博士論文（要約）

Thesis Abstract

# Eco-physiological study on efficient water use for production of rice genotypes under diverse management and environmental conditions
# (多様な管理・環境条件でのイネ遺伝子型の生産のための効率的な水利用に関する生理・生態学的研究)

**Deshmukh Vivek Vasant**
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# Eco-physiological study on efficient water use for production of rice genotypes under diverse management and environmental conditions
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**Deshmukh Vivek Vasant**

Department of Agricultural and Environmental Biology
Graduate School of Agricultural and Life Sciences
The University of Tokyo

**Supervisor: Associate Professor Akihiko Kamoshita**

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# Abstract

Worldwide increasing demand for freshwater resources limits the irrigation water for agriculture which necessitates reduction in rice water use with improved water productivity in many parts of Asia and also outside Asia. Reducing water supply often leads to yield reduction, and understanding water saving management for rice production and designing improved water saving rice cultivation are required. This study focuses on assessment of rice production under diverse range of environments to study effect of water saving on rice genotypes for grain yield and water productivity.

Choice of genotypes plays significant role in water saving rice cultivation. We tested rice genotypes with different root traits (Chapter 2) and heading dates (Chapter 3) under three water managements flooded lowland (FL), alternate wetting and drying lowland (AWD), and rainfed upland (UP) conditions in 2013 and 2014 in Japan. Three near-isogenic lines (NILs) of *Oryza sativa* subsp. *indica* cv. IR64 (Dro1-NIL, Sta1-NIL, Dro1+Sta1-NIL) with *DEEPER ROOTING 1* (*DRO1*), a novel gene for steeper root growth angle, and/or with *Stele Transversal Area 1* (*Sta1*), a QTL for wider stele area, were tested under three water managements. Dro1-NIL had 14% higher yield than that of IR64 across the three water conditions due to higher harvest index, aboveground biomass, leaf area index, and number of grains. Water productivity was higher for Dro1-NIL (0.51 kg $m^{-3}$) than background parent IR64 (0.44 kg $m^{-3}$) and Sta1-NIL (0.45 kg $m^{-3}$). Sta1-NIL not showed higher productivity compared to IR64 or Dro1-NIL but tended to reduce the carbon isotope composition ($\delta^{13}C$), leading to a higher harvest index than that of IR64. Dro1+Sta1-NIL had the highest fraction of intercepted radiation, cumulative radiation interception, and panicle number, with a small but insignificant yield improvement over IR64, but the combination of *DRO1* and *Sta1* did not surpass the increment from the effects of *DRO1* alone and also not showed improvement in water productivity over diverse range of water environments. For IR64 and NILs higher grain yield was attained in AWD in rainy year 2014 with higher water productivity and higher biomass, with significant water by year interaction for water productivity. Genotypic variation in water productivity was related with higher leaf area index and fraction interception, with Dro1-NIL larger than in IR64 and Sta1-NIL.

Koshihikari and four of its near isogenic lines (NILs) with early heading date Koshihikari-NIL (Hd1) and Koshihikari-NIL (Hd17) and late heading date Koshihikari-NIL (Hd6) and Koshihikari-NIL (Hd16), showed higher water saving can be achieved by early heading genotypes Hd1 and Hd17; however the yield and water productivity values were lowered in Hd1 compared to Koshihikari. Slightly later flowering Hd17 saved water but did not showed reduction in grain yield and water productivity. All Koshihikari NILs showed reduction in grain yield under AWD and UP conditions compared to FL. Reduction in available water in UP treatments showed significant reduction in leaf area index and lowered biomass and grain yield. Later heading genotypes Hd6 and Hd16 showed 19% and 52% lower grain yield in AWD and UP compared to FL. Water saving from FL to AWD resulted in improved water productivity without changing $\delta^{13}C$ values whereas higher water saving in UP treatment resulted in lowered biomass and higher $\delta^{13}C$ than FL and AWD. Alternate wetting and drying irrigation in Japan saved around 76% water with 10% lower grain yield compared to flooded lowland.

In rice ecosystems limited availability of water and variability of rainfall affects grain yield and water productivity. In South India two non-system tanks with different sizes (big tank- 28.5 ha and small tank- 16.5 ha) were studied from 2012 to 2014 for spatial variability of water distribution and grain yield (Chapter 4). Differences in tank size showed significant effect on tank water irrigations and borewell irrigations. More tank irrigations with relatively stable grain yield was observed in big tank; whereas small tank used more borewell irrigations with variable grain yield. Reduced rainfall in 2012 significantly affected number of tank irrigations, and small tank showed more borewell irrigations with more number of unharvested fields (35%) than big tank (7%). Location of fields in tank area showed significant effect on grain yield. Differences in water productivity was observed between two tanks. Genotypic differences were not observed among genotypes however JGL showed higher grain yield than BPT.

Improvement of resource use efficiency such as water and nitrogen fertilizer has not been studied for high input direct seeding system in tropical South America. Field experiment was conducted in Colombia with eight rice genotypes and five N levels under three sets of water managements with irrigation interval of 3 days in conventional irrigation (W1), 6 days in mild water saving (W2) and 8 days in risky water saving (W3) treatments (Chapter 5). On average of two seasons, increasing irrigation interval from W1 to W2 and W3 showed 16%

and 25% reduction in irrigation water with 9% and 18% reduction in grain yield, respectively. Higher water saving was achieved with improved grain yield and water productivity in wet season 2016. Reducing N application rate from 220 kgN $ha^{-1}$ to 140 kgN $ha^{-1}$ did not change grain yield and water productivity, indicating low N recovery and possible improvement of N use efficiency. Among genotypes short duration rice varieties FEDEARROZ473, Dro1-NIL and IR64 showed lower water use with higher water productivity. FEDEARROZ473 showed more stable grain yield (6.8 t $ha^{-1}$) on average of two seasons due to higher number of grains and thousand grain weight whereas FEDEARROZ67 yielded highest in wet season.

This study showed that 1) estimation of water productivity across different environmental conditions showed higher water saving can be achieved in wet season than dry season. Genotypic traits such as deeper rooting growth angle significantly improved water productivity across three water managements; and use of short duration varieties can save irrigation water for rice. $\delta^{13}C$ was more affected by plant physiological changes under upland conditions and also in marginal poor fields in small tank and indicated possible use of $\delta^{13}C$ as an indicator for guiding safe water saving management practices.

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## List of Abbreviations

| | |
|---|---|
| AGDM | Above ground biomass |
| ANOVA | Analysis of variance |
| AWD | Alternate wetting and drying |
| CEC | Cation exchange capacity |
| CIAT | Central institute for tropical agriculture |
| Cum RI | Cumulative radiation interception |
| $\delta^{13}C$ | Carbon isotope composition |
| DAP | Diammonium phosphate |
| DRO | Deeper rooting |
| EC | Electrical conductivity |
| FI | Fraction interception |
| FL | Flooded |
| K | Light extinction coefficient |
| kPa | kilo pascal |
| LAC | Latin American countries |
| LSD | Least significance difference |
| MJ | Mega joule |
| N | Nitrogen |
| NIL | Near isogenic lines |
| NUE | Nitrogen use efficiency |
| PAR | Photosynthetic active radiation |
| PU | Panchayat union |
| PWD | Public works development |
| QTL | Quantitative trait loci |
| RI | Radiation interception |
| RTA | Root transversal area |
| RUE | Radiation use efficiency |
| SLW | Specific leaf weight |
| STA | Stele transversal area |
| TE | Transpiration efficiency |
| WD | Water depth |
| WP | Water productivity |
| WUE | Water use efficiency |
| UP | Upland |

# Chapter 1

# Introduction

Task of increasing rice production for growing population will put more pressure on water demand and bringing additional area under rice cultivation. As popular lowland rice is cultivated with standing water for substantial duration during crop cultivation, rice needs large consumption of fresh water and has lower ratio of grain yield produced from unit amount of water input.

Water productivity respect to evapotranspiration ($WP_{ET}$) is lower in rice (0.5-1.1 kg $m^{-3}$) compared to other crops like wheat (0.6-1.9 kg $m^{-3}$), maize (1.2-2.3 kg $m^{-3}$) and forage sorghum (7-8 kg $m^{-3}$) and irrigation water productivity of rice is also varied from 0.05 to 0.6 kg $m^{-3}$ and is lower than that of maize (0.2 to 0.8 kg/$m^3$) (Sadeghi and Rahimi, 2003). The ratio of rice production to input water is often called as water productivity or water use efficiency, depending on the exact definition of production and water, but as biomass production and water consumption is known to be strongly linked at both plant physiological level of gas exchange and agronomic field level, saving of water often results in reduction in yield, without significant improvement in water productivity. However, rice water productivity or efficiency of rice yield production with relation to supplied water can be altered by the choice of crop genetic materials, water management and agronomic practices.

Changes in water management have great potential for water-saving and increasing of water productivity in rice. Several water-saving technologies and practices have been developed to help farmers to cope with water scarcity in irrigated environments such as saturated soil culture, aerobic rice, alternate wetting and drying (AWD) (Belder et al. 2005; Bouman et al. 2007) and raised bed system (Choudhury et al., 2007). Among the various methods, the most widely promoted one for rice is AWD irrigation (Tuong et al., 2005 and Cabangon et al., 2011) and practiced in many countries such as China (Cabangon et al. 2001; Moya et al., 2004) India (Singh et al., 1996), Bangladesh (Palis et al., 2014), Philippines (Rejesus et al., 2013; Lampayan et al., 2014), Myanmar (Lampayan et al., 2014), and Vietnam (Rejesus et al., 2013. “Safe AWD” (not dropping water table below -15 cm) can help to enhance root growth and improve grain yield and also save the irrigation and labor cost for farmers

(Bouman and Lampayan 2009; Yang et al., 2009). In AWD, the field is not continuously flooded, instead the soil is allowed to dry out for one to several days after the disappearance of ponded water before it is flooded again. It uses the same conventional lowland system but just change frequency or interval of irrigation, which has been in fact locally practiced among farmers of water scarce regions (Van der Hoek et al., 2001); this is considered as reasons why AWD popularly extended to Asian farmers. Application of AWD can substantially decrease the water use up to 35% compared to irrigated rice (Zhang et al. 2009). Yield could reduce in AWD depending on frequency and duration of drying cycles, soil hydrological properties and selection of genotypes (Tabbal et al. 1992; Bouman and Tuong, 2001), but successful AWD can maintain yield as conventional lowland water management, leading to increase in water productivity. Some AWD studies reported even increased grain yield because of the enhancement in nutrient uptake by rice plants, root growth, grain filling rate, and remobilization of carbon reserves from vegetative tissues to grains (Liu et al., 2013, Yao et al., 2012 and Zhang et al., 2012), so application of safe level (soil moisture not below -10 kPa, Belder et al., 2004) of water saving can help to maintain grain yield with reduced water saving.

Agronomic management is also important for water-saving rice production, affecting water productivity. For example, planting method substantially changes required amounts of water for rice production. Transplanting requires shorter duration in the paddy compared with wet direct seeding, but substantial water for puddling is required, whereas dry direct seeding does not require water for land preparation and can save much water during establishment stage when standing water is usually absent (Du and Tuong, 2002). Nitrogen fertilizer management could also interact with water-saving. When the magnitude of water-saving is high and soil moisture content declines lower under AWD conditions, N losses from the applied N fertilizer could increase in the form of $N_2O$ and N taken up by plants would decline. If cracks should be developed in AWD, it would rather increase water and N fertilizer lost through percolation, also reducing N use efficiency. It is necessary to test the interaction effect of N fertilizer management and water-saving.

Water saving rice varieties are developed to give higher yield with water saving. The varieties mostly have drought resistant traits. Hanyou 3 (HY3), which is an elite water-saving and drought-resistance rice (WDR) is having drought tolerance and water saving with relatively high yield (Yu et al., 2005; Liu et al., 2009). Some super hybrid rice varieties also performed well with ample water supply and they have increased sink size due to large and heavy panicles and improved biomass production due to great canopy light interception are responsible for high yield potential (Yao et al., 2012).

Adaptation of different rice genotypes to water-saving management is known and plant traits such as enhancement of root growth, grain filling rate and remobilization of carbon reserves from vegetative tissues to grains are considered advantageous in water-saving lowland ecosystem (Tuong et al., 2005; Zhang et al., 2008). Zhang et al., 2009 stated that moderate wetting and drying regime can enhance root growth which benefits physiological processes such as root oxidation activity, cytokinin concentrations in roots and shoots, leaf photosynthetic rate and result in higher grain yield and WUE.

Genotypic variation in adaptation to drought has been extensively studied (Babu et al., 2003, Kumar et al., 2008, 2008; Dixit et al., 2014), and there may be some similarity between adaptation to drought and adaptation to water-saving management, but they may also differ at some points. Recently, Uga et al. 2013 developed Dro1-NIL (having *DEEPER ROOTING 1* gene) which avoided drought by extracting water from deeper soil layers. QTL for wider stele transversal area has been discovered by Uga et al. (2008) which is involved in higher transport of water from root to shoot. Use of these near isogenic lines with deeper root angle and wider stele area can help to improve water productivity in rice. Differences in heading date or phenology could be interacted with water-saving in rice due to differences in heading dates, just as development of early or later heading NILs can provide options to escape from the negative effects of high temperature during summer. Study of these lines can help to know adaptation mechanism to water saving environment.

At plant physiological level, ratio of photosynthesis to transpiration, referred as transpiration efficiency (TE) is considered as similar indicator of efficiency of water use for assimilation at plant physiological scale. Carbon isotope discrimination (Δ) defined as $\Delta = R_{air}/R_p - 1$, where $R_{air}$ and $R_p$ stand for the $^{13}C/^{12}C$ ratio in air and the photosynthetic product, respectively (Farquhar et al., 1982). Carbon isotope discrimination is known to be related with TE and can be used as a selection criterion for genotypic improvement in rice (Zhao et al., 2004). For the most successful water-saving, both saving of water input based on irrigation management and agronomic intervention and utilization of plants with higher TE might be important, but they have not been studied systematically; the former water-saving is attained without plant physiological changes but reducing luxury consumption of water, while the latter water-saving is attained to maintain biomass production in spite of water deficit perceived physiologically by plants.

Water productivity can be calculated in both irrigated rice ecosystem and rainfed rice ecosystem, and both temperate and tropical countries with different climate conditions. It is possible to compare the productivity of different countries just same as yield comparison. Water productivity of rice in India ranged from 0.2 to 0.26 kg $m^{-3}$ which is lower than China (0.44-0.60 kg $m^{-3}$), Australia (0.41-0.44 kg $m^{-3}$) and USA (0.36-0.41 kg $m^{-3}$) (Cai and Rosegrant, 2003). South India is major rice growing area where rice ecosystem is diverse and ranging from irrigated lowland to upland. For rice three major irrigation water sources are used such as tanks, canals and/or underground water resources. Tanks are human made reservoirs where water comes from rainfall, rain water runoff, canals and rivers. However share of tank irrigated area in India declined from 16.5% in 1952-53 to 5.2% in 1999-2000 and ground water irrigation increased from 30.2% to 55.4% in this period. In Tamil Nadu between 1990-91 and 2000-01 the share of tank-irrigated area in net irrigated area was declined by 34% (Palanisami et al., 2010). Tanks are mainly divided in to two types 1) system tanks (connected to rivers or canals) and 2) non-system tanks (depend on rain water). Among these non-system tanks are more vulnerable to water scarcity due to reduced attention from government and village peoples, lack of management and climate variability. Assessment of non-system tanks with different size and capacity for variation in rice production and water productivity can help to understand the irrigation structure within tank, affected areas and further investigation may help to find new ways to improve non-system tanks in South India.

Rice is relatively new crop in Latin America and Caribbean (LAC) where rice is grown from irrigated lowland to rainfed upland and about 45% of rice production is under upland or non-irrigated conditions (Moncada et al., 2001). Total production of paddy rice in LAC increased from around 8 million tons in 1961 to more than 28 million tons in 2009, an increase of over 250% (Zorilla et al., 2012) however, there is still a negative balance between production and consumption in the region as a whole (Pulver, 2003). Cost of rice production is higher in some of the LAC e.g. Brazil (2,172 USD/ha) which is higher than other neighboring countries in Latin America (South and Central America) (Ricepedia Brazil- 2015) mainly due to higher use of fertilizers and water. Colombia is also major rice producing country among LAC and cost of rice production is also higher. Direct dry seeded rice is more dominant in Colombia. Colombian rice ecosystem is also affected due to uneven rainfall and lack of irrigation water which affects the rice production and total cultivation area (El-nino 2015-16). Reduced use of N fertilizer and irrigation water can help to reduce the total cost of rice production. In this study we assessed the possibility of reducing N fertilizer input and also quantified irrigation water use to assess water saving by increasing irrigation interval.

This study analyzed ways in which water-saving irrigation can help to meet these challenges at the field level. The objectives of the this thesis were 1) assessment of water productivity for rice under different seasons in different water management regimes across three regions (Japan, India, Colombia), 2) assessment of significant effects of traits of genotypes (root traits, phenology) for grain yield and water productivity and 3) Assessment of usefulness of $\delta^{13}C$ as an indicator for water productivity under various water saving rice cultivation Sequence of chapters were arranged under two main headings, genotypic variation for water productivity (Chapters 2, 3) and eco-physiology of rice cultivation (Chapters 4, 5). The series of field experiments were conducted as follows.

To understand genotypic variation for water productivity,

- Effect of water saving regimes on grain yield and water productivity was analyzed for IR64 NILs introgressed with *DRO1* and Sta1 (Chapter 2)
- Analyzed effect of heading date on water productivity and carbon isotope composition ($\delta^{13}C$) in Koshihikari NILs (Chapter 3)

To understand eco-physiology of rice cultivation

- Assessed spatial variation for rice production and water use within tank irrigated rice system in non-system tanks in South India (Chapter 4)
- Assessment of water saving in dry direct seeded rice cultivation in Colombia (Colombia 5)

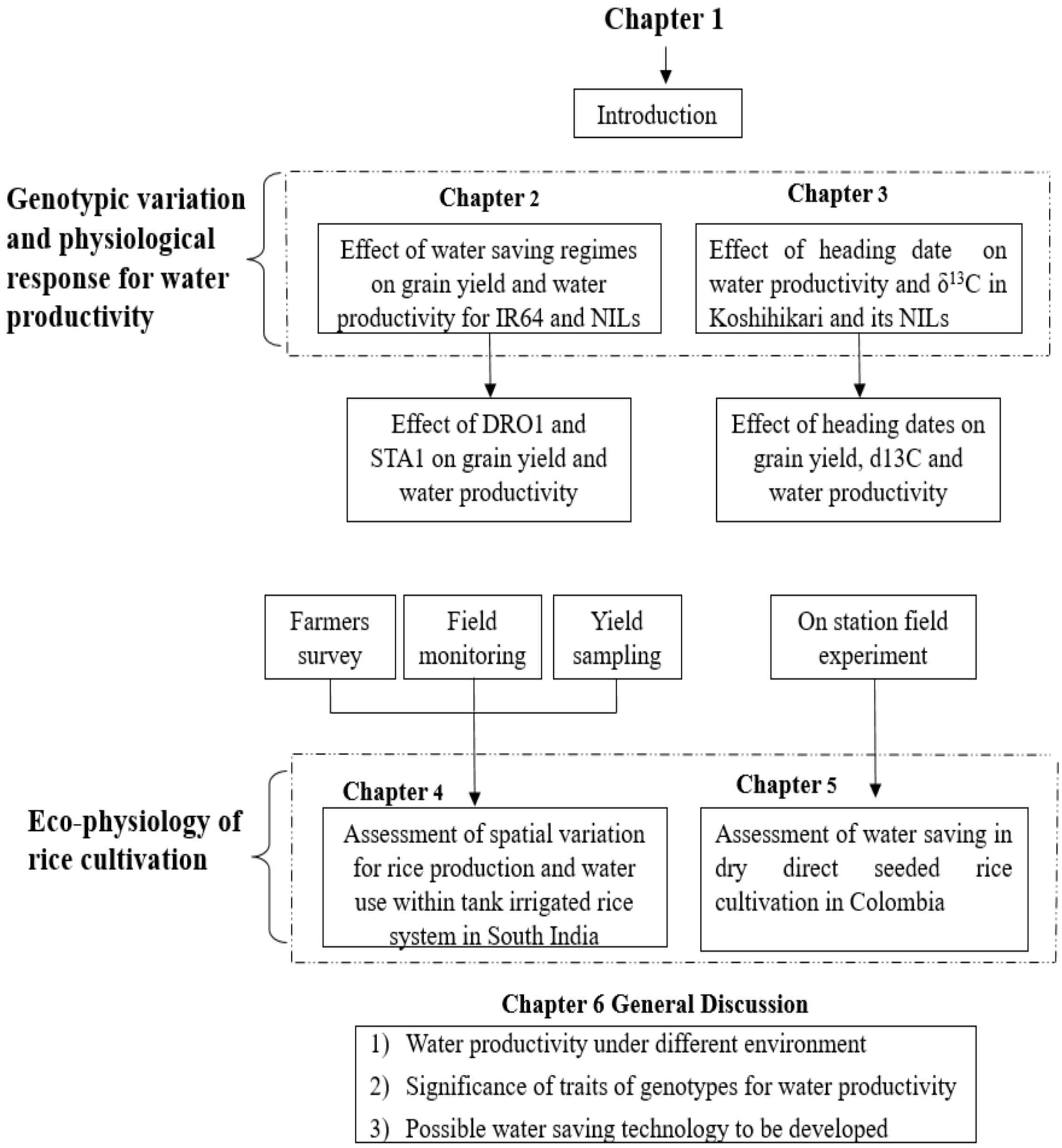


Fig. 1.1 Thesis Flow chart

# Chapter 2

## Near-isogenic lines of IR64 (*Oryza sativa* subsp. *indica cv.*) introgressed with *DEEPER ROOTING 1* and *STELE TRANSVERSAL AREA 1* improve rice yield formation over the background parent across three water management regimes

### 2.1 Introduction

Root traits can influence yield under resource-limiting conditions. In addition to morphological traits such as root diameter (Armenta-Soto et al., 1983), rooting depth (Kato et al., 2006a), and penetration ability (Clark et al., 2008), root growth angle and stele transversal area (STA) of root have been genetically dissected recently to identify their quantitative trait loci (QTLs) and/or genes. Uga et al., (2013) isolated the gene *DEEPER ROOTING 1 (DRO1)* from a deep-rooting rice variety, *Oryza sativa* subsp. *japonica cv*. Kinandang Patong. *DRO1* improved grain yield of a near-isogenic line (Dro1-NIL) in the genetic background of *O. sativa* subsp. *indica cv.* IR64, a shallow-rooting variety, under dry upland conditions. Superior yield of Dro1-NIL over IR64 was shown under well-watered paddy conditions in both low- and high-nitrogen fertilizer treatments (Arai-Sanoh et al., 2014). *DRO1* may improve rice yield across diverse water regimes, such as water-saving alternate wetting and drying lowland (AWD) conditions, but the extent of its interaction with water availability has not been studied. Understanding the effects of *DRO1* under various environmental conditions is necessary to broaden the scope of its use in breeding programs.

The stele in rice roots contains xylem, which is involved in the transport of water from root to shoot. The total area of late metaxylem vessels was strongly correlated with STA (Uga et al., 2008). Henry et al. (2012) reported that the proportion of cross-sectional area represented by stele increased under drought conditions, which would prioritize the retention of more water in vascular tissue. Stele size in rice root is genetically controlled by a QTL for STA detected on chromosome 9 in a mapping population derived from a cross between IR64 (with small STA) and Kinandang Patong (with large STA) (Uga et al., 2008). However, no studies have examined the effect of STA on yield under water-limiting conditions in rice.

The first objective of this research was to confirm the effects of *DRO1* on rice yield and water productivity across three water management regimes: rainfed upland (UP), AWD, and flooded lowland (FL) conditions (the effects of *DRO1* have never been tested under AWD conditions). We quantified the interactive effects of *DRO1* with these regimes and with various environmental conditions, as well as the degrees of improvement in water productivity by water-saving over different seasons. The second objective was to evaluate the usefulness of wider STA and its combined effects with *DRO1* on grain yield and water productivity.

## 2.2 Materials and methods

### 2.2.1 Plant materials

We used IR64, Kinandang Patong, and three near-isogenic lines (Dro1-NIL, Sta1-NIL, and Dro1+Sta1-NIL) for this study. IR64 is a modern lowland cultivar (subsp. *indica*) developed by the International Rice Research Institute (IRRI) in the Philippines and is widely grown in South and Southeast Asia. Kinandang Patong is a traditional upland cultivar (subsp. tropical *japonica*) that originated in the Philippines. Dro1-NIL is homozygous for the Kinandang Patong allele of *DRO1* and was developed by repeated backcrossing with IR64 and marker-assisted selection to eliminate non-target regions (Uga et al., 2013). In Dro1-NIL, the homozygous Kinandang Patong allele spans from 15.90 Mb (SSR marker RM24386) to 18.81 Mb (SSR marker RM242) on chromosome 9. Sta1-NIL ($BC_5F_4$) used in this study was developed from a cross between IR64 and Kinandang Patong by five repeated backcrosses with IR64 and marker-assisted selection to eliminate non-target regions. Sta1-NIL is homozygous for the Kinandang Patong allele between 14.59 Mb (InDel marker ID07_12) and 15.86 Mb (SSR marker RM24382) on chromosome 9. Dro1+Sta1-NIL ($BC_5F_3$) was selected while developing materials for Sta1-NIL. Dro1+Sta1-NIL is homozygous for the Kinandang Patong allele between 14.59 Mb (InDel marker ID07_12) and 18.81 Mb (SSR marker RM242) on chromosome 9.

### 2.2.2 Experimental site

Summer field experiments were conducted from April to late October in 2013 and 2014 at the Institute for Sustainable Agro-ecosystem Services, the University of Tokyo, in Nishitokyo, Japan (35°43′N, 139°32′E). The site has volcanic ash soil of the silty Kanto loam type (Humic Andosol). The topsoil layer (0–35 cm) is a dark humic silty loam, and the subsoil layer (below 35 cm) is a red-brown silty clay loam (Yamagishi et al., 2003). Average values for soil chemical properties from the fields (*N=9*) were pH 6.6±0.1, electrical conductivity 0.098±0.026 mS $cm^{-1}$, cation exchange capacity 37.6±1.6 meq 100 g, and bulk density 0.90 ±0.05 g $cm^{-3}$.

### 2.2.3 Trials and experimental design

One field of approximately 27 m × 36 m was divided into two parts, a lowland field and an upland field (each 27 m × 16 m) separated by a 4-m-wide mounted levee constructed with two plastic sheets inserted to 20-cm soil depth. Each of the two parts was further divided in half and separated by plastic sheets inserted to 20-cm soil depth. Half the lowland rice field (12 m × 16 m) was used for conventional flooded water management, and the other for alternate wet and dry irrigation management. Rice was grown on half the upland field (12 m × 16 m), and on the other half *Crotalaria juncea* (L.) was grown.

In each year, rice was grown under three water management regimes, FL, AWD, and UP conditions. Locations of the FL and AWD treatments were the same in both years, whereas the Crotalaria area in the upland field in 2013 was used as the experimental UP area in 2014. The five rice genotypes were arranged in a randomized block design with three replications in each water treatment. The genotypes were IR64 (recipient parent), Kinandang Patong (donor parent), and three NILs: Dro1-NIL (with a deeper root growth angle than that of IR64), Sta1-NIL (with a larger stele size than that of IR64), and Dro1+Sta1-NIL (with the combination of deep root angle and large stele size).

### 2.2.4 Plant cultivation

Seeds were soaked in water for 1 week and then sown in cup trays with one seed per cell on 22 April 2013 and 28 April 2014. All the genotypes were transplanted with hill spacing of 15 cm × 30 cm, with one plant per hill. Transplanting dates were 5 June for FL and AWD and 29 May for UP in 2013 and 23 May, 26 May, and 28 May, respectively, in 2014. In the FL and AWD treatments, flooded conditions with water depth around 5 cm were maintained for 2 weeks after transplanting to secure rooting and regrowth of seedlings. In the UP treatment, small holes similar to the cell size of the cup trays were made in soil (not flooded but containing moisture) and the seedlings were transplanted. Within a single plot, 18 and 50 plants were transplanted in 2013 and 2014, respectively, to achieve a homogeneous canopy across the experimental plots. Fertilizers ($P_2O_5$ and $K_2O$) were applied at 10 g $m^{-2}$ as a basal application on 2 May 2013 and 12 May 2014 before puddling the soil. Nitrogen was applied after transplanting and split into two doses of 3 g $m^{-2}$ each; the first and second doses were applied on 19 June and 3 July (18 and 32 days after transplanting) in 2013 and on 29 May and 3 July (4 and 38 days after transplanting) in 2014, respectively. Pre-emergence herbicides were applied to control weeds in the early growth stages, and plots were regularly hand weeded until the grain-filling stage to avoid damage by weeds.

### 2.2.5 Measurements

*2.2.5.1 Climate and soil moisture*

In both years, rainfall, solar radiation, minimum and maximum temperatures, and relative humidity were measured from June to October by a weather station with a 60-min logging interval (WatchDog 2900ET, Spectrum Technologies Inc., Aurora, IL, USA) installed 50 m away from the plots. Soil moisture potential at 5-cm soil depth was measured in AWD and UP plots by using a tensiometer (Water Mark WM-100, Spectrum Technologies Inc.). The daily mean (±SD) air temperature was 25.3 (±3.4) °C in 2013 and 24.0 (±3.3) °C in 2014. The daily minimum and maximum air temperatures were 21.1 (±3.3) and 30.2 (±4.4) °C in 2013 and 20.1 (±3.1) and 28.4 (±4.2) °C in 2014. Daily solar radiation was 15.9 (±6.5) in 2013 and 15.1 (±7.5) MJ $m^{-2}$ in 2014. Total seasonal rainfall was 659 in 2013 and 941 mm in 2014 (Fig. 2.1), resulting in higher soil water potential in 2014 (measured from June to August). In the 2013 cropping season, plants in the UP treatment encountered water scarcity due to less rainfall. Average soil water potential from June to August was –19.1 (±4.5) and –10.2 (±7.1) kPa in AWD in 2013 and 2014, respectively, and –36.4 (±15.7) and –16.1 (±5.7) kPa in UP. The minimum soil water potential in UP was lower in 2013 (–157.7 kPa) than in 2014 (–46.3 kPa), as was the case in AWD (–71.8 and –24.0 kPa, respectively). The cumulative evapotranspiration was comparable, 348 mm and 330 mm in 2013 and 2014, respectively, while cumulative rainfall was higher in 2014 (500 mm) than 2013 (449 mm), particularly during early to middle growth stage in July and August, with larger positive net water balance between rainfall and evapotranspiration (169 mm and 101 mm in 2014 and 2013, respectively) (Fig. 2.2). Cumulative irrigation was larger in 2013 (684 mm) than 2014 (427 mm).

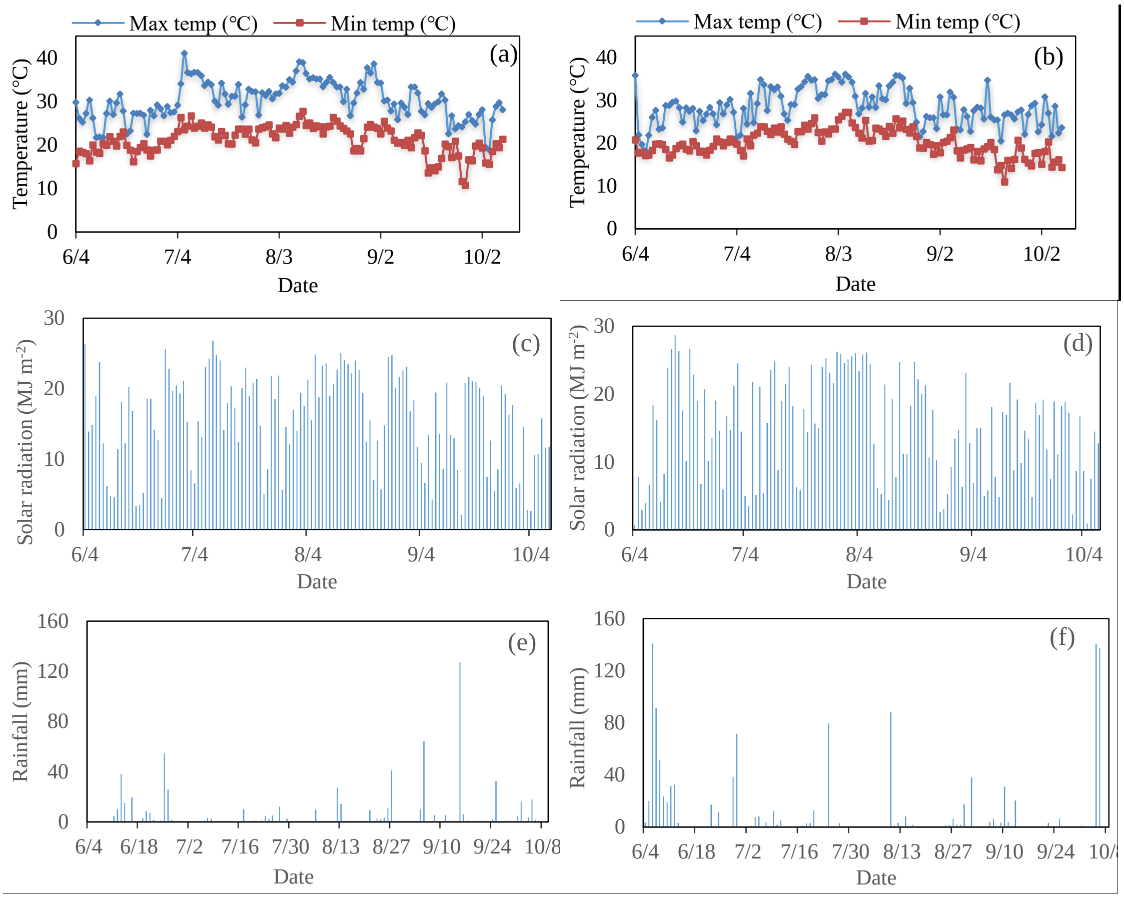


Fig. 2.1. Minimum and maximum daily air temperatures (°C) (a, b), daily solar radiation (MJ $m^{-2}$) (c, d), and daily rainfall (mm) (e, f) during the field experiments from June to October in 2013 (a–e) and 2014 (b–f), respectively.

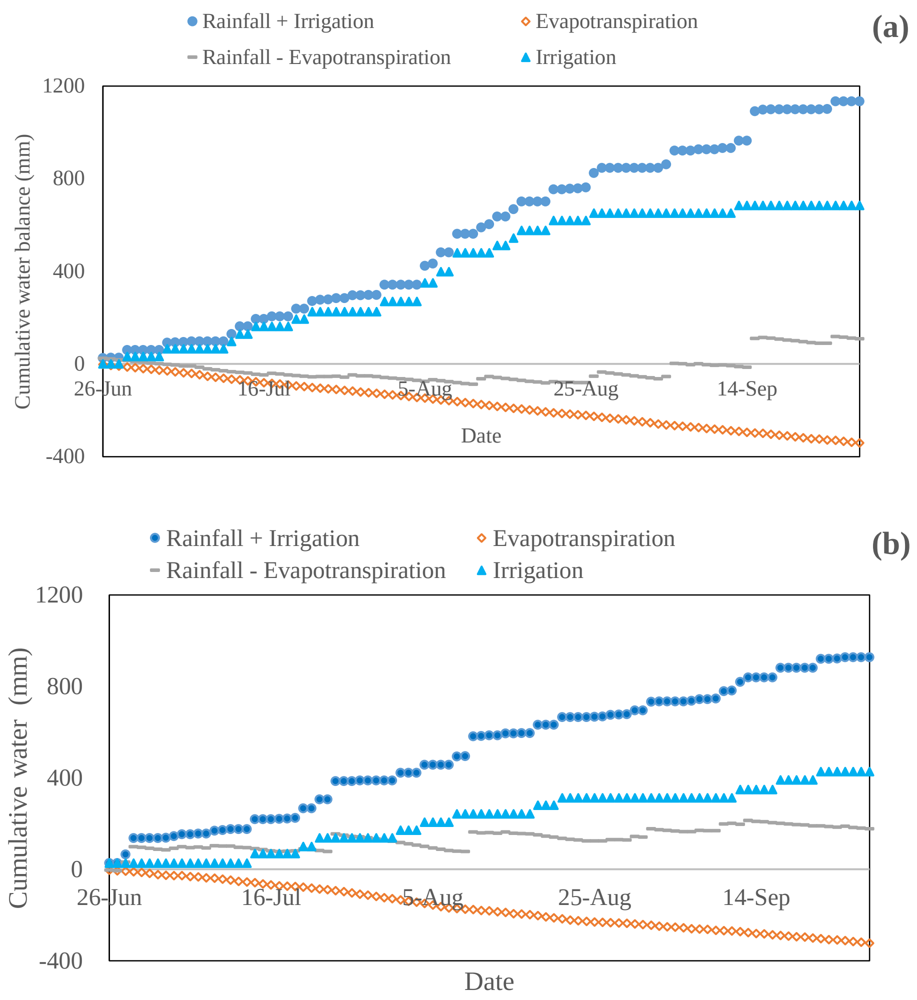


Fig. 2.2. Cumulative water supply (irrigation + rainfall) (●), cumulative irrigation (▲), evapotranspiration (◇), net water balance (rainfall – evapotranspiration) (–) in alternate wetting and drying lowland (AWD) conditions from 26 June to 30 September in 2013 (a) and 2014 (b).

### 2.2.5.2 Water depth monitoring and water balance

In the FL treatment, irrigation water was provided from the intake gate of the irrigation channel and the amount was calculated from the frequent recording of standing water depth. Water depth diver sensors (Baro Divers, Daiki Rika Kogyo, Saitama, Japan) were kept on the soil surface of FL plots to record hourly changes in water depth. The average percolation rate was calculated from changes in water depth during day and night from diver sensors and it was 40.1 and 38.9 mm $d^{-1}$ in 2013 and 2014 respectively. Average daily evapotranspiration calculated from Penman-Monteith method were 3.65 and 3.34 mm $d^{-1}$ in 2013 and 2014 respectively. Water depth was maintained at 3 to 5 cm throughout the season, except for the

1-week mid-season drainage each year. In the AWD treatment, irrigation was provided by a separate pump with a capacity of 105 and 80 L $min^{-1}$ in 2013 and 2014, respectively. The irrigation interval was set to 3 to 5 days, depending on the soil moisture potential reaching around –20 to –25 kPa. In the UP treatment, plants were completely rainfed until harvest, with no irrigation given.

*2.2.5.3 Phenology and leaf area index*

Phenology was recorded at the 50% flowering stage for each plot in all three treatments. One plant per plot was harvested on 24 August 2013 and 19 August 2014 to measure leaf area index and aboveground biomass. Leaf area index was measured with a leaf area meter (LI-3100, Li-Cor, Lincoln, NE, USA), and oven dried aboveground biomass was measured.

*2.2.5.4 Fraction interception*

Photosynthetically active radiation (PAR) was measured between 11:00 and 13:00 h on a clear sunny day during the 50% flowering stage with a line quantum sensor (LI-191, Li-Cor). In each plot, PAR above and below the canopy was measured by placing the sensor diagonal to rows below canopy. These PAR values were used to calculate the fraction of intercepted radiation (FI) as follows:

$$\text{FI (\%)} = (\text{PAR above canopy} - \text{PAR below canopy})/\text{PAR above canopy} \times 100. \qquad (1)$$

In 2014, three additional plants were harvested from each plot at 73, 86, 99, and 113 days after sowing for estimating aboveground biomass. To calculate daily FI during crop growth, PAR was also measured above and below the canopy on these four occasions. The cumulative radiation interception (RI) was calculated by summing daily incident solar radiation multiplied by daily FI, as follows:

$$\text{RI (MJ m}^{-2}) = \Sigma \text{ daily solar radiation} \times \text{daily FI} \qquad (2)$$

The aboveground biomass was plotted against cumulative RI, and radiation use efficiency (RUE) was obtained from the slope of the linear regression line by using both treatment average data and replicated data (Sinclair & Muchow, 1999). The natural logarithm of (1 – FI/100) was plotted against the leaf area index and the value of the light extinction coefficient (k) was obtained from the slope of the regression line forced through the origin for each replication.

*2.2.5.5 Photosynthetic parameters*

The photosynthetic parameters like photosynthetic rate, transpiration rate and stomatal conductance, intercellular $CO_2$ concentration were measured by portable photosynthetic system LI6400 (LI-COR, Lincoln, USA). The measurements were taken on flag leaf, on 16 Aug and 13 Aug in 2013 and 2014 respectively. Measurements were taken on clear sunny days during 09:00 to 11.30 hrs. Relative humidity was adjusted to 60% to 80%, $CO_2$ within the chamber was 400 µmol $CO_2$ $mol^{-1}$, and the block temperature set to 28 °C to 31 °C with the light source at 1500 µmol $m^{-2}$ $s^{-1}$. The values of reference and sample $CO_2$ concentrations were matched at 400 µmol $CO_2$ $mol^{-1}$ every 30 minutes.

*2.2.5.6 Carbon isotope composition*

In 2014, carbon isotope composition ($\delta^{13}C$) was analyzed from the stem and leaf sheath of three plants per treatment at the seedling, tillering, heading, and maturity stages. The plant samples were oven dried and ground into a very fine powder by using a fine mill (Heiko sample mill, TI 300, Fujiwara Seisakusho, Ltd. Tokyo, Japan). The $\delta^{13}C$ values of powdered samples (0.2 mg) were analyzed with an elemental analyzer/mass spectrometer (Flash 2000/Delta V Advantage, Thermo Fisher Scientific, Waltham, MA, USA).

*2.2.5.7 Yield and its components*

In both years, the plant height and number of tillers per hill were recorded for three plants per plot at maturity. Six plants per plot were harvested from 0.27 $m^2$ to calculate the aboveground biomass, panicle number per square meter, grain yield as grain dry weight, and yield components. The total number of panicles was counted, and panicles were hand threshed. All grains were soaked in tap water to separate fully filled grains from partially filled and empty grains. Grains that sank were considered to be fully filled and floating grains were again separated into empty (grain filling $<30\%$) and partially filled grains (grain filling $\geq30\%$), as checked by hand pressing. After separation, the grains were oven dried and weighed. For each grain category, 50 grains were counted manually and weighed, and thousand-grain weight and number of grains per square meter for each category was calculated. The number of grains per square meter includes only fully filled plus partially filled grains. Grain filling was calculated as the ratio of fully filled grains to total number of grains. The straw dry weight was recorded after oven drying to a constant weight. The harvest index was calculated as the ratio of grain yield to aboveground biomass.

*2.2.5.8 Water productivity*

Water used (irrigation plus rainfall) is presented as mega liters per hector (ML $ha^{-1}$). Water productivity (kg $m^{-3}$) was calculated as the ratio of grain yield at maturity to total water used (irrigation plus rainfall) from transplanting until physiological maturity.

2.2.5.9 Root and stele transversal area measurements

Roots in the UP treatment at the maturity stage were collected and immersed in FAA solution (5% formalin, 5% acetic acid, 45% ethanol, and 45% $H_2O$). Cross sections of nodal roots were taken at 1 cm from the root base and examined under a fluorescence and phase contrast microscope (BX51, Olympus, Hicksville, NY, USA). CellSens standard software (Olympus) was used to capture the microscopic images. By using ImageJ 1.50i software (National Institutes of Health, Bethesda, MD, USA) the dimensions of the root transversal area (RTA), STA, endodermis thickness, and number of xylem vessels were recorded. The STA ratio was calculated as STA/RTA × 100 (%). Rooting depth was not measured in this experiment.

**2.2.6 Statistical analysis**

Data were analyzed using GenStat 15th edition software (VSNi, Hemel Hempstead, UK). To assess genotypic variation and its interaction with water treatments and with environmental conditions, we performed a combined analysis of the entire dataset for the two years, separate yearly analyses, and Finlay-Wilkinson regression analysis. The combined analysis of variance (ANOVA) for two years was conducted among the four genotypes (IR64 and its three NILs) across the three water treatments to estimate the effect size of year, water treatments, genotypes, and their interactions. ANOVA was also performed each year for each water treatment and across the three water treatments to compare the performance of the NILs with that of the recipient genotype IR64. Multiple comparison analysis of the main effects (water treatment, genotypic variation) was done by using Tukey's test (significance set at $P<0.05$). The three water treatments in two years were regarded as six environments, and the genotype × environment interaction was analyzed by ANOVA for grain yield, aboveground biomass, harvest index, water productivity, and leaf area index. Finlay-Wilkinson regression analysis was performed between each NIL and IR64 across the six environments.

## 2.3 Results

### 2.3.1 Genotypic differences in yield and its components

Average two-year grain yield in the three water treatments was highest in Dro1-NIL (a significant increase of 14% over that of IR64); no significant increase was observed in Sta1-NIL or Dro1+Sta1-NIL in comparison with IR64 (Fig. 2.3a). Average aboveground biomass did not differ significantly between IR64 and any of the three NILs, although it was significantly higher in Dro1-NIL than in Sta1-NIL (Fig. 2.3b). Dro1-NIL and Sta1-NIL had significantly higher harvest indices than those of IR64 and Dro1+Sta1-NIL (Fig. 2.3c). ANOVA of data for both years among the four genotypes showed significant effects of genotype, water, year, and water × year and genotype × year (except for grain yield) interactions for grain yield, aboveground biomass, and harvest index, whereas the genotype × water treatment and genotype × water treatment × year interactions were not significant (Table 2.1). Based on average data from both years, the highest panicle number was in Dro1+Sta1-NIL and the highest grain number was in Dro1-NIL, whereas no significant differences were observed between other lines (Table 2.2). Grain filling percentage was highest in Dro1-NIL with a significant difference between this line and Dro1+Sta1-NIL. Thousand grain weight did not differ among IR64 and the three NILs. Plant height at maturity was significantly higher in Dro1-NIL and Dro1+Sta1-NIL than in Sta1-NIL, but was not significantly different from that of IR64.

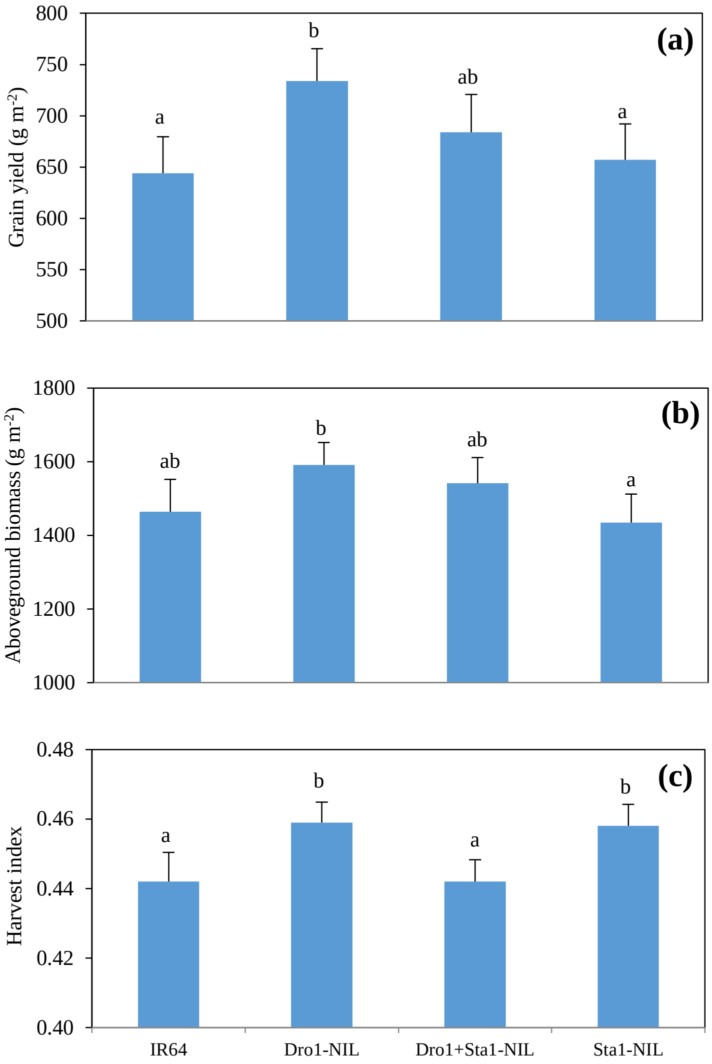


Fig. 2.3. Grain yield (g $m^{-2}$) (a), aboveground biomass (g $m^{-2}$) (b), and harvest index (c) averaged over three water management regimes in two years among IR64 and three of its near-isogenic lines (Dro1-NIL, Dro1+Sta1-NIL, Sta1-NIL). Different letters indicate significant differences at the 5% level (Tukey's test). Bars show standard error (*n*=18).

Table 2.1. Combined analysis of variance for grain yield, aboveground biomass, and harvest index across three water management regimes and two years among IR64 and its near-isogenic lines

| Factors | Grain yield | Aboveground biomass | Harvest index |
|---|---|---|---|
| Genotype (G) | *** | ** | ** |
| Water (W) | *** | *** | *** |
| Year (Y) | *** | *** | *** |
| G × W | NS | NS | NS |
| G × Y | NS (+) | ** | * |
| W × Y | *** | *** | *** |
| G × W × Y | NS | NS | NS |

Significance level NS- non significant *- significant at the P≤0.05 level, **- significant at the P≤0.010 level, ***- significant at the P≤0.001 level and NS (+)- significance at P<0.10 level.

Table 2.2. Panicle number, number of grains, grain filling percentage, thousand-grain weight, leaf area index at the 50% flowering stage, plant height, and number of tillers at maturity averaged over three water management regimes and two years among IR64 and its near-isogenic lines (NILs).

| Genotypes | Panicle number ($m^{-2}$) | Number of grains ($m^{-2} \times 10^3$) | Number of grains per panicle | Grain filling (%) | Thousand-grain weight (g) | Leaf area index | Plant height (cm) | Number of tillers ($hill^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| IR64 | 319 a | 26.9 a | 84.1 ab | 78.5 ab | 23.9 | 5.14 | 97.0 ab | 15.9 |
| Dro1-NIL | 346 ab | 30.5 b | 88.6 b | 80.8 b | 24.3 | 5.67 | 99.3 b | 16.1 |
| Dro1+Sta1-NIL | 353 b | 28.8 ab | 82.3 a | 76.6 a | 24.1 | 5.64 | 98.7 b | 16.9 |
| Sta1-NIL | 322 a | 27.4 a | 85.2 ab | 80.2 ab | 24.0 | 4.91 | 95.6 a | 15.5 |
| Average | 335 | 28.4 | 85.0 | 79.0 | 24.1 | 5.34 | 97.7 | 16.1 |
| LSD (5%) | 23 | 1.9 | 4.0 | 2.8 | - | 0.61 | 1.8 | - |
| Genotype (G) | ** | ** | * | * | NS | * | *** | NS |
| Water (W) | *** | *** | *** | *** | *** | *** | *** | *** |
| Year (Y) | * | ** | NS(+) | * | *** | NS | NS | * |
| G × W | NS | NS | NS | NS | * | NS(+) | NS | NS |
| G × Y | * | * | NS | NS | NS | NS | NS(+) | NS |
| W × Y | * | *** | *** | NS | *** | NS | * | NS |
| G × W × Y | NS | NS | NS(+) | * | NS | NS | NS | NS |

LSD: least significant difference

Means within each column followed by different letters indicate significant differences at the 5% level (Tukey's test). Significance level NS- non significant *- significant at the P≤0.05 level, **- significant at the P≤0.010 level, ***- significant at the P≤0.001 level and NS (+)- significance at P<0.10 level.

### 2.3.2 Environmental effects and its interactions with genotypes for yield and its components

Overall average grain yield was higher in 2014 (716 g $m^{-2}$) than in 2013 (643 g $m^{-2}$) (cf. Table 2.3). Grain yield and aboveground biomass averaged across the four genotypes were significantly higher in FL than AWD and UP in 2013, but there were no such differences between the FL and AWD treatments in 2014 (Table 2.3). Dro1-NIL had the highest grain yield in the FL treatment ($P<0.05$) in 2013. We found no significant genotypic variation in grain yield in any of the three water treatments in 2014. The genotype × water treatment interaction was not significant for grain yield and aboveground biomass in either year. The harvest index was significantly higher in FL and AWD than in UP in 2013. In 2014, Dro1-NIL had the highest harvest index in AWD ($P<0.05$), whereas the harvest index in UP was highest for Sta1-NIL and lowest for Dro1+Sta1-NIL. The genotype × water treatment interaction for harvest index was significant in 2014.

Table 2.3. Grain yield, aboveground biomass, and harvest index in three water management regimes (FL: flooded lowland, AWD: alternate wetting and drying lowland, UP: rainfed upland) among IR64 and its near-isogenic lines (NILs) in 2013 and 2014.

| Genotypes / Water | Grain yield (g m$^{-2}$) | | | Aboveground biomass (g m$^{-2}$) | | | Harvest index | | |
|---|---|---|---|---|---|---|---|---|---|
| | FL | AWD | UP | FL | AWD | UP | FL | AWD | UP |
| 2013 | | | | | | | | | |
| IR64 | 756 a | 589 | 371 | 1600 a | 1209 | 887 | 0.473 | 0.487 | 0.417 |
| Dro1-NIL | 946 b | 627 | 539 | 1919 b | 1338 | 1284 | 0.493 | 0.469 | 0.420 |
| Dro1+Sta1-NIL | 819 ab | 659 | 532 | 1723 ab | 1440 | 1281 | 0.475 | 0.458 | 0.414 |
| Sta1-NIL | 791 a | 640 | 447 | 1627 a | 1306 | 1012 | 0.487 | 0.489 | 0.443 |
| Average | 828 C | 629 B | 472 A | 1717 C | 1323 B | 1116 A | 0.482 B | 0.476 B | 0.424 A |
| LSD (5%) | 90** | - | 146+ | 158** | - | 316+ | 0.018+ | - | - |
| Genotype (G) | | ** | | | *** | | | NS(+) | |
| Water (W) | | *** | | | *** | | | *** | |
| G × W | | NS | | | NS | | | NS | |
| 2014 | | | | | | | | | |
| IR64 | 780 | 761 | 603 | 1900 | 1844 | 1344 | 0.411 | 0.413 a | 0.449 b |
| Dro1-NIL | 800 | 858 | 635 | 1764 | 1836 | 1408 | 0.454 | 0.469 b | 0.450 b |
| Dro1+Sta1-NIL | 745 | 782 | 566 | 1719 | 1762 | 1329 | 0.433 | 0.445 ab | 0.426 a |
| Sta1-NIL | 769 | 773 | 519 | 1790 | 1732 | 1142 | 0.430 | 0.447 ab | 0.454 b |
| Average | 774 B | 794 B | 581 A | 1793 B | 1794 B | 1306 A | 0.432 | 0.443 | 0.445 |
| LSD (5%) | - | - | - | - | - | - | - | 0.027* | 0.015* |
| Genotype (G) | | NS(+) | | | NS | | | *** | |
| Water (W) | | *** | | | *** | | | NS(+) | |
| G × W | | NS | | | NS | | | * | |

LSD: least significant difference among genotypes within a water treatment. **, *, + show P=0.01, 0.05 and 0.10, respectively.

Means in the same column followed by different lowercase letters indicate significant differences between genotypes within water treatments, and capital letters in the same row indicate differences among treatment averages at the 5% level (Tukey's test). Significance level NS- non significant *- significant at the P≤0.05 level, **- significant at the P≤0.010 level, ***- significant at the P≤0.001 level and NS (+)- significance at P<0.10 level.

Average leaf area index across genotypes was higher in FL treatment than AWD and UP (Table 2.4). Average specific leaf weight across genotypes was significantly higher in AWD and UP than FL in 2014 but no differences were recorded in 2013.

In 2013, the number of grains averaged across the four genotypes was highest in FL (33,200 m$^{-2}$) followed by AWD (26,500 m$^{-2}$) and UP (22,300 m$^{-2}$), whereas in 2014 AWD and FL had similarly high number of grains (32,200 and 31,600 m$^{-2}$) but that of UP was significantly lower (24,800 m$^{-2}$; Table 2.5). Average grain filling percentage across the genotypes under the three water treatments was higher in 2014 (80.3%) than in 2013 (77.8%), with the lowest value (75.7%) in FL in 2013 and the highest value (83.8%) in AWD in 2014. Dro1-NIL had the highest grain filling percentage in FL (80.0%; P<0.05). Thousand-grain weight was higher in FL followed by AWD and UP in 2013, with a significant genotype × water treatment interaction in 2013. Sta1-NIL had higher thousand-grain weight (24.5 g) than

Dro1+Sta1-NIL (24.0 g) in AWD (P<0.05) in 2013. Plant height was higher in 2014 than 2013, with FL higher than UP in both years and with AWD in between in 2013 but comparable to FL in 2014. In general, no genotype × water treatment interaction was detected, except for thousand-grain weight in 2013, grain filling in 2014, and plant height in 2014.

Table 2.4. Leaf area index and specific leaf weight at the 50% flowering stage in the experiments in 2013 and 2014.

| Genotypes/ water | Leaf area index | | | Specific leaf weight | | |
|---|---|---|---|---|---|---|
| | FL | AWD | UP | FL | AWD | UP |
| 2013 | | | | | | |
| IR64 | 5.8 | 5.1 | 3.7 | 0.00504 | 0.00501 | 0.00488 |
| Dro1-NIL | 7.2 | 4.6 | 5.9 | 0.00500 | 0.00496 | 0.00496 |
| Dro1+Sta1-NIL | 6.6 | 5.2 | 4.7 | 0.00491 | 0.00480 | 0.00487 |
| Sta1-NIL | 5.5 | 4.1 | 4.2 | 0.00486 | 0.00489 | 0.00482 |
| Average | 6.3 B | 4.7 A | 4.6 A | 0.00495 | 0.00491 | 0.00488 |
| LSD (5%) | - | - | - | - | - | - |
| G (separate) # | NS | NS | NS(+) | NS | NS | NS |
| G (combined)# | | * | | | NS | |
| W# | | *** | | | NS | |
| G × W# | | NS | | | NS | |
| 2014 | | | | | | |
| IR64 | 6.5 b | 5.5 | 4.2 | 0.00558 | 0.00574 | 0.00628 |
| Dro1-NIL | 6.0 ab | 5.0 | 5.4 | 0.00506 | 0.00608 | 0.00546 |
| Dro1+Sta1-NIL | 6.2 ab | 5.5 | 5.7 | 0.00498 | 0.00540 | 0.00525 |
| Sta1-NIL | 5.0 a | 5.8 | 4.9 | 0.00528 | 0.00538 | 0.00562 |
| Average | 5.9 A | 5.4 A | 5.1 A | 0.00522 A | 0.00565 B | 0.00565 B |
| LSD (5%) | 0.8 | - | - | 0.00051 | 0.00055 | 0.00081 |
| G (separate) # | * | NS | NS | NS(+) | NS(+) | NS(+) |
| G (combined)# | | NS | | | ** | |
| W# | | NS | | | ** | |
| G × W# | | NS | | | NS(+) | |

LSD: least significant difference

Means in the same column followed by different lowercase letters indicate significant differences between genotypes within water treatments, and capital letters indicate differences among treatment averages at the 5% level (Tukey's test). Significance level NS- non significant *- significant at the P≤0.05 level, **- significant at the P≤0.010 level, ***- significant at the P≤0.001 level and NS (+)- significance at P<0.10 level.

Table 2.5. Panicle number, number of grains, grain filling percentage, thousand-grain weight, plant height, number of tillers at maturity, and water productivity in the experiments in 2013 and 2014.

| Traits | Panicle number ($m^{-2}$) | | | Number of grains ($m^{-2}\times10^{3}$) | | | Grain filling (%) | | | Thousand-grain weight (g) | | | Plant height (cm) | | | Number of tillers ($hill^{-1}$) | | | Water productivity ($kg\ m^{-3}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotypes / Water | FL | AWD | UP | FL | AWD | UP | FL | AWD | UP | FL | AWD | UP | FL | AWD | UP | FL | AWD | UP | FL | AWD | UP |
| 2013 | | | | | | | | | | | | | | | | | | | | | |
| IR64 | 310.8 a | 288.6 a | 265.2 | 30.2 a | 24.5 | 17.7 | 72.3 a | 83.5 | 73.7 | 25.4 | 24.3 ab | 21.2 | 106.7 | 103 | 81 | 16 | 15 | 14 | 0.17 a | 0.45 | 0.56 |
| Dro1-NIL | 363.6 b | 319.4 ab | 350.3 | 38.1 b | 26.7 | 24.7 | 80.0 b | 78.9 | 80.2 | 25.3 | 24.1 ab | 22.1 | 109.1 | 104.3 | 81.9 | 18 | 14 | 17 | 0.21 b | 0.48 | 0.81 |
| Dro1+Sta1-NIL | 346.6 ab | 346.6 b | 367.5 | 33.1 a | 28.5 | 25.2 | 72.4 a | 76.2 | 75.2 | 25.5 | 24.0 a | 21.7 | 107.4 | 103.9 | 81 | 16 | 14 | 17 | 0.18 ab | 0.5 | 0.8 |
| Sta1-NIL | 325.6 ab | 318.2 ab | 300.9 | 31.3 a | 26.4 | 21.7 | 78.1 ab | 83.9 | 78.5 | 25.7 | 24.5 b | 20.9 | 105.4 | 103.6 | 81 | 16 | 14 | 15 | 0.18 a | 0.49 | 0.67 |
| Average | 336.6 | 318.2 | 321 | 33.2 C | 26.5 B | 22.3 A | 75.7 A | 80.8 B | 76.9 AB | 25.5 C | 24.3 B | 21.5 A | 107.2 C | 103.7 B | 81.2 A | 16.5 | 14.4 | 15.8 | 0.19 A | 0.48 B | 0.71 C |
| LSD (5%) | 29.9 | 37.4 | - | 2.5 | - | - | 4.9 | - | - | - | 0.3 | - | - | - | - | - | - | - | 0.02 | - | - |
| G (separate) [#] | * | * | NS | *** | NS | NS | * | NS | NS | NS | * | NS | NS | NS | NS | NS | NS | NS | ** | NS | NS |
| G (combined)[#] | | ** | | | *** | | | NS | | | NS | | | NS | | | NS | | | * | |
| W[#] | | NS | | | *** | | | * | | | *** | | | *** | | | NS | | | *** | |
| G × W[#] | | NS | | | NS | | | NS | | | * | | | NS | | | NS | | | NS | |
| 2014 | | | | | | | | | | | | | | | | | | | | | |
| IR64 | 400.8 | 326.8 | 323.1 | 31.6 | 31 | 26.4 | 76.4 | 81.6 | 83.7 | 24.9 | 24.6 | 23.0 | 104.3 a | 103.9 | 83.4 ab | 19 | 15 | 16 | 0.16 | 0.65 | 0.64 |
| Dro1-NIL | 384.8 | 321.9 | 333 | 32.2 | 34.8 | 26.6 | 81.7 | 86.4 | 77.7 | 25.1 | 24.8 | 24.1 | 109.0 b | 105.9 | 85.3 bc | 17 | 15 | 16 | 0.16 | 0.73 | 0.67 |
| Dro1+Sta1-NIL | 404.5 | 326.8 | 324.4 | 31.0 | 31.5 | 23.8 | 79.0 | 83.3 | 73.5 | 24.3 | 25.0 | 24.3 | 105.0 ab | 104.3 | 90.3 c | 21 | 16 | 17 | 0.15 | 0.67 | 0.6 |
| Sta1-NIL | 358.9 | 321.9 | 304.6 | 31.4 | 31.5 | 22.4 | 77.3 | 83.8 | 79.5 | 24.8 | 24.7 | 23.4 | 103.1 a | 102.7 | 77.5 a | 17 | 14 | 15 | 0.15 | 0.66 | 0.55 |
| Average | 387.3 B | 324.4 A | 321.3 A | 31.6 B | 32.2 B | 24.8 A | 78.6 A | 83.8 B | 78.6 A | 24.8 B | 24.8 B | 23.7 A | 105.4 B | 104.2 B | 84.1 A | 19 B | 15 A | 16 A | 0.16 A | 0.68 C | 0.62 B |
| LSD (5%) | - | - | - | - | - | - | - | - | - | - | - | 1.0 | 3.2 | - | 4.3 | - | - | - | - | - | - |
| G (separate) [#] | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | * | * | NS | ** | NS | NS | NS | NS | NS | NS |
| G (combined)[#] | | NS | | | NS | | | NS | | | NS | | | *** | | | * | | | * | |
| W[#] | | *** | | | *** | | | *** | | | *** | | | *** | | | *** | | | *** | |
| G × W[#] | | NS | | | NS | | | * | | | NS | | | * | | | NS | | | NS | |

LSD: least significant difference

Means in the same column followed by different lowercase letters indicate significant differences between genotypes within water treatments, and capital letters indicate differences among treatment averages at the 5% level (Tukey's test). Significance level NS- non significant *- significant at the P≤0.05 level, **- significant at the P≤0.010 level and ***- significant at the P≤0.001 level.

Among IR64 and its NILs, the effects of genotypes and six environments (i.e., three water treatments over two years) were significant for grain yield, aboveground biomass, and harvest index (Table 2.6). No genotype × environment interaction was detected for grain yield, above ground biomass and harvest index. ANOVA between the 2 groups of genotypes, one with *DRO1* (Dro1-NIL, Dro1+Sta1-NIL) and the other without *DRO1* (IR64, Sta1-NIL), showed significant effect for genotype for grain yield and above ground biomass, whereas genotype × environment interaction was significant only for above ground biomass. Harvest index was significant for genotype × environment interaction between the 2 groups. Between the other 2 groups of genotypes, one with *Sta1* (Sta1-NIL, Dro1+Sta1-NIL) and the other without *Sta1* (IR64, Dro1-NIL), genotype and genotype × environment interaction was non-significant for grain yield, above ground biomass and harvest index.

Table 2.6. Combined analysis of variance of grain yield, aboveground biomass, and harvest index across six environments (i.e., combination of three water management regimes and two years) among IR64 and three of its near-isogenic lines (NILs), between genotypes containing *DRO1* allele (*DRO1* plus) and genotypes without *DRO1* allele (*DRO1* minus) and genotypes containing *Sta1* allele (*Sta1* plus) and genotypes without *Sta1* allele (*Sta1* minus). LSD values are shown in bracket.

| Factors | Grain yield | Aboveground biomass | Harvest index |
|---|---|---|---|
| IR64 and NILs | | | |
| Genotype (G) | *** (44) | ** (99) | **(0.011) |
| Environment (E) | *** (54) | ** (121) | *** (0.014) |
| G × E | NS | NS(+) (242) | NS(+) (0.028) |
| *DRO1* plus and *DRO1* minus | | | |
| Genotype (G) | *** (32) | *** (69) | NS |
| Environment (E) | *** (56) | *** (119) | ***(0.008) |
| G × E | NS | * (168) | **(0.011) |
| *Sta1* plus and *Sta1* minus | | | |
| Genotype (G) | NS | NS | NS |
| Environment (E) | *** (62) | *** (138) | ***(0.017) |
| G × E | NS | NS | NS |

Significance level NS- non significant *- significant at the P≤0.05 level, **- significant at the P≤0.010 level, ***- significant at the P≤0.001 level and NS (+)- significance at P<0.10 level.

Comparison of the environmental mean yield response showed that Dro1-NIL yield was higher than that of IR64 across environments; the slope of the two linear regression lines looked similar but y-intercept was larger in Dro1-NIL (Fig. 2.4a). Grain yield was smaller in between Dro1-NIL and IR64 within medium yielding environments, but larger in the lower and higher yielding environments. Dro1+Sta1-NIL tended to have higher yield than IR64 under the lower yielding environment but their difference became small under higher yielding environment. IR64 and Sta1-NIL did not show differences across all environments in grain yield or aboveground biomass. Comparison of the environmental mean aboveground biomass response indicated that the advantage of the 2 NILs with *DRO1* (i.e., Dro1-NIL, Dro1+Sta1-NIL) over IR64 was largest in the lowest yielding environment (Fig. 2.4b). Dro1-NIL and Sta1-NIL had a higher harvest index across the six environments than that of IR64 and Dro1+Sta1-NIL (Fig. 2.4c).

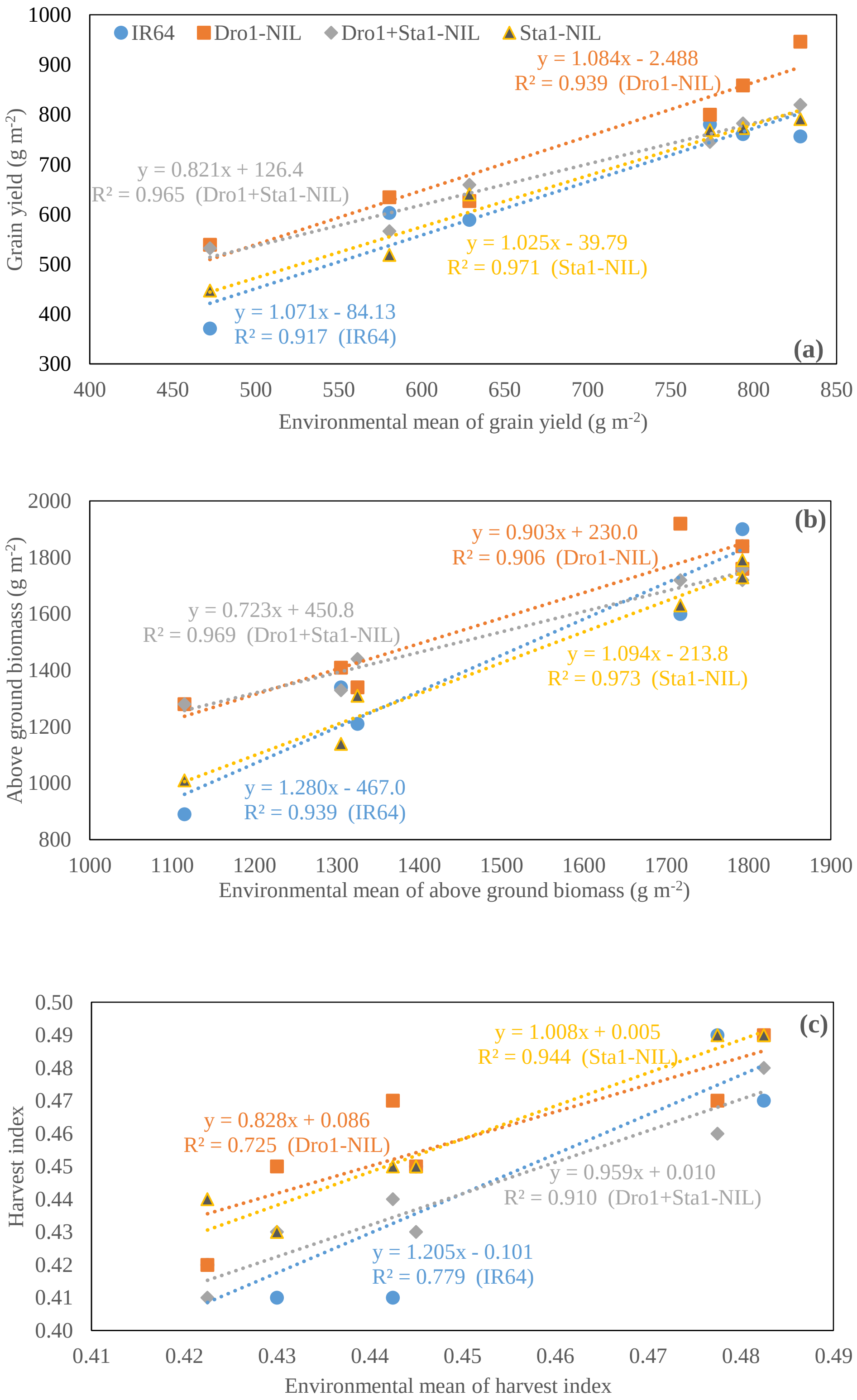


Fig. 2.4. Finlay-Wilkinson regression curves for analysis of genotype × environment interactions for grain yield (g $m^{-2}$) (a), aboveground biomass (g $m^{-2}$) (b), and harvest index (c) across six environments between average of four genotypes and IR64, Dro1-NIL, Dro1+Sta1-NIL and Sta1-NIL.

### 2.3.3 Light interception and RUE

The FI value was highest in FL, followed by AWD and UP. Dro1+Sta1-NIL had a significantly higher FI than that of IR64 (Table 2.7). We noted a significant genotype effect for FI between FL and UP treatments, but it was not significant for the AWD treatment. Combined ANOVA among the three water treatments showed significant effects of genotype, water, and genotype × water treatment interaction for FI and cumulative RI. In both the UP treatment and the three-treatment average, RI was higher in Dro1+Sta1-NIL than in Sta1-NIL. RUE was higher in AWD (1.55 g $MJ^{-1}$) and FL (1.50 g $MJ^{-1}$) than in UP (1.15 g $MJ^{-1}$), but we found no significant effect of genotype or genotype × water treatment interaction. There were no genotypic differences in extinction coefficient (k), which had an overall average value across all treatments and genotypes of 0.34. Finlay-Wilkinson regression showed a higher leaf area index in Dro1-NIL than in IR64, with a significant genotype × environment interaction (Fig. 2.5). The leaf area indices of Dro1+Sta1-NIL and Sta1-NIL were not significantly different from that of IR64.

Table 2.7. Fraction of intercepted radiation (FI), cumulative amount of intercepted radiation (RI), radiation use efficiency (RUE), and extinction coefficient (k) in three water management regimes (FL: flooded lowland, AWD: alternate wetting and drying lowland, UP: rainfed upland) among IR64 and its near-isogenic lines (NILs), with results of analysis of variance from the experiment in 2014.

| Traits | FI (%) | | | | RI (MJ m$^{-2}$) | | | | RUE (g MJ$^{-1}$) | | | | k | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotypes / Water | FL | AWD | UP | Average | FL | AWD | UP | Average | FL | AWD | UP | Average | FL | AWD | UP | Average |
| IR64 | 93.6 | 91.0 | 88.6 ab | 91.1 ab | 1242 | 1179 | 1203 ab | 1208 ab | 1.60 | 1.61 | 1.21 | 1.47 | 0.307 | 0.414 | 0.313 | 0.345 |
| Dro1-NIL | 95.2 | 92.9 | 90.9 ab | 93.0 bc | 1249 | 1205 | 1210 ab | 1221 ab | 1.46 | 1.57 | 1.26 | 1.43 | 0.339 | 0.341 | 0.317 | 0.332 |
| Dro1+Sta1-NIL | 95.2 | 92.1 | 92.5 b | 93.3 c | 1232 | 1209 | 1288 b | 1243 b | 1.41 | 1.48 | 1.07 | 1.32 | 0.365 | 0.345 | 0.374 | 0.361 |
| Sta1-NIL | 93.4 | 91.7 | 85.1 a | 90.1 a | 1228 | 1202 | 1161 a | 1197 a | 1.49 | 1.52 | 1.05 | 1.35 | 0.375 | 0.291 | 0.273 | 0.313 |
| Average | 94.4 C | 91.9 B | 89.3 A | | 1238 B | 1199 A | 1216 AB | | 1.50 B | 1.55 B | 1.15 A | | 0.347 | 0.348 | 0.319 | |
| LSD (5%) | 1.5* | - | 4.4* | 1.3** | - | - | 86+ | 28* | - | - | - | - | - | - | - | - |
| Genotype (G) | *** | | | | * | | | | NS | | | | NS | | | |
| Water (W) | *** | | | | * | | | | *** | | | | NS | | | |
| G × W | * | | | | * | | | | NS | | | | NS | | | |

LSD: least significant difference among genotypes within a water treatment. **, *, + show P=0.01, 0.05 and 0.10, respectively.
Means in the same column followed by different lowercase letters indicate significant differences between genotypes within water treatments, and different capital letters indicate differences among treatment averages at the 5% level (Tukey's test). Significance level NS- non significant *- significant at the P≤0.05 level, **- significant at the P≤0.010 level, ***- significant at the P≤0.001 level and NS (+)- significance at P<0.10 level.

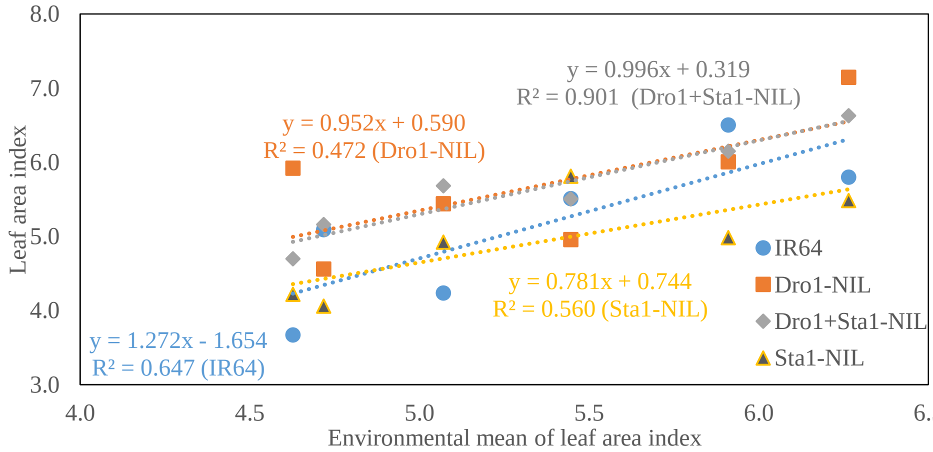


Fig. 2.5. Finlay-Wilkinson regression curves for analysis of genotype × environment interaction for leaf area index across six environments between average of four genotypes and IR64, Dro1-NIL, Dro1+Sta1-NIL and Sta1-NIL.

### 2.3.4 Genotypic differences for stele area, meta xylem and carbon isotope composition ($\delta^{13}C$)

IR64 and its NILs including donor parent Kinandang Patong not showed significant differences for root transversal area (Table 2.8, Fig. 2.6). Stele transversal area was significantly higher for Kinandang Patong than IR64 and its NILs. Stele transversal area, ratio of stele to root transversal areas, meta xylem numbers, meta xylem area and endodermis thickness were all larger or tend to be larger in Kinandang Patong than IR64 and its NILs. Sta1-NIL tended to show higher endodermis thickness compared to IR64 but statistically non-significant.

Table. 2.8 Root transversal area (RTA, $mm^2$), stele transversal area (STA, $mm^2$), ratio of transversal area of stele to root (%), meta xylem number, meta xylem area ($mm^2$) and endodermis thickness (μm) among five genotypes at maturity.

| Genotypes | RTA ($mm^2$) | STA ($mm^2$) | STA ratio | Meta xylem number | Meta xylem area ($mm^2$) | Endodermis thickness (μm) |
|---|---|---|---|---|---|---|
| IR64 | 680 a | 56.6 a | 8.3 bc | 5.0 a | 6.6 a | 9.4 a |
| Dro1-NIL | 697 a | 52.8 a | 7.7 ab | 5.3 a | 6.1 a | 11.1 ab |
| Dro1+Sta1-NIL | 693 a | 58.3 a | 8.5 bc | 5.0 a | 6.3 a | 11.8 ab |
| Sta1-NIL | 767 a | 48.4 a | 6.3 a | 5.0 a | 6.1 a | 12.7 ab |
| Kinandang Patong | 979 a | 92.9 b | 9.7 c | 6.7 b | 12.4 b | 13.5 b |
| Average | 763 | 61.8 | 8.1 | 5.4 | 7.5 | 11.7 |
| LSD (5%) | - | 13.6 | 1.8 | 1.1 | 1.8 | 3.2 |

Means followed by different letters indicate significant differences at 5% level (Tukey's test).

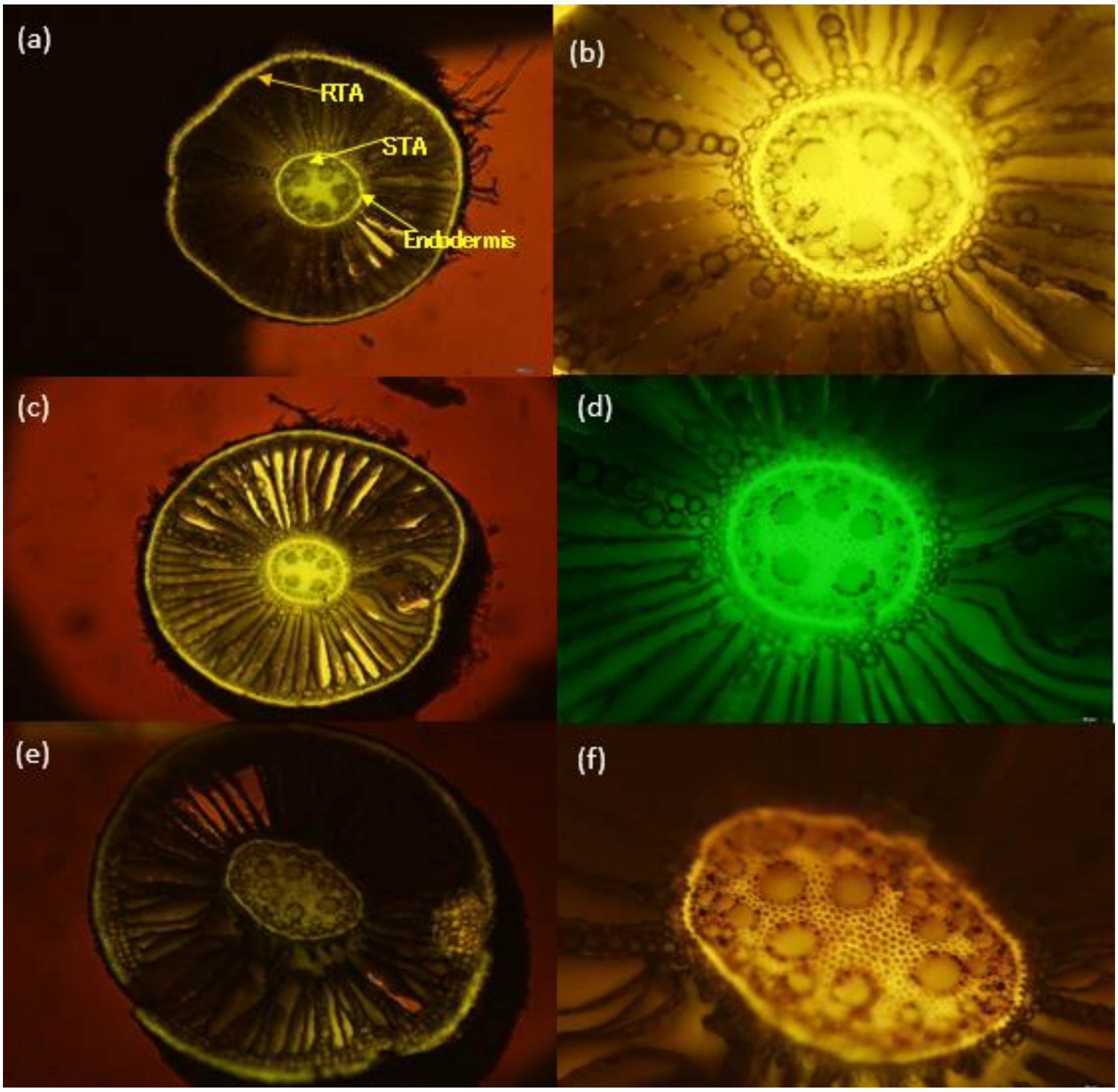


Fig. 2.6. Differences in root transversal area, stele transversal area, meta xylem vessels and endodermis thickness of rice plants stained with comassie brilliant blue at 4X and 10X zoom for IR64 (a, b), Sta1-NIL (c, d) and Kinandang Patong (e, f) respectively.

Combined ANOVA considering six water treatments as six environments showed lower $\delta^{13}C$ values (averaged across four genotypes) in UP treatment (-30.09‰) in 2014 while no differences were found between remaining water treatments in both years (Table 2.9). Significant difference for genotype was found (p value 0.073) and Sta1-NIL tended to show lower $\delta^{13}C$ values. We noted significant water × year interaction, however year, genotype × water and genotype × year effect was non-significant. When additional data from the all growth stages were combined, Sta1-NIL had the lowest $\delta^{13}C$ value (–29.54‰), followed by IR64 (–29.27‰), Dro1+Sta1-NIL (–29.19‰), and Dro1-NIL (–29.09‰).

Table 2.9. Carbon isotope composition ($\delta^{13}C$) of straw at maturity in the three water management regimes (FL: flooded lowland, AWD: alternate wetting and drying lowland, UP: rainfed upland) in two years (2013, 2014) and among IR64 and its near-isogenic lines (NILs).

| Water × Year | $\delta^{13}C$ ( ‰) |
|---|---|
| FL 2013 | -29.49 C |
| AWD 2013 | -29.70 B |
| UP 2013 | -29.62 B |
| FL 2014 | -29.38 D |
| AWD 2014 | -29.55 C |
| UP 2014 | -30.09 A |
| LSD water × Year (5%) | 0.11 |
| Genotype | |
| IR64 | -29.61 |
| Dro1-NIL | -29.56 |
| Dro1+Sta1-NIL | -29.59 |
| Sta1-NIL | -29.79 |
| - | - |
| ANOVA | |
| Genotypes (G) | NS(+) |
| Water (W) | *** |
| Year (Y) | NS |
| G × W | NS |
| G × Y | NS |
| W × Y | *** |
| G × W × Y | NS |

LSD: least significant difference
Means in the same column followed by capital letters indicate significant differences by LSD values at 5% level. Significance level NS- non significant *- significant at the P≤0.05 level, **- significant at the P≤0.010 level, ***- significant at the P≤0.001 level and NS (+)- significance at P<0.10 level.

UP treatment in 2013 had highest rate of photosynthesis and UP treatment in 2014 had highest rate of transpiration (Fig. 2.7a, b). Treatment effect was visible for stomatal conductance as in both years FL treatment maintained higher conductance than AWD and lowest in UP treatment (Fig. 2.7c). $\delta^{13}C$ values were increased with increasing stomatal conductance. Intercellular $CO_2$ concentration was lowest in UP treatment in 2013 however no differences were recorded among other treatments in both years (Fig. 2.7d).

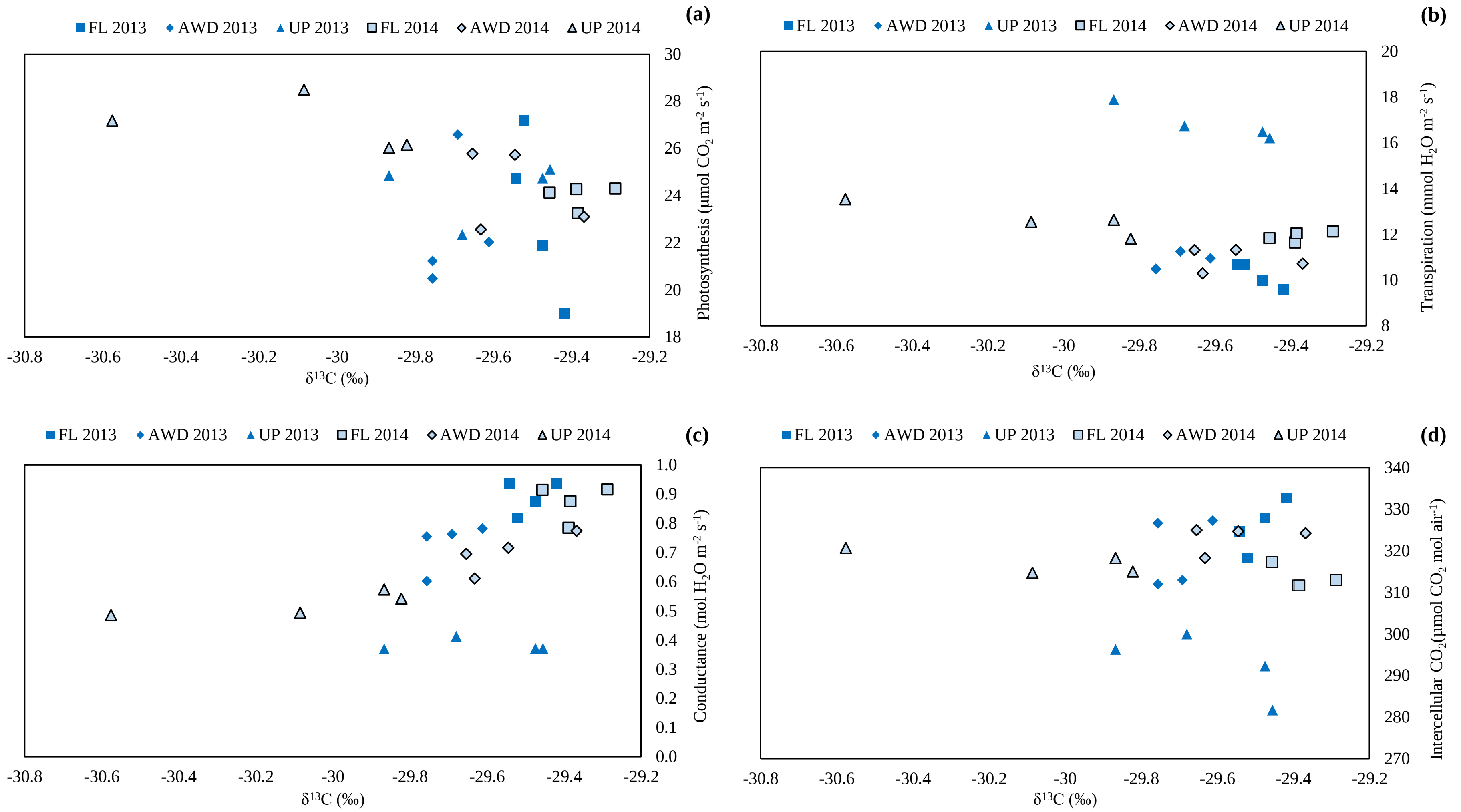


Fig. 2.7. Relationship of $\delta^{13}C$ with photosynthesis rate (a), transpiration rate (b), conductance (c) and intercellular $CO_2$ concentration. Genotypes are combined across two years under three water treatments.

### 2.3.5 Water use and water productivity

The average total amount of water (irrigation + rainfall) used in FL, AWD, and UP per hectare was 44.5, 13.0, and 6.6 ML $ha^{-1}$ in 2013 and 49.8, 11.7, and 9.4 ML $ha^{-1}$ in 2014, respectively. On average, over the two years AWD and UP required much less, with 74% and 83% of water saved compared with FL. Total water used was similar among IR64 and the three NILs, as their growth durations were similar (Table 2.10). Water productivity averaged over two years was highest in UP (0.66 kg $m^{-3}$), followed by AWD (0.58 kg $m^{-3}$) and then by FL (0.17 kg $m^{-3}$) Significant interaction was found between water management and year, with the value of AWD in 2014 equally highest as that of UP in 2013 and higher than AWD in 2013. Higher above ground biomass was associated with smaller water productivity in general among the six environments of combination of 3 water managements and 2 years, but the exception was AWD in 2014 that had attained both highest level of biomass production and water productivity (Fig. 2.8). Water productivity in AWD increased compared with FL without large changes in $\delta^{13}$C (Fig. 2.9). The differences in water productivity between AWD and UP was small but there was large variation in $\delta^{13}$C values.

Water productivity was highest in Dro1-NIL followed by Dro1+Sta1-NIL and lowest in Sta1-NIL and IR64 (Table 2.10). Dro1-NIL had the highest water productivity in FL in 2013 (0.21 kg $m^{-3}$; Table 2.5). Genotype and water management effects were significant in each year while genotype by water interaction was not significant at 5% level. Relationship of water used and grain yield showed higher grain yield in Dro1-NIL than IR64 and Sta1-NIL in higher and lower yielding environments (Fig. 2.10), indicating that the introgression of *DRO1* would improve productivity to a greater extent. There was on the other hand no difference in grain yield between IR64 and Sta1-NIL in almost all environments.

Genotypic variation in water productivity was positively correlated with various components of yield formation such as above ground biomass, leaf area index, fraction interception, panicle number, number of grains and thousand grain weight (Table 2.11). Cumulative radiation interception was positively correlated with leaf area index and fraction interception but radiation use efficiency was not positively correlated with water productivity nor with any of the components of yield formation. There was no correlation between water productivity and carbon isotope composition among the 4 genotypes.

Table 2.10. Average total amount of water used per hectare (rainfall + irrigation) and water productivity in the three water management regimes (FL: flooded lowland, AWD: alternate wetting and drying lowland, UP: rainfed upland) in two years (2013, 2014) and among IR64 and its near-isogenic lines (NILs) averaged over two years.

| | Water used (ML $ha^{-1}$) | Water productivity (kg $m^{-3}$) |
|---|---|---|
| Water x Year | | |
| FL 2013 | 44.5 E | 0.19 A |
| AWD 2013 | 13.1 D | 0.48 B |
| UP 2013 | 6.6 A | 0.71 D |
| FL 2014 | 49.8 F | 0.16 A |
| AWD 2014 | 11.7 C | 0.68 CD |
| UP 2014 | 9.4 B | 0.62 C |
| LSD water × year (5%) | 0.001 | 0.025 |
| Genotype | | |
| IR64 | 22.6 | 0.44 a |
| Dro1-NIL | 22.5 | 0.51 b |
| Dro1+Sta1-NIL | 22.6 | 0.48 ab |
| Sta1-NIL | 22.5 | 0.45 a |
| LSD genotype (5%) | - | 0.041 |
| Average | 22.5 | 0.47 |
| Genotypes (G) | NS | ** |
| Water (W) | *** | *** |
| Year (Y) | *** | NS |
| G × W | NS | NS |
| G × Y | NS | NS (+) |
| W × Y | *** | *** |
| G × W × Y | NS | NS(+) |

LSD: least significant difference

Means in the same column followed by different letters indicate significant differences at the 5% level (Tukey's test). Significance level NS- non significant *- significant at the P≤0.05 level, **- significant at the P≤0.010 level, ***- significant at the P≤0.001 level and NS (+)- significance at P<0.10 level.

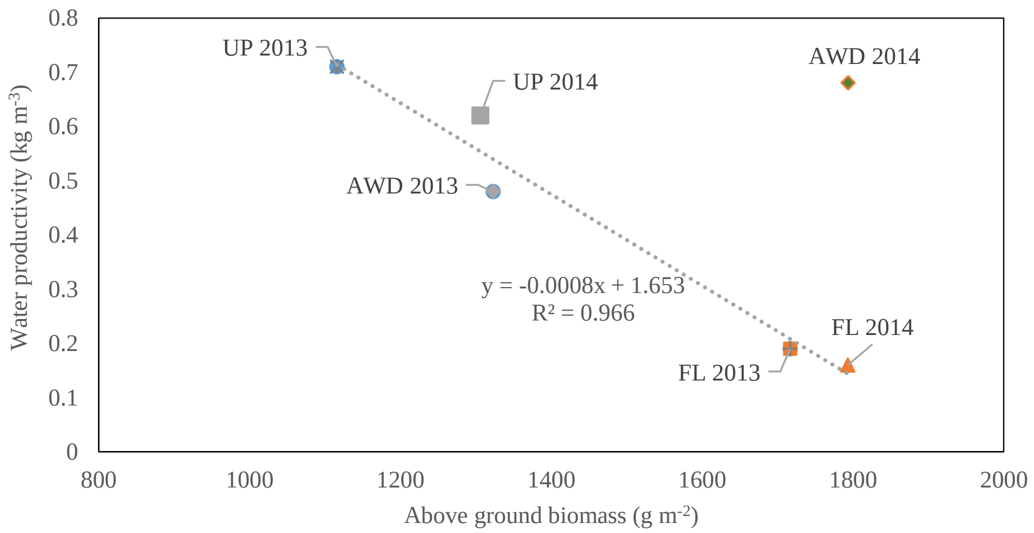


Fig. 2.8. Relationship between above ground biomass (g $m^{-2}$) and water productivity (kg $m^{-3}$) across three water managements ((FL: flooded lowland, AWD: alternate wetting and drying lowland, UP: rainfed upland) in two years (2013, 2014)). The regression line is drawn excluding AWD 2014.

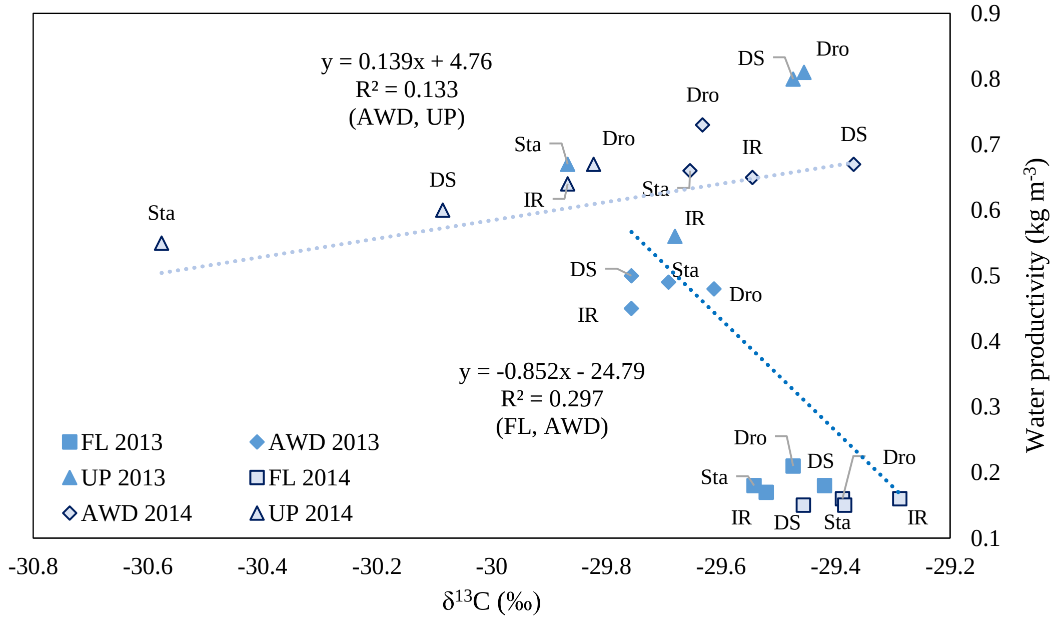


Fig. 2.9. Relationship between $\delta^{13}C$ (‰) and water productivity (kg $m^{-3}$) across three water managements ((FL: flooded lowland, AWD: alternate wetting and drying lowland, UP: rainfed upland) in two years (2013, 2014)). Abbreviations of 4 genotypes were labelled as IR64 (IR), Dro1-NIL (Dro), Dro1+Sta1-NIL (DS), Sta1-NIL (Sta). The regression lines were drawn for FL and AWD and for AWD and UP.

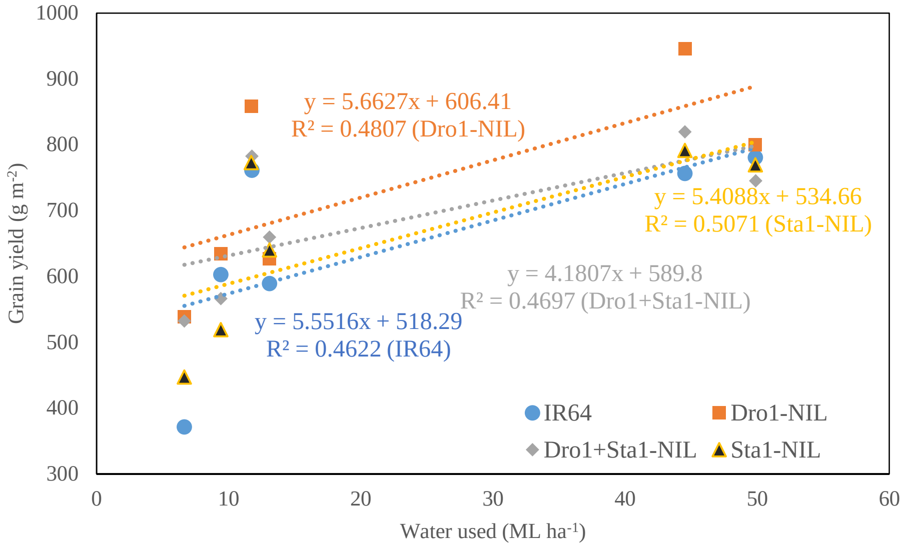


Fig. 2.10. Relationship of water used and grain yield across six environments in 2013 and 2014 among IR64 and its NILs.

Table 2.11. Correlation analysis of grain yield and yield components averaged across two years and three water treatment with fraction interception, cumulative radiation interception, radiation use efficiency and extinction coefficient from 2014 (*n=4*).

| | Grain yield | Above ground biomass | Harvest index | Leaf area index | Specific leaf weight | Panicle number | Number of grains $pan^{-1}$ | Number of grains $m^{-2}$ | Grain filling | Thousand grain weight | Water productivity | $\delta^{13}C$ | FI | RI | RUE | *K* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Grain yield | 1 | | | | | | | | | | | | | | | |
| Above ground biomass | 0.92* | 1 | | | | | | | | | | | | | | |
| Harvest index | 0.48 | 0.11 | 1 | | | | | | | | | | | | | |
| Leaf area index | 0.81* | 0.97** | -0.11 | 1 | | | | | | | | | | | | |
| Specific leaf weight | -0.23 | -0.19 | -0.09 | -0.28 | 1 | | | | | | | | | | | |
| Panicle number | 0.75 | 0.87* | -0.06 | 0.93* | -0.61 | 1 | | | | | | | | | | |
| Number of grains $pan^{-1}$ | 0.66 | 0.40 | 0.82* | 0.16 | 0.33 | 0.01 | 1 | | | | | | | | | |
| Number of grains $m^{-2}$ | 0.99** | 0.94** | 0.42 | 0.85* | -0.30 | 0.82* | 0.58 | 1 | | | | | | | | |
| Grain filling | 0.35 | 0.01 | 0.90* | -0.24 | 0.34 | -0.33 | 0.91* | 0.26 | 1 | | | | | | | |
| Thousand grain weight | 0.97** | 0.89* | 0.47 | 0.81* | -0.45 | 0.83* | 0.53 | 0.99** | 0.24 | 1 | | | | | | |
| Water productivity | 0.98** | 0.94** | 0.38 | 0.87* | -0.39 | 0.86* | 0.51 | 0.99** | 0.18 | 0.99** | 1 | | | | | |
| $\delta^{13}C$ | 0.56 | 0.81* | -0.38 | 0.85* | 0.21 | 0.62 | 0.12 | 0.58 | -0.29 | 0.46 | 0.57 | 1 | | | | |
| FI | 0.75 | 0.93** | -0.20 | 0.99** | -0.33 | 0.94** | 0.05 | 0.80 | -0.35 | 0.76 | 0.83* | 0.85* | 1 | | | |
| RI | 0.47 | 0.73 | -0.46 | 0.87* | -0.51 | 0.91* | -0.33 | 0.55 | -0.67 | 0.55 | 0.61 | 0.70 | 0.92* | 1 | | |
| RUE | -0.01 | 0.02 | -0.01 | -0.09 | 0.97** | -0.44 | 0.47 | -0.08 | 0.40 | -0.24 | -0.17 | 0.37 | -0.15 | -0.39 | 1 | |
| *K* | 0.05 | 0.42 | -0.85* | 0.62 | -0.12 | 0.56 | -0.59 | 0.12 | -0.86* | 0.07 | 0.17 | 0.73 | 0.69 | 0.84* | -0.08 | 1 |

*correlation is significant at 0.05 level and **correlation is significant at 0.01 level

### 2.4 Discussion

### 2.4.1 Improvement of water productivity by water-saving

Water-saving could often sacrifice biomass production (Fig. 2.8), as photosynthesis and transpiration are physiologically linked together, but our study pointed the greatly improved water productivity by water-saving through AWD in 2014, without reducing yield; with total water supply of 1.17 $m^3$ from rainfall and irrigation for 1 ha (i.e., 1170 mm) which was a dramatic saving as much as 77% of water supplied in flooded management (FL) (Table 2.10), AWD in 2014 maintained yield as high as FL in 2014. AWD in 2014 recorded higher water productivity (0.68 kg $m^{-3}$) than AWD in 2013 (0.48 kg $m^{-3}$) with the former having less yield penalty, because of the higher rainfall and lower evapotranspiration rate in 2014 (Fig. 2.1e, f; Fig. 2.2), and higher soil water potential in 2014 (-10.1 kPa) than in 2013 (-19.1 kPa). With significant year by water management interaction for yield and water productivity, our results indicate AWD is more successful (i.e., maintaining yield under water-saving cultivation) when water (i.e., rainfall, soil water) is more available in the crop production environments. Bouman et al. (2005) showed greater advantage of water-saving for increasing water productivity and minimizing yield reduction during wet season compared with dry season, under upland cultivation with soil water potential at 15 cm depth maintained over -30 kPa (i.e., aerobic rice). Kato et al. (2006b) also showed water-saving aerobic rice could maintain yield in a year with frequent and sufficient rainfall (794 mm) but not in a year with less rainfall (622 mm).

Our study also showed that choice of genotype can improve water productivity, as Dro1-NIL showed significantly higher water productivity than IR64 (by 16% on average, Table 2.10). Superior leaf area index which was related with higher fraction interception and larger radiation interception rather than RUE (data available only in 2014), contributed greater biomass production and larger water productivity for Dro1-NIL among the 4 genotypes. Modified root system by *DRO1* would have depleted greater soil moisture from deeper soil (A. Kamoshita, unpublished data) and enabled greater capture of water and nitrogen (cf. Arai-Sanoh et al., 2014) which sustained more vigorous vegetative growth for higher biomass production. Superior water productivity of a deeper rooting variety to a shallower rooting variety across different water availability in upland fields has been reported (e.g., Kato et al., 2006a), but our study showed that deeper root angle conferred by *DRO1* could be effective to improve water productivity across different water regimes, namely flooded lowland, alternate wet and dry lowland, and upland environments. However the superiority of Dro1-NIL over IR64 was more evident in drier year 2013 than in wetter year 2014 (Table 2.5).

### 2.4.2 Effect of *DRO1* on grain yield under alternate wetting and drying conditions

This was the first study as far as we know which tested the effect of introgression of *DRO1* on rice yield under alternate wetting and drying (AWD) conditions. Since the combined analysis of the six environments showed higher yield of Dro1-NIL than IR64 (Fig. 2.3a, 2.4a), *DRO1* was considered to be advantageous to higher yield for IR64 including under AWD conditions. However, such a superior yield performance was not detected at significant level within a single year analysis under AWD, suggesting the modest effect of *DRO1* under AWD.

In our study, we top-dressed the plots with 6 g $m^{-2}$ of nitrogen fertilizer, which might have reduced the potential advantage of *DRO1* to exploit more nitrogen from the deeper soil layer over IR64 (the soil nitrogen profile was not measured in this study). Under AWD conditions, in which the soil surface was exposed to aerobic conditions, the profile of $NO_3$-N and $NH_4$-N would have been altered too, which might have further complicated plant nitrogen uptake dynamics, resulting in smaller differences in nitrogen uptake and growth (e.g., see panicle number, leaf area index, and plant height in Table 2.5) between Dro1-NIL and IR64. Arai-Sanoh et al. (2014) reported the whole-plant nitrogen content at the maturity stage was higher for Dro1-NIL than IR64 under FL conditions, where all the nitrogen was incorporated as a basal control-released fertilizer and both the 0–10 and 10–20 cm soil layers contained similar amounts of available nitrogen (5.0 and 4.4 mg/100 g, respectively). They also reported larger grain yield of Dro1-NIL than IR64 in both low and high-nitrogen fertilizer treatments under FL conditions, showing no *DRO1* × nitrogen level interaction and enhancement of cytokinin production, nitrogen uptake, and grain yield by *DRO1* regardless of fertilizer treatments.

Our study is the first to have quantified the *DRO1* × water management interaction with the range of yield at a practical production level; the result was no significant interaction across FL, AWD and UP. Modification of root growth angle may contribute to better resource acquisition (e.g., nitrogen, water) and result in superior performance of Dro1-NIL across different water regimes both lowland and upland environments (Arai-Sanoh et al., 2014, Uga et al., 2013). Uga et al. (2013) showed superior grain dry weight per plant in Dro1-NIL as compared to that of IR64 under mild and severely dry upland conditions and no difference under sub-optimal level of irrigated upland conditions with yield level ca. 3 t $ha^{-1}$ (i.e., far below potential yield).

The higher grain yield of Dro1-NIL in our study resulted from a higher harvest index and number of grains per unit area, but also from panicle number, number of grains per panicle, grain filling, and leaf area index as well (Figs. 2.3c, 2.4b, 2.4c, 2.5; Table 2.2). Grain filling under FL and thousand-grain weight under UP also contributed to the yield advantage of Dro1-NIL. Previous studies also revealed the contribution of grain filling under dry upland conditions (Uga et al., 2013) and thousand-grain weight and number of ripened grains under FL conditions (Arai-Sanoh et al., 2014).

### 2.4.3 Performance of STA

Sta1-NIL did not produce higher yield or greater aboveground biomass than those of IR64 (Figs. 2.3a, 2.3b, 2.4a, 2.4b; Table 2.3). However, its harvest index was higher (Figs. 2.3c, 2.4c, Table 2.3), indicating better assimilate partitioning to grain in Sta1-NIL than in IR64. Aboveground biomass and leaf area index in higher yielding environments were lower in Sta1-NIL compared with those of IR64 and other NILs. Dixit et al. (2012) found a negative effect of a donor segment including qDTY9.1, which was associated with grain yield under moderate and severe stress conditions and was located close to one of the QTLs detected for stele and xylem structures on chromosome 9 (Uga et al., 2010). Sta1-NIL contained this segment from chromosome 9, which might have prevented the positive effect of *Sta1* for higher harvest index from contributing to higher yield in our study.

Among IR64 and its NILs, Sta1-NIL tended to show the lowest carbon isotope composition ($\delta^{13}C$) measured at maturity stage (Table 2.9), which indicates higher discrimination for $^{13}C$ during photosynthesis. Lowered leaf area index in UP and AWD increased the specific leaf weight (Table 2.4) which might have increased photosynthesis rate and lowered the discrimination for $^{13}CO_2$ under UP and AWD treatments compared to FL (Table 2.9). Kinandang Patong allele of *Sta1* increased stele transversal area (STA) by 25% as compared with that of IR64 (Uga et al., 2010) and the larger proportion of STA in the root transversal area (RTA) in Sta1-NIL (Uga et al., 2008) could be advantageous for retaining water in vascular tissues (Henry et al., 2012), which may result in greater stomatal opening and a higher intercellular carbon dioxide concentration (not measured in this study) than in IR64. Kinandang Patong, the donor parent of Sta1, not only had larger RTA, STA, and proportion of STA, with larger metaxylem number and area, but also greater endodermis thickness than those of IR64 (Table 2.8). Radial and transverse walls of endodermis have a Casparian strip that contains suberin and is impermeable to water (Schreiber et al., 2005). Thick endodermis and/or greater suberization in endodermis (Henry et al., 2012) might minimize the loss of water from the xylem to the cortex and contribute to better retention of water within vascular tissues. Sta1-NIL may have inherited genetic loci for root anatomical structure from Kinandang Patong, which might result in a stronger apoplastic barrier in root tissues. Genetic variation in apoplastic barrier with regard to the Casparian strip has been reported among rice varieties with different levels of salt resistance (Krishnamurthy et al., 2009). Sta1-NIL may be a beneficial genetic material for further studies of plant water movement and physiological responses to water-limiting conditions.

### 2.4.4 Effect of combination of *DRO1* and *Sta1* on grain yield and water productivity

This is the first study to investigate the combination of *DRO1* and *Sta1* across different water conditions by comparing IR64 and its NILs with a single gene/QTL inserted. Dro1+Sta1-NIL had the highest panicle number, FI, and RI (Tables 2.2, 2.7) among the genotypes, leading to higher aboveground biomass in the lower yielding environment (Fig. 2.3b) and slightly higher yield than that of IR64 (Table 2.3, Fig. 2.3a). However, the yield advantage of Dro1+Sta1-NIL was smaller than that of Dro1-NIL, and the combination of the two genes was not additive or synergetic. The performance of Dro1+Sta1-NIL was more similar to Dro1-NIL than to that of Sta1-NIL, which may be due to the stronger function of *DRO1* than *Sta1* in field experiments with different water treatments. The grain filling ratio of Dro1+Sta1-NIL was lower than those of other genotypes, which limited final grain yield. The negative effect of qDTY9.1 or other QTLs located near *Sta1* might have reduced yield in Dro1+Sta1-NIL (as was the case in Sta1-NIL). Water productivity was higher in Dro1+Sta1-NIL than in IR64 and Sta1-NIL (but less than in Dro1-NIL), and the difference was larger in environments with higher water productivity (e.g., UP; Fig. 2.4). Thus, from the viewpoint of field-level water savings, Dro1+Sta1-NIL was also considered as a suitable option compared with IR64, as was Dro1-NIL. Our results suggest the greater importance of resource acquisition by developing a deeper root system via *DRO1* than by manipulating hydraulic conductivity by stele thickness via Sta1, but they do not exclude the possibility of utilizing both genes where their combination can better sustain rice production than *DRO1* alone. It will be worthwhile to further examine the combined effects of *DRO1* and *Sta1* using NILs without the negative genomic locus nearby qDTY9.1 under various water-limiting conditions.

# Chapter 6

# General discussion

### 6.1 Water productivity under different environmental conditions in three countries

In our studies of on-station and on-farm experiments across three countries (Japan, India, Colombia) amounts of water supply for rice production were estimated for quantitative relationship with yield (Fig. 6.1) and also for calculation of water productivity. Reducing water input tended to sacrifice yield on average, but examination of each cases would clarify the conditions for more successful water saving rice cultivation technique.

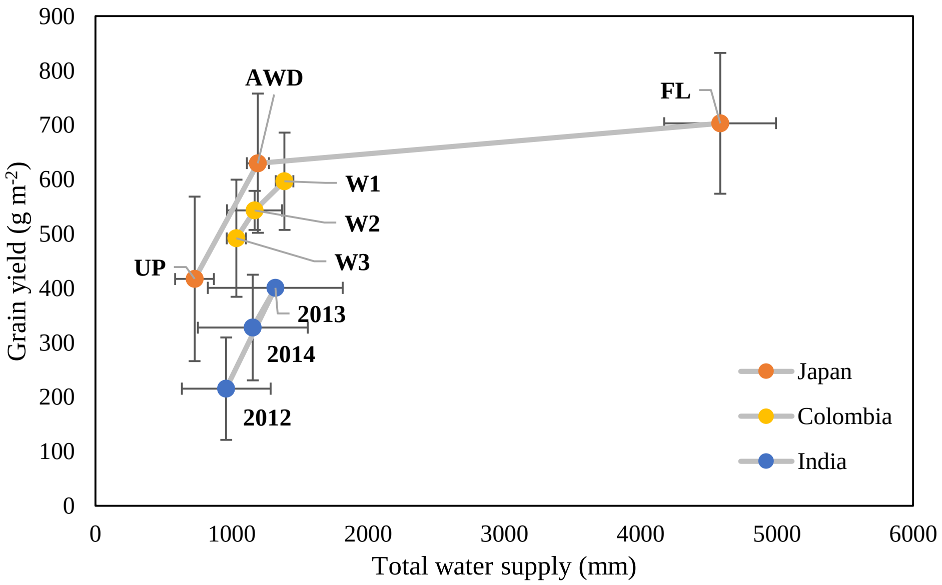


Fig. 6.1. Relationship of total water supply with grain yield in three water managements in Japan and Colombia across two years, and in India across three years. The bars indicates the standard deviation for grain yield on Y axis and total water supply on X axis.

Average water productivity values under different water regimes ranged from 0.13 to 0.71 kg $m^{-3}$ (Table 6.1). This range was comparable to the reported values of world water productivity in a review paper ranging from 0.15 to 0.60 kg $m^{-3}$ (Cai and Rosegrant, 2003). Among the three countries India showed lower water productivity of 0.27 kg $m^{-3}$ ranging from 0.19 to 0.41 kg $m^{-3}$ where the average grain yield surveyed in the tank irrigation ecosystem was 1.5 to 4.1 t $ha^{-1}$ with the average N fertilizer application rate of 97 kgN $ha^{-1}$. The South Indian farmers in the target area of this study prefer the locally adapted fine grain rice varieties which are not the highest yield potential among the available varieties

(Venkatesan et al., 2008); if higher yielding varieties such as Indian hybrid rice should have been used, water productivity could have increased.

Due to absence of large river and limited and variable availability of fresh water resources in South India, farmers depend largely on rainfall brought by monsoon during kuruwai season (Sep-Feb). Which may be another reason why farmers would not go with hybrid rice varieties. The soil in both tanks was brown and black colored sandy clay with slightly alkaline to neutral pH which may not be suitable for hybrid or other high yielding varieties. Our survey highlighted the drought year 2012 when seasonal rainfall was only 221 mm; and tank water storage capacity and irrigation water supply were greatly reduced (Chapter 4, Table 4.14); water productivity in 2012 reduced further in small tank (0.19 kg $m^{-3}$, Table 4.13). Yearly and spatial variation in water productivity was larger in small tank than in big tank (Table 4.12, 4.13). However water productivity was varied from year to year in both tanks and still lower values. There would be large scope to improve the water productivity for Indian tank rice cultivation system by variety improvement, agronomic manipulation and application of water saving irrigation techniques. Improving water productivity can help to reduce the competition to irrigation water, and also can save the cost by reducing ground water usage and helps to improve the income.

In Colombia, high yielding rice genotypes were used with high N application rate, 180 kgN $ha^{-1}$ or higher N dose (Chapter 5, Table 5.1) resulting in higher water productivity than India and Japanese flooded lowland systems. Lack of puddling can increase the percolation rate for rice fields, however dry direct seeding method in Colombia showed higher water productivity with relatively lower water use. Dry direct seeded rice can save irrigation water for land preparation which shares 30% of required water in transplanting method (Chauhan and Opeña, 2012). It is also reported that dry direct seeded rice reached up to 10.3 Mg $ha^{-1}$ in Missouri, USA with 700 mm water input (Stevens et al., 2012) and in Japan also yielded up to 9.6 t $ha^{-1}$ with 800-1300 mm of water input (Kato et al., 2009) or with 723-802 mm of water input (rainfall plus irrigation) (Katsura and Nakaide 2011). Zhao et al. (2007) reported 5.3 % higher grain yields and about 25–50% lower water use in dry direct-seeded rice than transplanted-flooded rice. As was discussed in Chapter 5, it was possible to further improve water productivity by lengthening irrigation interval. Conventional interval of 3 days (W1) can be extended to 5 or 6 days (W2) without yield penalty depending on weather conditions; our data showed water saving was more easier during wet season than dry season, and also improved water productivity in mild water saving treatment (W2) (0.41 kg $m^{-3}$ in dry season and improved up to 0.54 kg $m^{-3}$ in wet season). Rate of evapotranspiration was high (4.4 and 4.5 mm $d^{-1}$ in wet and dry seasons, respectively) and little rainfall after establishment to late growth stage in dry season resulted large negative water balance (rainfall – evapotranspiration) (Fig. 5.3), while frequent and sufficient rainfall during establishment to vegetative stage helped comparable growth for W2 in wet season (Fig. 5.1c, d).

In Japan the field experiment was conducted with three water management regimes and average water productivity ranged from 0.13 to 0.71 kg $m^{-3}$. The FL treatment with continuous standing water showed lowest water productivity (0.16 kg $m^{-3}$) as the experimental paddy was artificially constructed in diluvium terrace with high percolation rate (40 mm $d^{-1}$) (Kamoshita et al., 2007) while UP, water saving by rainfed aerobic system, showed highest values (0.57 kg $m^{-3}$), but significant reduction in biomass and grain yield was accompanied. Another water saving treatment, alternate wetting and drying (AWD) showed higher water productivity values (0.53 kg $m^{-3}$) than FL (0.16 kg $m^{-3}$) with only smaller reduction in above ground biomass and grain yield compared to FL treatment. For FL the water productivity was greatly reduced mainly due to higher water use (higher percolation rate). The water productivity values under AWD could become lower as in the dryer year 2013 with 1240 mm of total water input (0.45 kg $m^{-3}$) or higher as in the wetter year 2014 with 1145 mm of total water input (0.62 kg $m^{-3}$), because of lower average soil water potential in 2013 (-19.1 kPa) than in 2014 (-10.1 kPa). Belder et al. (2004) reported that the soil water potential higher than -10 kPa should not reduce rice growth and yield formation when safe AWD was practiced in Hubei Province in China. Together with the experimental comparison between wet and dry seasons in Colombia, as mentioned in the previous paragraph, wetter conditions would be favorable for successful implementation of water-saving. Earlier flowering genotypes also can save irrigation water compared to later flowering ones, however the grain yield and water productivity values were lowered for early flowering genotypes (Chapter 3).

Table 6.1. Average above ground biomass, grain yield, total water supply and water productivity across water regimes and years in three countries with average total N application rate and climate factors. Values in brackets are range across years and field experiments conducted in all three countries.

| Factors/Country | Japan (2013-2014) (Chapters 2 and 3) | India (2012-2014) (Chapter 4) | Colombia (2015-2016) (Chapter 5) |
|---|---|---|---|
| Above ground biomass (g $m^{-2}$) | 1328 (580-1794) | 913 (903-998) | 1829 (1697-1974) |
| Grain yield (g $m^{-2}$) | 583 (222-828) | 313 (149-406) | 544 (396-699) |
| Water used ($m^3$) | 2.17 (0.64-0.50) | 1.15 (0.73-1.67) | 1.20 (0.99-1.47) |
| Water productivity (kg $m^{-3}$) | 0.42 (0.13-0.71) | 0.27 (0.19-0.41) | 0.46 (0.35-0.61) |
| Irrigation method | Furrow | Furrow | Furrow |
| N rate (kg $ha^{-1}$) | 60 | 97 (79-109) | 180 (140-220) |
| Min temp (°C) | 20.6 | 22.5 | 24.3 |
| Max temp (°C) | 29.3 | 33.9 | 34.6 |
| Rainfall (season) (mm) | 800 | 615 | 286 |
| Daily solar radiation (MJ $m^{-2}$) | 15.5 | 21.0 | 20.4 |
| Evapotranspiration (mm $day^{-1}$) | 3.65 | *3.36 | 4.45 |
| Growing season and #growth duration (range) | April – October 130-160 | August – January 115-125 | May – September (dry season), February – June (wet season) 91-125 |
| Cultivar type (Ind/Jap) | Ind/jap | Indica | Indica |

Ind- *Indica,* Jap- *Japonica.* Rainfall is calculated only during rice growing season
*-Evapotranspiration rate is for two months (19 Nov 2014- 18 Jan 2015)
#- Growth duration range is from sowing to maturity

Improvement of N recovery with lower N consumption (without improving yield) would be important to attain higher water productivity. In other word, we could improve water productivity by better N and other agronomic management. Best N application is related with water availability as was seen no positive response of N fertilizer under W3 water-saving treatment in Colombia. Wang et al. (2003) reported that there were significant interactions between N fertilizer and water management, and when water stress increased, N application increased nitrogen uptake but decreased efficiency for dry matter production. In Indian tank irrigation system the application of N fertilizer was lowered depending on tank water availability (Fig. 4.6 e.g. ST 2012). Linear regression line showed increase in grain yield with increased N fertilizer application rate in small tank however no relation was found in big tank. The efficiency of N fertilizer was very low in both tank and can be improved to greater extend by effective water management (e.g. AWD) or agronomical practices.

### 6.2 $\delta^{13}C$ as an indicator for water availability

We hypothesized water saving should contain two stages; (i) reduction of higher water input (water management stage), and (ii) plant response to reducing available water (physiological change stage). Measurement of $\delta^{13}C$ values was intended to distinguish if the rice plants should be in stage (i) or stage (ii). In Chapter 3 using *japonica* ecotype Koshihikari NILs, water saving to AWD did not affect $\delta^{13}C$ values and improved water productivity, whereas further water saving to UP resulted in increased $\delta^{13}C$ values without further improvement in water productivity. However, in Chapter 2 we used *indica* ecotype IR64 NILs; $\delta^{13}C$ values did not increase but rather tended to a bit decrease in response to AWD and to UP compared with FL, while water productivity increased to AWD and further to UP (0.71 and 0.62 kg $m^{-3}$, in 2013 and 2014, respectively) (Fig. 6.2). Reducing $\delta^{13}C$ was apparently related with increasing yield for the *japonica* but with decreasing yield for the *indica* ecotype (Fig. 6.3). IR64 NILs showed lower $\delta^{13}C$ values than Koshihikari NILs, as reported difference between *indica* and *japonica* ecotype (Dingkuhn et al., 1991; Zhao et al., 2004). The reason may be a thinner leaf of IR64 (smaller specific leaf weight (SLW)) which could allow higher conductance of $CO_2$ from stomata to intercellular space maintaining higher Ci values and lowered conductance compared with Koshihikari (Fig. 6.4). Kondo et al. (2004) found negative association between genotypic variation in carbon isotope discrimination and specific leaf weight. Differences in discrimination due to genotypic variation also can be explained by photosynthesis capacity, stomatal conductance (Zhao et al., 2004), response of stomata to soil stress and variation in photosynthetic capacity (Condon et al., 1992).

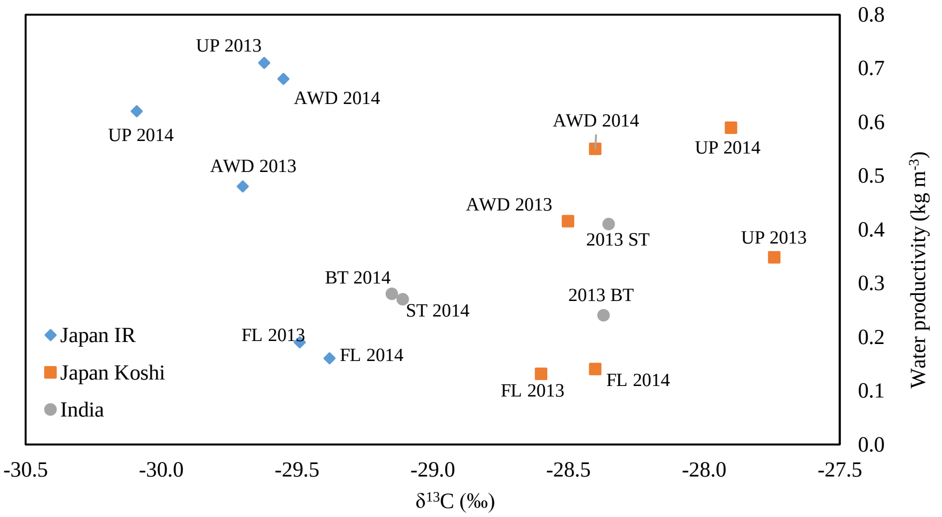


Fig. 6.2. Relationship of water productivity and $\delta^{13}C$ (‰) across Japan and India (Japan- three water treatments; FL- flooded, AWD- Alternate wetting and drying and UP- Upland in 2013 and 2014; India- two tanks (Big tank- BT and Small tank- ST in 2013 and 2014). Regression line is drawn leaving FL treatments in Japan.

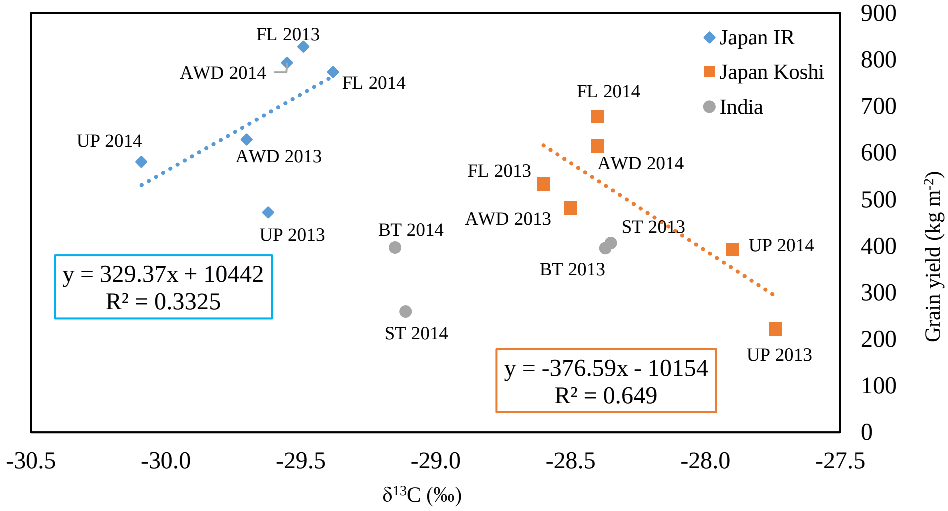


Fig. 6.3. Relationship of grain yield and $\delta^{13}C$ across Japan and India (Japan- three water treatments; FL-flooded, AWD-Alternate wetting and drying and UP-Upland in 2013 and 2014; India- two tanks BT (big tank) and ST (small tank) in 2013 and 2014).

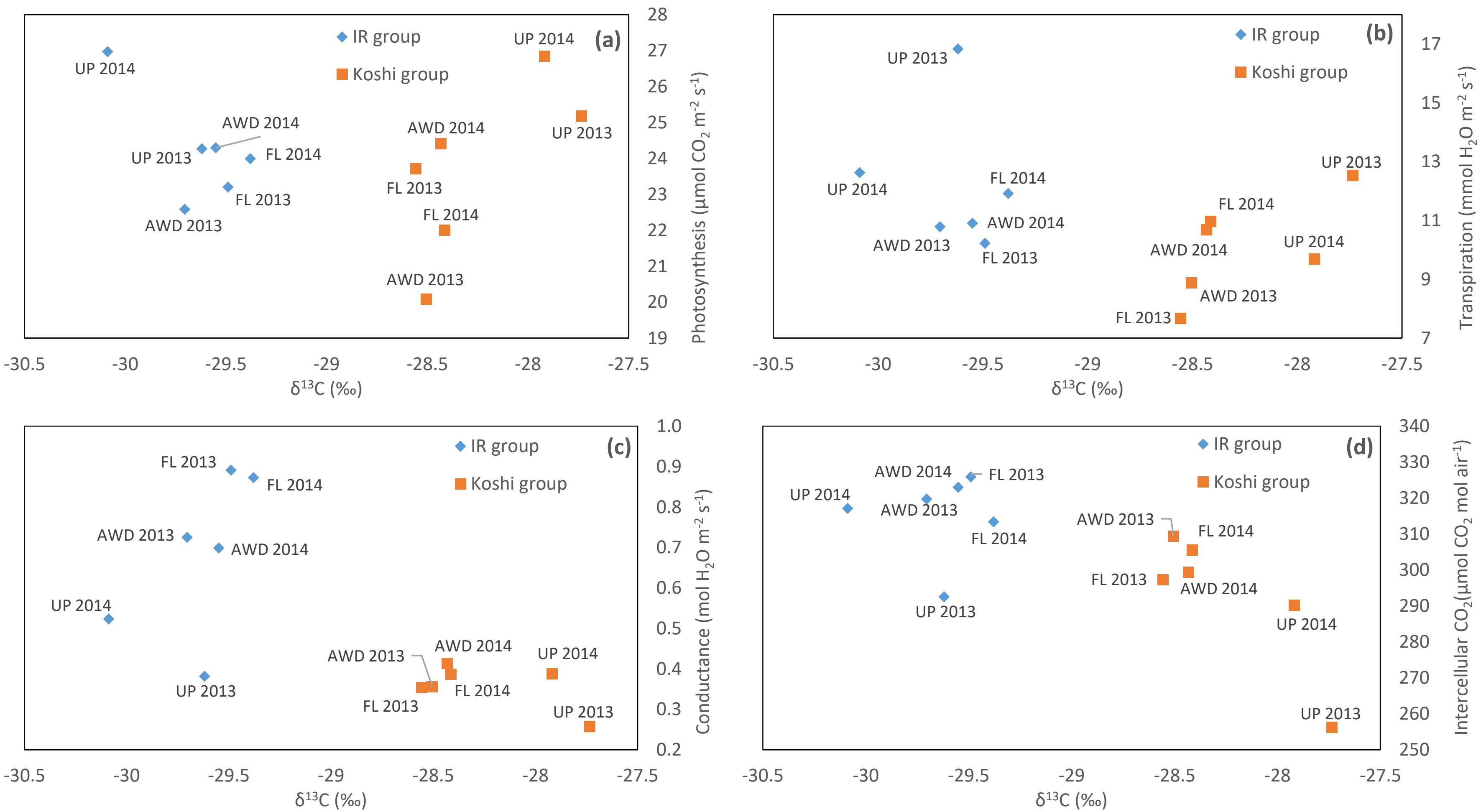


Fig. 6.4. Relationship of δ$^{13}$C with photosynthesis (a), transpiration (b), conductance (c) and intercellular $CO_2$ concentration (d) among IR-group and Koshihikari group.

Water saving beyond the "safe" limit, such as in UP treatment, affected plant physiological conditions such as water status and its consequent responses. Leaf extension and leaf area development is known to be sensitive to water stress (Okami et al., 2013; Lilley and Fukai, 1994), and this could lead to increased SLW. Larger SLW reduced the discrimination (higher $\delta^{13}C$) in Koshihikari NILs as the pass way of $CO_2$ from atmosphere to chloroplast is lengthened during which chance of physical discrimination of $^{13}C$ also decreased. This was not the case in IR64 NILs which had lower SLW than Koshihikari NILs (thinner leaves) and increment of SLW had no association with $\delta^{13}C$ and discrimination was higher in UP 2014 (Fig. 6.5). In UP 2014 the stomatal conductance was lower with higher photosynthetic rate which might help to maintain higher intercellular $CO_2$ concentration with more discrimination (Fig. 6.5). This also may be related to higher stomatal density in *indica* than *japonica* cultivar (Chen et al., 1990). Plants with higher SLW or lower SLA has lower stomata number (Xu and Zhou, 2008) and higher rate of photosynthesis, which might allow the plants for more discrimination of $^{13}C$ during carbon assimilation process.

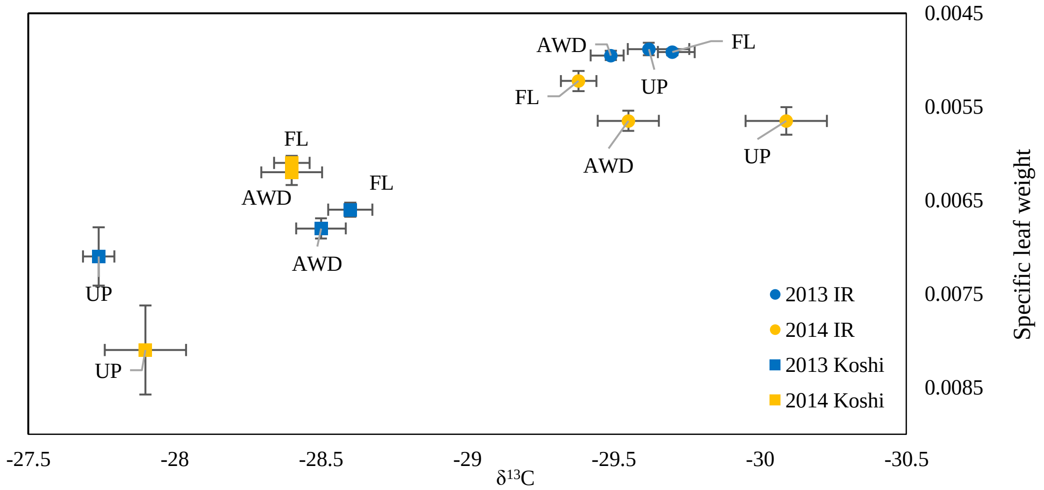


Fig. 6.5. Relationship between specific leaf weight and $\delta^{13}C$ across three water managements (FL: flooded lowland, AWD: alternate wetting and drying lowland, UP: rainfed upland) in two years (2013, 2014) for IR64 group and Koshihikari group. Bars represents standard error.

### 6.3 Significance of traits of genotypes for water saving

Choice of genotype is also important to determine water productivity for rice. Genotypes having higher water extraction capacity (higher transpiration) and short duration of growth or maturity showed higher water productivity. In our experiment in Japan Dro1-NIL with deeper root angle showed higher water productivity than other genotypes over three water managements (Chapter 2). The deeper root growth helps to extract more water and nutrients from deeper soil layers which significantly increased above ground biomass and grain yield in our experiment. In Japan and Colombia experiments we used set of genotypes with varying heading dates under three water management regimes. Among Koshihikari NILs (Chapter 3) Hd1 showed earlier flowering than Hd16 and saved higher amount of water, however water productivity was lowered due to lower grain yield potential. In Colombian experiment short duration varieties FEDEARROZ473 (higher yield potential) and Dro1-NIL showed higher water productivity than other genotypes over two seasons.

Under the tropical environment with direct seeding system with ample N fertilization, earliness may not be so disadvantageous. Number of grains and grain filling was higher for earlier heading genotypes which might have contributed for higher grain yield. Use of high yielding early heading genotypes can save total water supply in Colombian environments and can help to reduce the resource cost. However, the data of wet season which growth duration was shortened due to high temperature during early season (cf. due to El Nino) showed that late heading FEDEARROZ67 yielded highest.

The Japanese experiment using near-isogenic lines of IR64 with similar phenology clearly showed that root growth angle can improve both grain yield across different water management and water productivity (Chapter 2). Compared with the effects of *DRO1*, *Sta1* had no positive effects on water productivity. The deeper root angle promotes deeper root growth which helps for extracting more water from deeper soil layers (Uga et al., 2013). Use of such genotypes can yield higher even if frequency of irrigation is reduced and can help in water saving rice cultivation. However, it is known that effects of *DRO1* reduced under hard soil compaction of 1.8 MPa (Ramalingam et al., 2017), and Dro1-NIL in Colombian experiments did not perform better than IR64. The reasons may not be clear, but there might be interactions with management and environmental conditions which should be further clarified. In India, although underlying basis was not clear, JGL and ANNA4 had higher water productivity and lower $\delta^{13}C$ values than BPT, showing the potentail for variety improvement and importance of its choice.

In Colombian experiments, FEDEARROA174 and FEDEARROZ473 in dry season and FEDEARROZ67 in wet and high temperature season showed higher yield and water productivity; FEDEARROZ174 and FEDEARROZ473 showed high bottom root ratio, but not significantly different from other Colombian genotypes (data not presented).

FEDEARROZ67 is regarded as to be better adapted to high temperature among Colombian rice researchers (D. Pineda, personal communication). However, the detailed physio-morphological mechanisms of these three varieties are not yet clarified. Further works would be needed.

### 6.4 Possible water-saving technologies to be developed

Water productivity can be improved by irrigation methods; in our studies of the three countries, the irrigation method was all furrow irrigation. Sprinkler irrigation and drip irrigation are known to be higher in field-level water productivity (Evans and Sadler, 2008), but these may not be practical in rice. Levelling of the fields enable shallower water management that could reduce consumption of water and improve water productivity, which would be possible in Japan. If “safe AWD” would be more disseminated which reduces frequency of irrigation without lowering soil moisture potential below -20 kPa, more water saving and higher water productivity would be possible, but as our analysis highlighted (6.1), AWD may be more smooth in wetter environments but could not attain more in dryer environments.

In Indian tank rice system, community based irrigation managing villager (usually one person) need to take care of all the paddy fields, and shallower and more saving irrigation management may increase his labors and may not be feasible. Bore-well based irrigation managed by family unit may be more efficient and better implement water-saving management (i.e., AWD, system of rice intensification (SRI) Prabha et al., 2011), if bore-well could keep sufficient fresh water resources, and if farmers could afford for pumping.

In Colombia, levelled paddy and sloped paddy co-existed, the former’s water productivity is known to be higher (Pineda et al., 2016). New irrigation method of using multiple inlet irrigation system (MIRI), which are practiced some part of USA (Vories et al., 2005; Massey et al., 2014), is now being introduced in Colombia, which could improve water productivity. Improving water productivity is needed in areas where water is scarce and competition for fresh water is increasing e.g. India and Colombia. Improving water productivity in rice will help to save the water and resource cost for the farmers. Saved water can be used for next rice growing season or cultivation of other crops e.g. India. Japan is temperate country and water scarcity is very rare, however reducing the water input without affecting rice yield can save resource cost and increase a chance of alternate use of freshwater resources for other purposes.

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Deshmukh Vivek Vasant
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