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

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**ABSTRACT**

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 flooded lowland (FL), alternate wetting and drying lowland (AWD), and rainfall upland (UP) conditions in 2013 and 2014 to compare the effects of DRO1 and Sta1 on yield across different water management regimes. Genotypic variation and water management effects were significant for grain yield, aboveground biomass, and harvest index, as well as their interactions with year, but no significant genotype × water interaction was detected. 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. Sta1 tended to reduce the carbon isotope composition (δ¹³C), leading to a higher harvest index of Sta1-NIL than that of IR64, but grain yield was not increased. 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. AWD in the more rainy year 2014 attained both 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.

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, Abe, et al., 2006), and penetration ability (Clark et al., 2008), root growth angle and root stele transversal area (STA) 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 degree 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.

1. Materials and methods

1.1. 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 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 (BC5F3) 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 (BC5F3) 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.

1.2. 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 ± 1, electrical conductivity .098 ± .026 mS cm−1, cation exchange capacity 37.6 ± 1.6 meq 100 g m−1, and bulk density .90 ± .05 g cm−3.

1.3. 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, Sta1-NIL, and Dro1+Sta1-NIL.

1.4. 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 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 the 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 (P2O5 and K2O) 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.
1.5. Measurements

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 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) MJ m⁻² in 2013 and 15.1 (±7.5) MJ m⁻² in 2014. Total seasonal rainfall was 659 in 2013 and 941 mm in 2014 (Supplementary Figure 1). 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).

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. 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:

\[
FI(\%) = \frac{(PAR \text{ above canopy} - PAR \text{ below canopy})}{PAR \text{ above canopy}} \times 100
\]  

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:

\[
RI \text{ (MJ m}^{-2}\text{)} = \sum \text{daily solar radiation} \times \text{daily FI}
\]

The aboveground biomass was plotted against cumulative RI, and radiation use efficiency (RUE) was obtained from the slope of the linear regression line 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 extinction coefficient (k) was obtained from the slope of the regression line forced through the origin for each replication.

In 2014, carbon isotope composition (δ¹³C) 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 using a fine mill (Heiko sample mill, TI 300, Fujiwara Seisakusho, Ltd. Tokyo, Japan). The δ¹³C values of powdered samples (.2 mg) were analyzed with an elemental analyzer/mass spectrometer (Flash 2000/Delta V Advantage, Thermo Fisher Scientific, Waltham, MA, USA).

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% H₂O). Cross sections of nodal roots were taken at 1 cm from the root base and examined under a fluorescence and phase contrast microscope (BXS1, Olympus, Hicksville, NY, USA). CellSens standard software (Olympus) was used to capture the microscopic images. 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.

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 .27 m² 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 ≥ 30%), 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 included 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.

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 the Penman-Monteith method was 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. 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.

1.6. 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 data-set 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 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 \(\times\) 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 for IR64 and its three NILs across the six environments.

2. Results

2.1. 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 (Figure 1(a)). 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 (Figure 1(b)). Dro1-NIL and Sta1-NIL had significantly higher harvest indices than those of IR64 and Dro1+Sta1-NIL (Figure 1(c)). ANOVA of data for both years among the four genotypes showed significant effects of genotype, water, year, and water \(\times\) year and genotype \(\times\) year (except for grain yield) interactions for grain yield, aboveground biomass, and harvest index, whereas the genotype \(\times\) water treatment and genotype \(\times\) water treatment \(\times\) year interactions were not significant (Table 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). Grain filling percentage was highest in Dro1-NIL with a significant difference between this line and Dro1+Sta1-NIL.
2013, the genotype × water treatment interaction for harvest index was significant in 2014. 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; Supplementary Table 1). 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 < .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 < .05) in 2013. Leaf area index and plant height were also 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.

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 4). 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 a significant

| Table 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) | <.001 | <.001 | <.001 |
| Water (W) | <.001 | <.001 | <.001 |
| Year (Y) | <.001 | <.001 | <.001 |
| G × W | .742 | .375 | .303 |
| G × Y | .073 | .005 | .034 |
| W × W × Y | <.001 | <.001 | <.001 |
| G × W × Y | .249 | .697 | .113 |

Notes: LSD: least significant difference. Means within each column followed by different letters indicate significant differences at the 5% level (Tukey’s test).

2.2. Environmental effects and its interactions with genotype 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 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 3). Dro1-NIL had the highest grain yield in the FL treatment (P < .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. In addition, the genotype × water treatment interaction for grain yield analyzed by combining pairs of water treatments (i.e. FL and AWD, FL and UP, AWD and UP) was not significant (data not shown). 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 < .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. 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 X 10³) | 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                     | 78.3 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           | 98.7 b           | 16.1                   |
| Dro1+Sta1-NIL | 353 b       | 28.8 ab                     | 82.3 a                      | 76.6 a            | 24.1                     | 6.49           | 96.3 a           | 15.5                   |
| Sta1-NIL  | 322 a               | 27.4 a                      | 85.2 ab                     | 80.2 b            | 24.0                     | 6.91           | 95.6 a           | 15.5                   |
| Average   | 335                 | 28.4                        | 85.0                        | 79.0              | 24.1                     | 6.34           | 97.7             | 16.1                   |
| LSD (5%)  | 23                  | 1.9                         | 4.0                         | 2.8               | 3                        | 1.8            | 1.8              | 1.3                    |
| Genotype (G) | .008                | .002                        | .021                        | .022              | .165                     | .038           | <.001            | .169                   |
| Water (W) | <.001               | <.001                       | <.001                       | <.001             | <.001                    | <.001          | <.001            | <.001                  |
| Year (Y)  | .021                | .002                        | .100                        | .012              | <.001                    | .212           | .402             | .028                   |
| G × W     | .698                | .936                        | .789                        | .492              | .021                     | .076           | .172             | .755                   |
| G × Y     | .026                | .032                        | .752                        | .443              | .718                     | .306           | .063             | .311                   |
| W × Y     | .026                | <.001                       | <.001                       | .819              | <.001                    | .115           | .014             | .244                   |
| G × W × Y | .814                | .196                        | .069                        | .047              | .308                     | .651           | .112             | .604                   |

Notes: LSD: least significant difference. Means within each column followed by different letters indicate significant differences at the 5% level (Tukey’s test).
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 was similar but the y-intercept was larger in Dro1-NIL (Figure 2(a)). It was also possible to use a quadratic regression line for Dro1-NIL with larger $R^2$ values ($0.9779$ (not shown)) compared with that of linear regression ($0.9392$), which indicated smaller difference in yield 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 the difference became small under higher yielding environments (Figure 2(a)). 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 (Figure 2(b)). An additional ANOVA under the two lowest yielding environments in 2013 (i.e. UP and AWD) showed significantly higher yield of Dro1-NIL as compared to that of IR64 (data not shown). Dro1-NIL and Sta1-NIL had a higher harvest index across the six environments than that of IR64 and Dro1+Sta1-NIL (Figure 2(c)).

### Table 3. Grain yield, aboveground biomass, and harvest index in three water management regimes 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 | 589 | 371 | 1600| 1209| 887 | 0.473 | 0.487 | 0.417 |
| Dro1-NIL        | 946 | 627 | 539 | 1919| 1338| 1284| 0.493 | 0.469 | 0.414 |
| Dro1+Sta1-NIL   | 819 | 659 | 532 | 1723| 1440| 1281| 0.475 | 0.458 | 0.414 |
| Sta1-NIL        | 791 | 640 | 447 | 1627| 1306| 1012| 0.487 | 0.489 | 0.443 |
| Average         | 828 | 629 | 472 | 1717| 1323| 1116| 0.482 | 0.476 | 0.424 |
| LSD (5%)        | 90**| 125 | 146*| 158**|223 | 316*| 0.018*| 0.042 | 0.041 |
| Genotype (G)    | .003|     |     |     | <.001|    |     | .086 |     |
| Water (W)       | <.001|     |     |     | <.001|    |     | <.001|     |
| G × W           | .257|     |     |     | .357|    |     | .530 |     |
| 2014            |     |     |     |     |     |     |     |     |     |
| IR64            | 780 | 761 | 603 | 1900| 1844| 1344| 0.411 | 0.413 | 0.449 |
| Dro1-NIL        | 800 | 858 | 635 | 1764| 1836| 1408| 0.454 | 0.469 | 0.450 |
| Dro1+Sta1-NIL   | 745 | 782 | 566 | 1719| 1762| 1329| 0.433 | 0.445 | 0.426 |
| Sta1-NIL        | 769 | 773 | 519 | 1790| 1732| 1142| 0.430 | 0.447 | 0.454 |
| Average         | 774 | 794 | 581 | 1793| 1794| 1306| 0.432 | 0.443 | 0.445 |
| LSD (5%)        | 133 | 120 | 107 | 288 | 350 | 229 | 0.033 | 0.027*| 0.015*|
| Genotype (G)    | .085|     |     |     | <.001|    |     |     |     |
| Water (W)       | <.001|     |     |     | <.001|    |     | .081 |     |
| G × W           | .793|     |     |     | .736|    |     | .036 |     |

Notes: FL: flooded lowland, AWD: alternate wetting and drying lowland, UP: rainfed upland, LSD: least significant difference among genotypes within a water treatment. **, *, + show $P = .01, .05, and .10$, 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).

### Table 4. 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.

| Genotypes/water | Grain yield Aboveground biomass Harvest index |
|-----------------|-----------------------------------------------|
|                 | IR64 and NILs |                               |
| Genotype (G)    | <.001 (44)   | .010 (99)                      | .002 (0.111)      |
| Environment (e) | <.001 (54)   | <.001 (121)                    | <.001 (0.104)      |
| G × E           | .256 (109)   | .089 (242)                     | .054 (0.286)      |
| $DRO1$ plus and $DRO1$ minus |        |                               |
| Genotype (G)    | <.001 (32)   | .001 (69)                      | .001 (0.04)       |
| Environment (e) | <.001 (56)   | <.001 (119)                    | <.001 (0.008)      |
| G × E           | .188 (79)    | .012 (168)                     | .005 (0.011)      |
| $Sta1$ plus and $Sta1$ minus |       |                                 |
| Genotype (G)    | .306 (36)    | .329 (80)                      | .926 (0.010)      |
| Environment (e) | <.001 (62)   | <.001 (138)                    | <.001 (0.017)      |
| G × E           | .348 (88)    | .442 (195)                     | .893 (0.025)      |

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 was similar but the y-intercept was larger in Dro1-NIL (Figure 2(a)). It was also possible to use a quadratic regression line for Dro1-NIL with larger $R^2$ values ($0.9779$ (not shown)) compared with that of linear regression ($0.9392$), which indicated smaller difference in yield 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 the difference became small under higher yielding environments (Figure 2(a)). 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 (Figure 2(b)). An additional ANOVA under the two lowest yielding environments in 2013 (i.e. UP and AWD) showed significantly higher yield of Dro1-NIL as compared to that of IR64 (data not shown). Dro1-NIL and Sta1-NIL had a higher harvest index across the six environments than that of IR64 and Dro1+Sta1-NIL (Figure 2(c)).

### 2.3. 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...
genotype × environment interactions for grain yield (g m⁻²) (a), Figure 2. Finlay–Wilkinson regression curves for analysis of aboveground biomass (g m⁻²) (b), and harvest index (c) across FL (1.50 g MJ⁻¹) than in UP (1.15 g MJ⁻¹), but we found dro1-nil, dro1+Sta1-nil, and Sta1-nil.

six environments between average of four genotypes and ir64, than in Sta1-NIL. RUE was higher in AWD (1.55 g MJ⁻¹) and three-treatment average, RI was higher in Dro1+Sta1-NIL FL 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 .34. Finlay–Wilkinson regression showed a higher leaf area index in Dro1-NIL than in IR64, with a significant genotype × environment interaction (Figure 3). The leaf area indices of Dro1+Sta1-NIL and Sta1-NIL were not significantly different from that of IR64.

2.4. Carbon isotope composition

Combined ANOVA showed lower δ¹³C values (averaged across four genotypes) in UP than FL, with significantly lower value in UP in 2014 (–30.09‰) which resulted in significant water × year interaction (Table 6). Sta1-NIL tended to show lower δ¹³C values (P = .073), while genotype × water and genotype × year interactions were non-significant. When additional data from the all growth stages were combined, Sta1-NIL had the lowest δ¹³C value (–29.54‰), followed by IR64 (–29.27‰), Dro1+Sta1-NIL (–29.19‰), and Dro1-NIL (–29.09‰) (P = .05).

2.5. Water use and water productivity

The average total amount of water (irrigation + rainfall) used in FL, AWD, and UP per hectare was 4.45, 1.30, and .66 m³ in 2013 and 4.98, 1.17, and .94 m³ in 2014, respectively. On average, over the two years the AWD and UP treatments required much less water, with 74% and 83% water savings compared with FL. Total water used was similar among IR64 and the three NILs, as their growth durations were similar (Table 7). Water productivity was highest in UP (.66 kg m⁻³), followed by AWD (.58 kg m⁻³) and then by FL (.17 kg m⁻³), and a significant interaction was found between water management and year, with the value of AWD higher than that of UP in 2014. Higher above ground biomass was associated with lower water productivity in general among the six environments with the three water management treatments and 2 years combined, but the exception was AWD in 2014 that had attained both the highest level of biomass production and water productivity (Figure 4). Water productivity in the AWD treatment increased compared with FL without large changes in δ¹³C (Figure 5). The differences in water productivity between AWD and UP were small but there was large variation in δ¹³C values.

Water productivity was highest in Dro1-NIL followed by Dro1+Sta1-NIL and lowest in Sta1-NIL and IR64 (Table 7). Dro1-NIL had the highest water productivity in FL in 2013 (.21 kg m⁻³; Supplementary Table 1). Genotype and water management effects were significant in each year while genotype by water interaction was not significant at the 5% level. The slopes of Finlay–Wilkinson regression lines over the six environments were steeper in Dro1-NIL and Dro1+Sta1-NIL than IR64 and Sta1-NIL (Figure 5), indicating that the introgression of DRO1 would improve productivity to a greater extent under water saving conditions. There

![Figure 2](image-url)
through AWD in 2014, without reducing yield; with a total water supply of 1.17 m³ 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 6), the AWD treatment maintained yield as high as that of the FL treatment in 2014. AWD in 2014 recorded higher water productivity (.68 kg m⁻³) than AWD in 2013 (.48 kg m⁻³) with the latter having a yield penalty, because of the higher rainfall and lower evapotranspiration rate in 2014 (Supplementary Figures 1(e) and (f), 2), and higher soil water potential in 2014 (–10.1 kPa) than in 2013 was on the other hand no difference in water productivity between IR64 and Sta1-NIL.

Table 5. Fraction of intercepted radiation (Fl), 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 | Genotypes/Water | FL (%) | AWD | UP | Average FL | AWD | UP | FL | AWD | UP | Average FL | AWD | UP | k |
|--------|-----------------|--------|-----|----|------------|-----|----|-----|-----|----|------------|-----|----|----|
| Fl     | 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| .307 | .414 | .313 | .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| .339 | .341 | .317 | .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| .365 | .345 | .374 | .361 |
|       | Sta1-NIL        | 94.4 C| 91.7| 85.1 a| 90.1 a| 1228 | 1202 | 1161 a| 1197 a| 2.87| 1.52| 1.05| 1.35| .375 | .291 | .273 | .313 |
| Average|                 | 94.4 C| 91.9| 89.3 A| 93.7 b| 1238 | 1199 A| 1216 AB| 1.50 B| 1.55 B| 1.15 A| .347 | .398 | .319 |
| LSD (5%)|                | 1.5*  | 1.6 | 4.4* | 3.9** | 41   | 43   | 86+| 28* | 26 | 30   | .26 | .12 | .216 | .179 | .093 | .075 |
| Genotype (G)|          | <.001 | .046 | .029  | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | .031 | .051 | .082 | .060 |
| Water (W)    |                | .01   | .01  | .01   | .01   | .01   | .01   | .01   | .01   | .01   | .01   | .01   | .01   | .01   | .01   | .01   |
| G × W |                 | .01   | .01  | .01   | .01   | .01   | .01   | .01   | .01   | .01   | .01   | .01   | .01   | .01   | .01   | .01   |

Notes: LSD: least significant difference among genotypes within a water treatment. **, *, + show P = .01, .05, and .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).

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 8). 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.

3. Discussion

3.1. Improvement of water productivity by water saving

Water saving could result in trade-off for biomass production (Figure 4), as photosynthesis and transpiration are physiologically linked together, but our study pointed to greatly improved water productivity by water saving through AWD in 2014, without reducing yield; with a total water supply of 1.17 m³ 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 6), the AWD treatment maintained yield as high as that of the FL treatment in 2014. AWD in 2014 recorded higher water productivity (.68 kg m⁻³) than AWD in 2013 (.48 kg m⁻³) with the latter having a yield penalty, because of the higher rainfall and lower evapotranspiration rate in 2014 (Supplementary Figures 1(e) and (f), 2), and higher soil water potential in 2014 (–10.1 kPa) than in 2013.
and nitrogen (cf. Arai-Sanoh et al., 2014) which sustained unpublished data) and enabled greater capture of water greater soil moisture from deeper soil (A. Kamoshita, DRO1 would have depleted a modified root system by larger water productivity among the four genotypes. 2014), contributed to greater biomass production and radiation interception rather than RUE (data available only in related with higher fraction interception and larger radiative production level; the result was no significant interaction for yield and water productivity, our results interaction for yield and water productivity, our results means in the same column followed by different letters indicate significant

| Water × Year | Water used (m³) | Water productivity (kg m⁻³) |
|--------------|----------------|----------------------------|
| FL 2013     | 4.45           | .19 A                      |
| AWD 2013    | 1.31           | .48 B                      |
| UP 2013     | .66            | .71 C                      |
| FL 2014     | 4.98           | .16 A                      |
| AWD 2014    | 1.17           | .68 C                      |
| UP 2014     | .94            | .62 B                      |
| LSD water × year (5%) | .001 | .025                      |
| Genotype    |                |                            |
| IR64        | 2.26           | .44 a                      |
| Dro1-NIL    | 2.25           | .51 b                      |
| Dro1-Stat1-NIL | 2.26 | .48 ab                     |
| Sta1-NIL    | 2.25           | .45 a                      |
| LSD genotype (5%) | .001 | .041                      |
| Average     | 2.25           | .47                       |
| Genotypes (G) | .112   | .003                      |
| Water (W)   | <.001         | <.001                      |
| Year (Y)    | <.001         | .106                      |
| G × W       | .457          | .139                      |
| G × Y       | .112          | .054                      |
| W × Y       | <.001         | <.001                      |
| G × W × Y   | .457          | .077                      |

Notes: LSD: least significant difference. Means in the same column followed by different letters indicate significant differences at the 5% level (Tukey’s test).

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 7). Superior leaf area index which was related with higher fraction interception and larger radiation interception rather than RUE (data available only in 2014), contributed to greater biomass production and larger water productivity among the four genotypes. A 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, Kamoshita, et al., 2006), but our study showed that a deeper root angle as reported to be 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.

### 3.2. Effect of Dro1 on grain yield under alternate wetting and drying conditions

This was the first study to our knowledge to have 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 (Figures 1(a) and 2(a)), Dro1 was considered to be advantageous to higher yield for IR64 including under AWD conditions. However, such superior yield performance was not detected at a significant level within a single year analysis under AWD, suggesting a 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 NO₃-N and NH₄-N would have been altered, 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 Supplementary Table 1) between Dro1-NIL and IR64. Arai-Sanoh et al. (2014) reported that the whole-plant nitrogen content at 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 (Figures 1(c) and 2(b) and (c), 3; Table 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).

3.3. Performance of STA

Sta1-NIL did not produce higher yield or greater aboveground biomass than those of IR64 (Figures 1(a) and (b), 2(a) and (b); Table 3). However, its harvest index was higher (Figures 1(c), 2(c), Table 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 had the lowest carbon isotope composition (δ¹³C) averaged across the entire growth stage (data not shown, cf. Table 6), which indicates higher discrimination against ¹³C during photosynthesis. The Kinandang Patong allele of Sta1 increased STA by 25% as compared with that of IR64 (Uga et al., 2010) and the larger proportion of STA in the 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 openness and a higher intercellular carbon dioxide concentration (not measured in this study) than in IR64.
Table 8. Correlation analysis of grain yield averaged across two years and three water treatment among 4 genotypes ($n = 4$) with other traits from 2014 experiment.

| Traits | GY | AGB | HI | LAI | SLW | PN | GN pan$^{-1}$ | GN m$^{-2}$ | GF | TGW | WP | δ$^{13}$C | FI | RI | RUE | $k$ |
|--------|----|-----|----|-----|-----|----|--------------|-------------|----|-----|----|----------|----|-----|-----|------|
| GY     | 1  |     |    |     |     |    |              |             |    |     |    |          |    |     |     |      |
| AGB    | .92* | 1  |    |     |     |    |              |             |    |     |    |          |    |     |     |      |
| HI     | .48 | .11 | 1  |     |     |    |              |             |    |     |    |          |    |     |     |      |
| LAI    | .81* | .97** | −11 | 1  |     |    |              |             |    |     |    |          |    |     |     |      |
| SLW    | −.23| −.19| −.09| −.28| 1  |    |              |             |    |     |    |          |    |     |     |      |
| PN     | .75 | .87* | −.06| .93*| −.61| 1  |              |             |    |     |    |          |    |     |     |      |
| GN pan$^{-1}$ | .66 | .40 | .82*| .16 | .33 | .01 | 1            |             |    |     |    |          |    |     |     |      |
| GN m$^{-2}$ | .99** | .94** | .42 | .85*| −.30| .82* | .58 | 1            |     |     |    |          |    |     |     |      |
| GF     | .35 | .01 | .90*| −.24| .34 | −.33| .91*         | .26         | 1  |     |    |          |    |     |     |      |
| TGW    | .97**| .94* | .47 | .81*| −.45| .83*| .53          | .99**       | .24| 1    |    |          |    |     |     |      |
| WP     | .98**| .94**| .38 | .87*| −.39| .86*| .51          | .99**       | .18| .99**| 1  |          |    |     |     |      |
| δ$^{13}$C | .56 | .81*| −.38| .85*| .21 | .62 | .12          | .58         | −.29| .46 | .57| 1        |    |     |     |      |
| FI     | .75 | .93**| −.20| .99**| .33 | .94**| .05          | .80         | −.35| .76 | .83*| .85*| 1  |    |     |      |
| RI     | .47 | .73 | −.46| .87*| −.51| .91*| −.33         | .55          | −.67| .55 | .61 | .70 | .92*| 1  |    |      |
| RUE    | −.01| .02 | −.01| −.09| .97***| −.44| .47          | −.08         | .40 | −.24| −.17| .37 | −.15| −.39| 1  |      |
| $k$    | .05 | .42 | −.85*| .62 | −.12 | .56 | −.59         | .12          | −.86*| .07 | .17 | .73 | .69 | .84*| −.08| 1  |

Note: GY (grain yield); AGB (aboveground biomass); HI (harvest index); LAI (leaf area index); SLW (specific leaf weight); PN (panicle number); GN (grain number); GF (grain filling ratio); TGW (thousand grain weight); WP (water productivity); $δ^{13}$C (carbon isotope composition); FI (fraction of intercepted radiation); RI (cumulative amount of intercepted radiation); RUE (radiation use efficiency); $k$ (extinction coefficient).

*p < .05; **p < .01.
3.4. 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 and 5) among the genotypes, leading to higher aboveground biomass in the lower yielding environment (Figure 2(b)) and slightly higher yield than that of IR64 (Table 3, Figure 2(a)). 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 synergistic. 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; Figure 6). 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.

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Disclosure statement

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