochem 39:173–177

Ishimaru K, Yano M, Aoki N, Ono K, Hirose T, Lin SY, Monna L, Sasaki T, Ohsugi R (2001b) Toward the mapping of physiological and agronomic characters on a rice function map: QTL analysis and comparison between QTLs and expressed sequence tags. Theor Appl Genet 102:793–800

Johnson RC (1993) Carbon isotope discrimination, water relations, and photosynthesis in Tall Fescues. Crop Sci 33:169–174

Juenger TE, McKay JK, Hausmann N, Keurentjes J, Sen S, Stowe KA, Dawson TE, Simms EL, Richards JH (2005) Identification and characterization of QTL underlying whole-plant physiology in *Arabidopsis thaliana*: delta C-13, stomatal conductance and transpiration efficiency. Plant Cell Environ 28:697–708

Keeling CD (1958) The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas. Geochim Cosmochim Acta 13:322–334

Knight JD, Livingston NJ, van-Kessel C (1994) Carbon isotope discrimination and water-use efficiency of six crops grown under wet and dryland conditions. Plant Cell Environ 17:173–179

Kondo M, Pablico PP, Aragones DV, Agbisit R (2004) Genotypic variations in carbon isotope discrimination, transpiration efficiency, and biomass production in rice as affected by soil water conditions and N. Pl Soil 267:165–177

Lambrides CJ, Chapman SC, Shorter R (2004) Genetic variation for carbon isotope discrimination in sunflower: association with transpiration efficiency and evidence for cytoplasmic inheritance. Crop Sci 44:1642–1653

Larcher W (1995) Physiological plant ecology: ecophysiology and stress physiology of functional groups, 3rd edn. p xvi + 506p

Laza MR, Kondo M, Ideta O, Barlaan E, Imbe T (2006) Identification of quantitative trait loci from d13C and productivity in irrigated lowland rice. Crop Sci 46:763–773

Lin SY, Sasaki T, Yano M (1998) Mapping quantitative trait loci controlling seed dormancy and heading date in rice, *Oryza sativa* L, using backcross inbred lines. Theor Appl Genet 96:997–1003

Lu H, Redus MA, Coburn JR, Rutger JN, McCouch SR, Tai TH (2005) Population structure and breeding patterns of 145 US rice cultivars based on SSR marker analysis. Crop Sci 45:66–76

Mae T (1997) Physiological nitrogen efficiency in rice: nitrogen utilization, photosynthesis, and yield potential. Pl Soil 196:201–210

Makino A (2003) Rubisco and nitrogen relationships in rice: leaf photosynthesis and plant growth. Soil Sci Plant Nutr 49:319–327

Martin B, Nienhuis J, King G, Schaefer A (1989) Restriction fragment length polymorphisms associated with water use efficiency in tomato. Science (Wash DC) 243:1725–1728

Masle J, Gilmore SR, Farquhar GD (2005) The ERECTA gene regulates plant transpiration efficiency in *Arabidopsis*. Nature 436:866–870

McKay JK, Richards JH, Mitchell-Olds T (2003) Genetics of drought adaptation in *Arabidopsis thaliana*: I. Pleiotropy contributes to genetic correlations among ecological traits. Mol Ecol 12:1137–1151

Minhas JS, Khurana SMP, Sheshshayee MS, Kumar MU (2003) Potato varieties show genetic variability in water use efficiency based on carbon isotope discrimination. J Ind Potato Assoc 30:193–194

Monneveux P, Reynolds MP, Trethowan R, Gonzalez-Santoyo H, Pena RJ, Zapata F (2005) Relationship between grain yield and carbon isotope discrimination in bread wheat under four water regimes. Europ J Agron 22:231–242

Passioura JB (1977) Grain yield, harvest index, and water use of wheat. J Aust Inst Agric Sci 42:117–120

Peng S, Laza RC, Khush GS, Sanico AL, Visperas RM, Garcia FV (1998) Transpiration efficiencies of indica and improved tropical japonica rice grown under irrigated conditions. Euphytica 103:103–108

Price AH, Cairns JE, Horton P, Jones HG, Griffiths H (2002) Linking drought-resistance mechanisms to drought avoidance in upland rice using a QTL approach: progress and new opportunities to integrate stomatal and mesophyll responses. J Exp Bot 53:989–1004

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Rosegrant MW (1998) Water and irrigation policy: prospects for the future and implications for rice production. In: Pingali P, Hossain M (eds) Impact of Rice Research Thailand Development Research Institute and International Rice Research Institute. Bangkok, Thailand, pp 83–112

Saranga Y, Flash I, Paterson AH, Yakir D (1999) Carbon isotope ratio in cotton varies with growth stage and plant organ. Pl Sci 142:47–56

Saranga Y, Jiang CX, Wright RJ, Yakir D, Paterson AH (2004) Genetic dissection of cotton physiological responses to arid conditions and their inter-relationships with productivity. Plant Cell Environ 27:263–277

Semon M, Nielsen R, Jones M, McCouch S (2005) The population structure of African cultivated rice *Oryza glaberrima* (Steud.): evidence for elevated levels of LD caused by admixture with *O. sativa* and ecological adaptation. Genetics 169:1639–1647

Sparks JP, Ehleringer JR (1997) Leaf carbon isotope discrimination and nitrogen content for riparian trees along elevational transects. Oecologia (Berlin) 109:362–367

Specht JE, Chase K, Macrander M, Graef GL, Chung J, Markwell JP, Germann M, Orf JH, Lark KG (2001) Soybean response to water: a QTL analysis of drought tolerance. Crop Sci 41:493–509

Stiller WN, Read JJ, Constable GA, Reid PE (2005) Selection for water use efficiency traits in a cotton breeding program: cultivar differences. Crop Sci 45:1107–1113

Tai TH, Tanksley SD (1990) A rapid and inexpensive method for isolation of total DNA from dehydrated tissue. Plant Mol Biol Rep 8:297–303

Takai T, Fukuta Y, Sugimoto A, Shiraiwa T, Horie T (2006) Mapping of QTLs controlling carbon isotope discrimination in the photosynthetic system using recombinant inbred lines derived from a cross between two different rice (*Oryza sativa* L.) cultivars. Plant Prod Sci 9:271–280

Teulat B, Merah O, Sirault X, Borries C, Waugh R, This D (2002) QTLs for grain carbon isotope discrimination in field-grown barley. Theor Appl Genet 106:118–126

Thumma BR, Naidu BP, Chandra A, Cameron DF, Bahnisch LM, Liu CN (2001) Identification of causal relationships among traits related to drought resistance in *Stylosanthes scabra* using QTL analysis. J Exp Bot 52:203–214

Turner NC (1993) Water use efficiency of crop plants: potential for improvement. In: Buxton DR, Shibles R, Forsberg RA, Blad BL, Asay H, Paulsen GM, Wilson RG (eds) International crop science. Crop Science Society of America, Madison, pp 75–82

Wang S, Basten CJ, Zeng Z-B (2006) Windows QTL Cartographer 2.5, 2.5 edn. Dept. of Statistics. North Carolina State University, Raleigh

Wong SC, Cowan IR, Farquhar GD (1979) Stomatal conductance correlates with photosynthetic capacity. Nature (London) 282:424–426

Wright GC, Hubick KT, Farquhar GD, Nageswara RC (1993) Genetic and environmental variation in transpiration efficiency and its correlation with carbon isotope discrimination and specific leaf area in peanut. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant carbon-water relations. Academic Press, San Diego, pp 247–267

Yan J, Zhu J, He C, Benmoussa M, Wu P (1999) Molecular marker-assisted dissection of genotype × environment interaction for plant type traits in rice (*Oryza sativa* L). Crop Sci 39:538–544