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RESEARCH

Transgressive Variation for Yield Components Measured throughout the Growth Cycle of Jefferson Rice (*Oryza sativa*) × *O. rufipogon* Introgression Lines

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ABSTRACT

Previous studies demonstrated that alleles introduced into elite rice (*Oryza sativa* L.) cultivars from the wild, ancestral species *Oryza rufipogon* Griff. enhanced yield as a result of transgressive variation. A study was conducted to unveil the phenological and agronomic mechanisms that underlie increased yields in introgression lines (ILs) developed through backcrossing an *O. rufipogon* accession [International Rice Germplasm Collection (IRGC) 105491] with the recurrent parent, 'Jefferson', a U.S. long-grain variety. Phenological development and agronomic and yield component traits of Jefferson and eight ILs, each carrying a major introgression for one of six quantitative trait loci (QTLs) for yield (*yld 1.2*, *yld 2.1*, *yld 3.2*, *yld 6.1*, *yld 8.1*, and *yld 9.1*) were determined in greenhouse studies over 2 yr. A novel method to estimate aboveground biomass nondestructively with digital images was used. The higher yielding ILs had slower phenological development and produced more biomass than Jefferson. Comparison of the yield component traits at maturity revealed longer flag leaves and panicles in all ILs than in Jefferson, with differences for five wild QTLs (*yld 1.2*, *yld 2.1*, *yld 3.2*, *yld 8.1*, and *yld 9.1*) introgressions being significant. Three ILs with introgressions from *yld 6.1* and *yld 9.1* had significant increases in primary panicle branch number. Six of the ILs had more florets per panicle and introgressions for three of the wild QTLs (*yld 2.1*, *yld 6.1*, and *yld 8.1*) produced significantly more seed. These results support an additive model of transgressive variation, where the inferior wild donor introgressions contribute variation in traits (e.g. growth rate, flag leaf length, and panicle size) that increase yield in an elite genetic background.

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Abbreviations: AB, advanced backcross; GSOR, Genetic Stocks—*Oryza*; IL, introgressed line; IRGC, International Rice Germplasm Collection; logistic 3P, logistic three-parameter; QTL, quantitative trait locus.

Improving yield is one of the most important goals for most breeding programs of crop plants. Yield is a complex trait that is quantitatively inherited and controlled by many genes with small effects that are subject to environmental variability and thus it is often dissected into several component traits that have much higher heritability and are more effective breeding targets for improving productivity (Gravois and McNew, 1993). For cultivated Asian rice, the source of calories for much of the world's population, yield components include, among others, days to heading, plant height, number of panicles, panicle length, florets per panicle, and seeds per panicle (Gravois and McNew, 1993; Samonte et al., 1998).

Phenotypes measured at a single time point may be insufficient to assess complex dynamic traits like yield (Wu and Lin, 2006). For example, in rice, the number and magnitude of significant QTLs detected differs at various developmental stages for plant height (Yan et al., 1998) and tiller number (Wu et al., 1999). Repeated measurements of phenological or agronomic traits that change over time (i.e., time-dependent or longitudinal traits) are best analyzed by fitting a growth curve model to estimate the mathematical parameters that describe the curve (Yang et al.,

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2006). The parameter estimates can then be used as traits in statistical tests (Ma et al., 2002). Nondestructive measurements of yield components over time such as plant height, tiller number, and panicle number are straightforward but biomass measurements based on dry weight are destructive. However, with recent advances in image analysis, aboveground biomass can be estimated using image analysis pixel count methods (Paruelo et al., 2000), with only a small difference between the actual and estimated shoot dry weights (Golzarian et al., 2011).

Gaining a better understanding of how the rice plant grows and develops to achieve grain yield is only part of the equation. Introducing new variation by making crosses between diverse cultivars, as well as wild ancestral species, to introduce new combinations of alleles and increase the variation available for selection opens new breeding opportunities. Plant breeders working with inbred crops like rice have often observed transgressive variation caused by additive interactions between the genotypes of the parents, resulting in offspring that perform better than either parent (McCouch, 2004; Tanksley and McCouch, 1997). In rice, several studies have reported valuable alleles donated and introgressed from the ancestral species, *O. rufipogon* (*O. nivara* Sharma et Shastry), as well as other closely related *Oryza* species (Brar and Singh, 2011; summarized by Shakiba and Eizenga, 2014). These alleles introduced from rice wild species have not only improved resistance to a number of biotic and abiotic stresses but also enhanced yield performance in elite rice cultivars, as measured by agronomic and yield component traits, including yield per plant (McCouch et al., 2007).

As part of an international study to evaluate the yield-enhancing effects of *O. rufipogon* in the background of cultivated rice, collaborating institutions in China, South Korea, Indonesia, Colombia, Ivory Coast, and the United States provided one or two elite cultivars, either inbred or hybrid, for crossing with the wild, weedy accession *O. rufipogon* (IRGC 105491) from Malaysia (McCouch et al., 2007). This accession was selected from a collection of 34 closely related wild *Oryza* species accessions (*O. barthii* A. Chev., *O. glaberrima* Steud., *O. glumaepatula* Steud., *O. nivara*, *O. rufipogon*, and *O. sativa* f. *spontanea* Roshev.) on the basis of principal component analysis with 25 restriction fragment length polymorphism markers indicating that it clustered closely with *O. sativa* and because it easily hybridized with both *O. sativa* ssp. *indica* and ssp. *japonica* cultivars (Xiao et al., 1998). The advanced backcross (AB)-QTL strategy as described by Tanksley and Nelson (1996) was used to develop the populations by backcrossing to selected elite cultivars for two generations to produce a series of 200 to 300 BC_2F_2 families or, in the case of hybrids, a series of BC_2F_1 test cross families.

The AB population was developed with the elite inbred U.S. long-grain *tropical japonica* rice cultivar Jefferson as a recurrent parent. Subsequent QTL analysis of agronomic traits, yield components, and yield was reported by Thomson et al. (2003). The study by Thomson et al. (2003) revealed that alleles from the wild *O. rufipogon* donor significantly enhanced several agronomic and yield component traits in Jefferson, including days to heading, plant height, panicle number, panicle length, spikelets (florets) per panicle, and grains (seed) per panicle. Five yield QTLs were identified, with the yield component trait attributed to *O. rufipogon* in parenthesis, *yld 2.1* (number of spikelets per panicle, number of grains per panicle, grain weight per plant), *yld 3.2* (days to heading, number of spikelets per panicle, number of grains per panicle, grain weight per plant), *yld 6.1* (grain weight per plant), *yld 8.1* (number of grains per panicle), and *yld 9.1* (panicle length, number of spikelets per panicle, number of grains per panicle, tiller type, grain weight per plant). The sixth QTL, *yld 1.2*, was associated with increased number of spikelets per panicle and number of grains per panicle attributed to the Jefferson parent. To verify the impact of these major QTLs, lines carrying one of the six yield-related QTLs were backcrossed for an additional generation. Fourteen BC_3F_2 families were used to produce 50 BC_3F_3 ILs that were evaluated for yield and 14 agronomic, grain quality, and yield component traits in multi-environment field trials conducted in the southern United States (Imai et al., 2013). Twelve ILs, each carrying one of the six wild QTLs and having a 15.4 to 27.7% yield increase over the Jefferson parent and the fewest background introgressions, were made publicly available through the Genetic Stocks—*Oryza* (GSOR) collection (http://www.ars.usda.gov/Main/docs.htm?docid=23562, accessed 27 May 2016). Although these were documented to have stable yield increases relative to the recurrent parent, it was not known which specific yield component traits had been altered as a result of the QTL introgressions. The objectives of this study were to (i) determine the phenological changes of the ILs in a controlled greenhouse environment over different planting dates throughout the year, (ii) compare the effect of the six wild QTL introgressions on the growth and development of the ILs compared with the Jefferson parent, and (iii) determine the relationships between the yield component traits and the six yield QTLs first identified in the AB population (Thomson et al., 2003).

MATERIALS AND METHODS

Introgression Lines

The ILs used in this study were developed through backcrossing using Jefferson (Reg. no. CV-103, PI593892, GSOR 303013), an elite, long-grain, blast-disease-resistant, semidwarf variety developed for the U.S. market (McClung et al., 1997), as the recurrent parent and an *aus*-like accession of *O. rufipogon* (IRGC 105491, GSOR 303014) from Malaysia as the donor parent (Thomson et al., 2003; Imai et al., 2013).

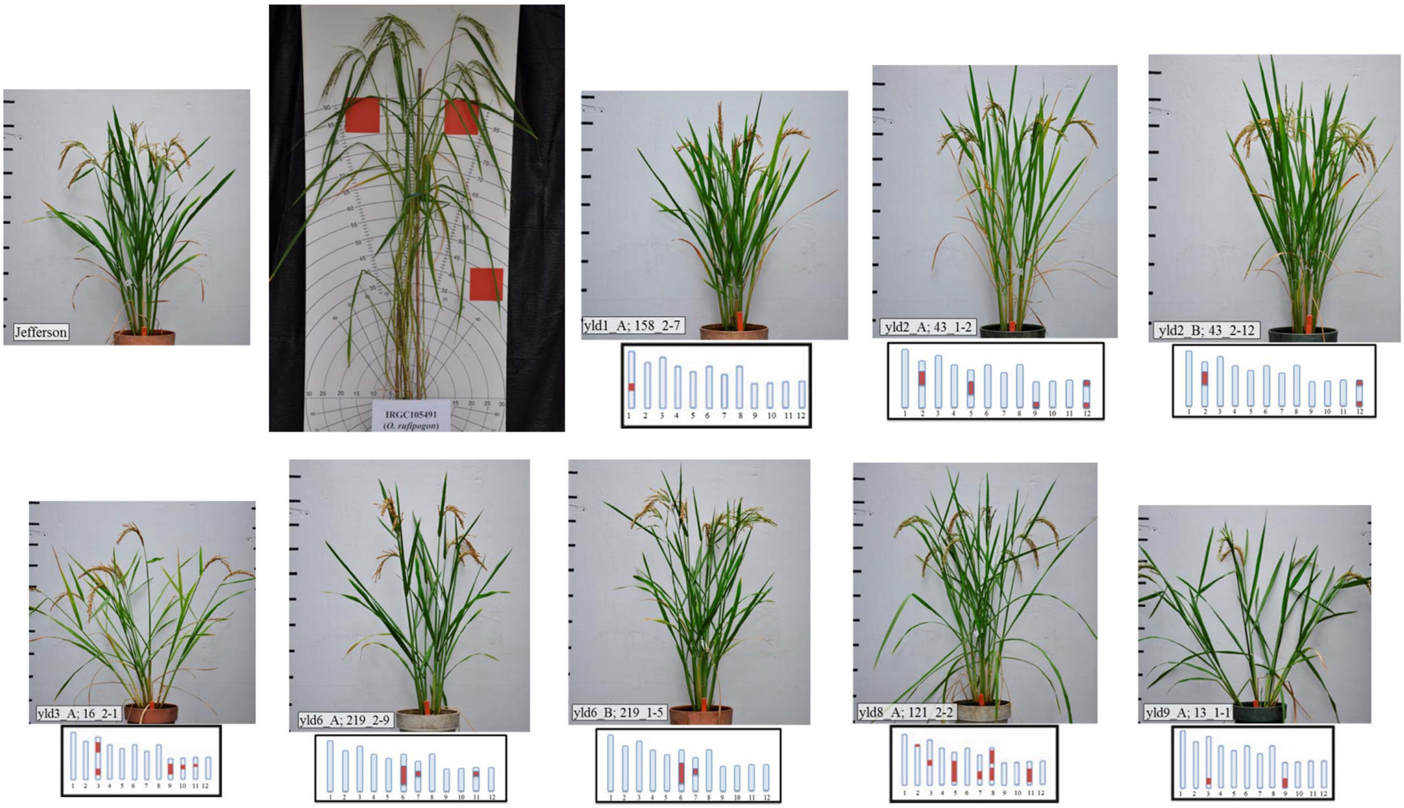

Fig. 1. Eight Jefferson introgression lines (ILs) of rice with introgressions from *Oryza rufipogon* for the six targeted yield quantitative trait loci (QTLs) (*yld 1.2, yld 2.1, yld 3.2, yld 6.1, yld 8.1*, and *yld 9.1*) and the parents, Jefferson and *O. rufipogon* (IRGC 105491). The introgressions are on chromosome 1, (yld1_A), chromosome 2 (yld2_A, yld2_B), chromosome 3 (yld3_A), chromosome 6 (yld6_A, yld6_B), chromosome 8 (yld8_A), and chromosome 9 (yld9_A). The ILs and Jefferson parent were planted on 21 May 2014 in the greenhouse and were at or near the R9 growth stage, which occurred when all the developed grain had a brown hull (Counce et al., 2000), when these photographs were taken (3 Sept. 2014). *Oryza rufipogon* was planted in February 2015 and photographed in August 2015. The bars (or semicircles for *O. rufipogon*) represent 10-cm intervals. The karyotypes of the ILs are based on Imai et al. (2013), with red representing the homozygous introgressions from *O. rufipogon.*

The selected ILs had *O. rufipogon* introgressions for one of the six targeted yield QTLs, (*yld 1.2*, *yld 2.1*, *yld 3.2*, *yld 6.1*, *yld 8.1*, and *yld 9.1*), which were identified by Thomson et al. (2003), and a few background introgressions (Imai et al., 2013). Seed was obtained from the GSOR collection for the ILs identified as yld1_A (158_2–7; GSOR 303001) with the targeted chromosome 1 introgression, yld2_A (43_1–2; GSOR 303002) and yld2_B (43_2–12; GSOR 303003) with the chromosome 2 introgression, yld3_A (16_2–1; GSOR 303006) with the chromosome 3 introgression, yld6_A (219_2–9; GSOR 303008) and yld6_B (219_1–5; GSOR 303009) with the chromosome 6 introgression, yld8_A (121_2–2; GSOR 303011) with the chromosome 8 introgression, and yld9_A (13–1_1; GSOR303012) with the chromosome 9 introgression. The ILs were genotyped by Imai et al. (2013) with a custom-designed 1536-SNP Illumina (Illumina Inc., San Diego, CA) Golden Gate assay (Zhao et al., 2010), a custom-designed 384-SNP BeadXpress assay (Illumina Inc.) identified as OPA6.0 (Thomson et al., 2012) and The Kompetitive Allele Specific PCR genotyping assay (LGC Genomics, Teddington, Middlesex, UK) using SNPs selected from the 44K-SNP Affymetrix rice chip (Affymetrix, Thermo-Fisher Scientific Inc., Santa Clara, CA)_(Zhao et al., 2011). This genotyping revealed both the extent of the target introgressions and the locations of any background introgressions (Fig. 1).

Phenological and Yield Component Data Collection

A greenhouse experiment was conducted at the Dale Bumpers National Rice Research Center, Stuttgart, AR during 2013 and 2014. Initially, a single seed was planted in a 5 cm by 5 cm by 5 cm cell filled with Baccto potting soil (Michigan Peat Company, Houston, TX). At the four-leaf stage, one heathy, vigorous seedling was transplanted to a 20.5 cm by 19.0 cm (~6 L) pot filled with field soil. The pots were kept flooded and plants were fertilized monthly with Osmocote Plus (The Scotts Company, Marysville, OH; N/P/K 15:9:12) patterned release fertilizer throughout the growing cycle. The study was conducted using a randomized complete block design with five different planting dates. At each planting date, the number of replications varied, with the first planting on 17 Apr. 2013 having four pots per experimental unit, the second on 31 May 2013 having six, the third on 18 Sept. 2013 having five, the fourth on 27 Jan. 2014 having four, and the fifth on 21 May 2014 having four. The location of the experimental units (individual pots) was rotated on a 7- to 10-d basis to avoid any edge effects in the greenhouse. Starting at the second or third vegetative growth stage (V2–V3; Counce et al., 2000), phenological data were collected on a weekly basis throughout the vegetative stage (V2–V13) and the reproductive stage (R2–R9). Plant height was measured from the soil surface to the recently emerged leaf collar and the number of tillers, the number of panicles, and the growth stage were recorded. The main tiller was tagged at about the V6 to V7 stage. The days to heading measurement was determined as the days between planting the seed and when the main panicle began to shed pollen (R4).

Final plant height was measured from the soil surface to the top of the extended main panicle. The main panicle and flag leaf were harvested separately to measure the panicle length and flag leaf length and width. The number of primary branches, total florets, unfilled florets, and total seed were counted for the main panicle. Seeds from the main panicle were weighed separately and this weight was added to the weight of seed from the remaining tillers on the plant for individual plant total grain weight.

Image Analysis of Aboveground Biomass

For the first and second planting dates, digital images were taken every 7 to 10 d to estimate aboveground biomass during the growth of the plant. To take the photographs, each plant was placed on a table with a white backboard that included a ruler and a 10 cm by 10 cm black square for calibration of the pixel area. A Nikon D90 digital camera (Nikon Corporation, Shinagawa, Tokyo, Japan) was placed on a tripod set approximately 1.8 m from the table. The images were processed with the GNU Image Manipulation Program version 2.8 (http://www.gimp.org/, accessed 27 May 2016), using the fuzzy select tool to remove background noise and set the background to white. To obtain estimates of aboveground biomass, the images were processed in two ways, the first to remove all background so only the pixels of the plant tissue was recorded and the second to record only the pixels within the calibration square. The number of pixels in each image was obtained in a GNU/Linux environment with bash scripts that use the ImageMagicK (http://www.imagemagick.org/, accessed 27 May 2016) software suite [scripts available at https://gist.github.com/jeremyde/60aef4aac90958ec7c31a6631dd0585f (accessed 2 June 2016)] to count all nonwhite pixels. The number of pixels for the plant image was divided by the number of pixels in the calibration box to obtain the estimates of aboveground biomass.

Statistical Analysis

Statistical analyses were performed using R (R Core Team, 2015; http://www.r-project.org/, accessed 27 May 2016). For the measurements taken at maturity, considered to be the R9 growth stage (plant height, number of tillers, number of panicles, main panicle yield components, and flag leaf length and width) and days to reach each growth stage (V2–R9), each trait was used as a response vector in a linear model incorporating line (ILs and Jefferson), planting date effects, and their interaction as predictor terms and experimental replicate as a random effect (Bates et al., 2015). Dates of specific growth stages that were not directly recorded for a given replication (pot) were estimated from the growth stages it was between. Least squares means were calculated (Lenth, 2016) and the contrast command of the least squares means package was used to calculate contrast coefficients for each IL compared with the Jefferson control using Dunnett's method. The means of each IL at a given planting date were used to determine Pearson's correlation coefficients among the traits (Harrell and Dupont, 2015). Quantile box-and-whisker plots were made with the trait values for each line grouped by planting date with Dunnett's tests (Supplemental Fig. S1) using JMP software (SAS Institute, 2015).

The traits measured over time were observed to be classical sigmoid growth curves for each of the lines (ILs and Jefferson) (Fig. 2) and were evaluated following the process outlined by Archontoulis and Miguez (2015) to select the particular model. The growth curve functions for each line (pot) × planting date were estimated using nonlinear regression models in the fit curve personality of the JMP software (SAS Institute, 2015). The estimated fit parameters of the selected growth model were evaluated for significance between the lines using the restricted maximum likelihood method within the fit model personality, where lines was a fixed effect and planting date and the line × planting date interaction were random (block) effects. Post-hoc Dunnett's comparison of each IL with the Jefferson control (IL minus Jefferson) were calculated for the significant fit parameters (growth rate, inflection point, and asymptote) (Keuls and Garretsen, 1982).

RESULTS

Phenological Changes

The difference in days, compared with Jefferson, for the ILs to reach 12 vegetative and nine reproductive growth stages is presented in Table 1. Interestingly, there was a trend for the high-yielding ILs to be delayed (positive values) in reaching specific vegetative and reproductive growth stages compared with Jefferson, although these differences were significant for only four of the eight ILs (Table 1; Supplemental Fig. S2). Both of the *yld 2.1* lines (yld2_A and yld2_B) and the yld9_A line were significantly delayed in reaching the final vegetative stages, V12 and V13, compared with Jefferson. Subsequently, these three ILs reached the booting stage (R3), anthesis (R4), and the grain-filling stages (R5, R6, R7, and R8) more slowly than Jefferson. Delayed anthesis and grain filling also were noted in in yld1_A. In contrast, in both *yld 6.l* lines, yld6_A and yld6_B, the transition from the vegetative phase to the initiation of the reproductive (R1, R2) phase occurred more quickly than in Jefferson. Figure 1 demonstrates the differences in plant architecture at R9, just prior to harvest, of the ILs and the *O. rufipogon* and Jefferson parents.

Growth Curve Analysis

Several nonlinear models were examined for their fit to the repeated measurement growth data (height, tiller number, panicle number, and aboveground biomass) including the logistic three-, four-, and five-parameter and the Gompertz three- and four-parameter models (Keuls and Garretsen, 1982; Archontoulis and Miguez, 2015; SAS Institute, 2015). The logistic three-parameter (logistic 3P) model consistently produced a good fit for all traits and planting dates. The logistic 3P model provided parameter estimates for growth rate (*a*), inflection point (*b*), and the asymptote (*c*) (Eq. [1]):

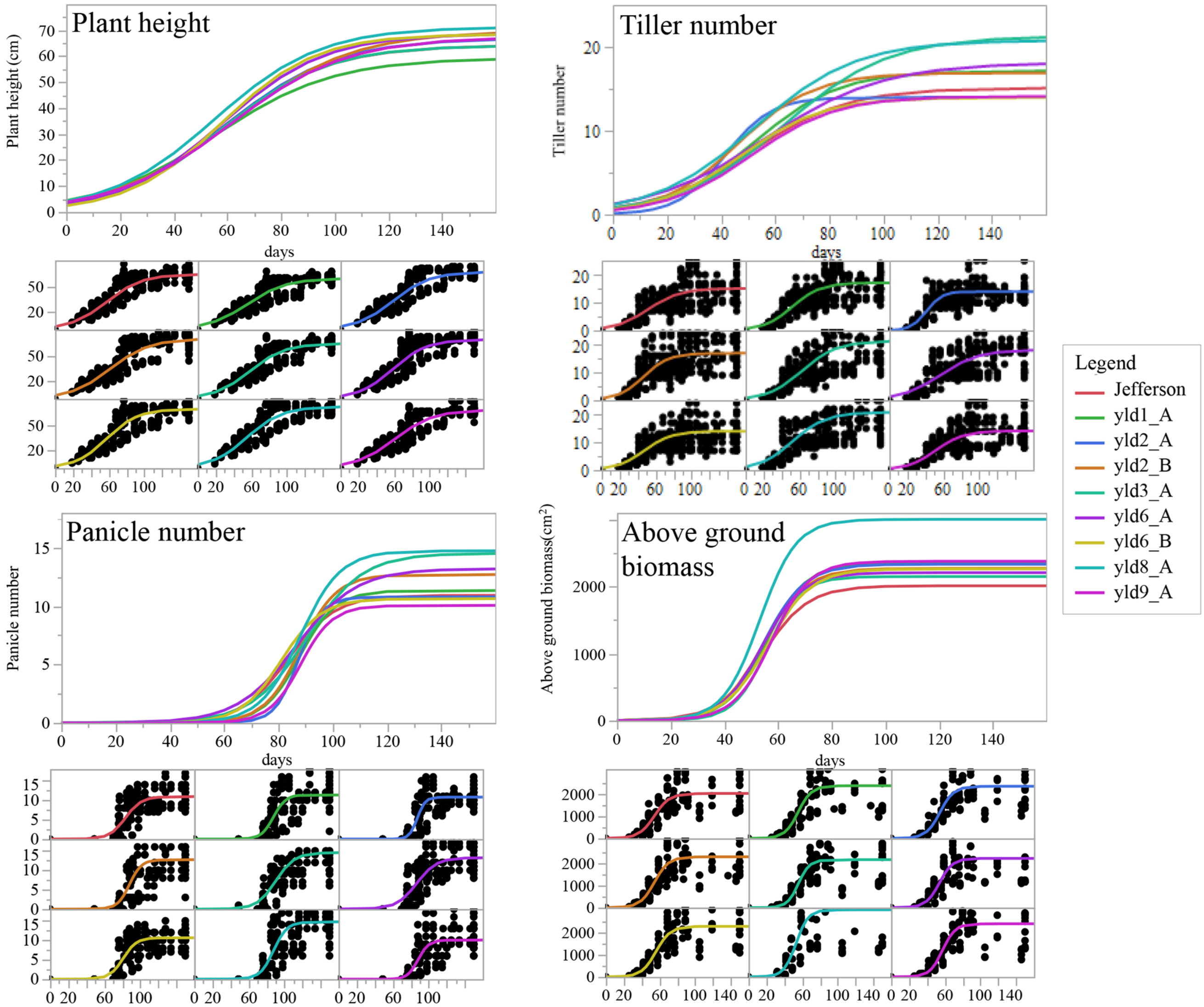

Fig. 2. Growth traits and yield components measured over time and fitting of logistic three-parameter nonlinear models for each rice line across all planting dates. Significant parameter estimates for each introgression line (IL) are in Table 2. Graphs were created with JMP software (SAS Institute, 2015).

$$\frac{c}{1+\exp\left[-a\left(x-b\right)\right]}. \qquad [1]$$

Figure 2 shows a logistic 3P model fit for all repeated data measured across all plantings. However, in evaluating the significance of the predictor variables, the logistic 3P models were fitted separately for each pot (individual experimental unit). The growth parameters with a significant line effect F-test ranged between $p < 0.0002$ and $p < 0.0180$, except for aboveground biomass growth rate, which had $p < 0.3090$ (Table 2). Post-hoc Dunnett's comparisons between the ILs and the recurrent parent revealed significant differences in logistic 3P model parameters (Table 2). Two significant comparisons for plant height growth rate were detected: a decrease in *yld 1.2* and an increase for *yld 6.1* (yld6_A and yld6_B) compared with Jefferson. No significant differences were found in the tiller number and panicle number growth rates. However, for *yld 2.1*, the inflection point difference was −12.8, indicating earlier tiller development than in Jefferson. The ILs with the *yld 3.2* and *yld 8.1* introgressions had significantly higher asymptotes than Jefferson for both tiller and panicle number, indicating that more tillers and panicles were produced. By contrast, for the *yld 1.2*, *yld 2.1*, and *yld 9.1* introgressions, the inflection point difference was positive, suggesting that panicle development was later than Jefferson. For aboveground biomass, *yld 3.2* had a significantly faster growth rate than Jefferson. The biomass asymptote parameters for *yld 1.2*, *yld 2.1*, *yld 9.1*, and especially *yld 8.1* were greater than Jefferson, indicating greater final aboveground biomass for these ILs.

Table 1. Differences in least squares means between each introgression line (IL) of rice and the recurrent parent, Jefferson (ILs minus Jefferson), for the length of time (days) to reach the 12 vegetative and nine reproductive growth stages. These growth stages are illustrated in Supplemental Fig. S2.

IL	yld1_A	yld2_A	yld2_B	yld3_A	yld6_A	yld6_B	yld8_A	yld9_A
QTL‡	*yld 1.2*	*yld 2.1*	*yld 2.1*	*yld 3.2*	*yld 6.1*	*yld 6.1*	*yld 8.1*	*yld 9.1*
V2†	−1.38	0	−2.39	0.31	−0.11	−0.15	0	0
V3	−0.73	0	−0.73	0.18	−0.18	−0.09	5.04	0
V4	0.08	0.44	−0.65	1.12	0.43	0.21	5.28	1.13
V5	0.5	−1.24	−2.03	0.58	−1.49	−2.57	−0.07	1.25
V6	−0.49	−3.32	−3.61	8.56	−2.53	−1.90	−4.57	2.38
V7	−0.1	−1.3	−3.07	0.31	2.88	−3.83	−3.78	1.95
V8	0.85	−1.13	−2.09	0.86	−2.11	−2.56	−2.52	1.21
V9	0.73	−0.77	−1.39	0.25	−1.51	−2.79	−1.07	1.8
V10	0.51	−0.69	−0.08	−0.19	−2.19	−3.3	−1.32	2.34
V11	−0.53	3.22	1.95	2.83	−1.59	−1.87	−0.97	5.1*
V12	5.1	11.13*	10.67*	5.96	1.1	2.59	5.04	11.38*
V13	2.97	10.75*	10.59*	4.43	−2.5	0.49	4.04	11.94*
R1	1.89	1.02	1.58	−2.36	−5.4	−5.72	−4.38	2.38
R2	1.7	3.13	2.21	0.19	−4.33*	−4.69*	−0.96	3.49
R3	2.12	5.92**	4.61*	1.11	−1.9	−2.51	−0.06	4.19*
R4	2.96*	6.21***	5.14***	0.63	−0.94	−0.92	0.45	4.96***
R5	1.88	5.65***	4.84***	−0.22	−0.1	−1	−0.12	5.52***
R6	4.9*	8.09***	6.99***	0.66	0.91	−0.81	0.61	6.77**
R7	3.49*	5.75***	4.04*	1.68	−0.17	−1.45	1.37	4.71**
R8	2.76	5.82*	4.12	0.68	1.05	−0.69	0.31	5.11*
R9	−1.71	1.67	−0.45	−0.34	−1.44	−2.15	−0.67	0.98

* Significant at the 0.05 probability level.

** Significant at the 0.005 probability level.

*** Significant at the 0.0005 probability level.

† Growth stages determined according to Counce et al. (2000).

‡ QTL, quantitative trait locus.

Table 2. Differences in least squares means between each introgression line (IL) of rice and the recurrent parent, Jefferson (ILs minus Jefferson), for significant logistic three-parameter regression parameter estimates for four growth traits using non-destructive measurements taken over the entire lifecycle. Figure 2 shows the curves for all growth traits over all replications.

Trait	Parameter estimates	Line F-test†	IL and yield QTL‡							
			yld1_A	yld2_A	yld2_B	yld3_A	yld6_A	yld6_B	yld8_A	yld9_A
			yld 1.2	*yld 2.1*	*yld 2.1*	*yld 3.2*	*yld 6.1*	*yld 6.1*	*yld 8.1*	*yld 9.1*
Plant height (cm)§	Growth rate	0.0002	−0.005*	−0.003	−0.001	0.001	0.005*	0.005*	−0.003	−0.003
	Asymptote	0.0180	−4.453	2.565	1.709	0.391	4.474	5.011	9.851*	3.664
Tiller number	Inflection point	0.0007	−1.463	−12.780**	−7.418	7.770	−4.814	−2.503	−3.619	4.082
	Asymptote	0.0016	1.822	−1.335	1.119	6.873**	0.996	−0.783	4.882*	−0.107
Panicle number	Inflection point	0.0012	5.150*	4.934*	4.452*	1.837	−0.284	−1.773	2.771	5.819**
	Asymptote	0.0080	1.206	0.482	2.175	2.986*	1.377	−0.034	3.895**	−0.402
Aboveground biomass (cm^2)¶	Growth rate	0.3090	0.018	0.013	0.012	0.038*	0.020	0.007	0.022	0.020
	Asymptote	0.0103	486.53*	526.03*	377.20	362.03	366.78	364.76	1756.95***	591.56*

* Significant at the 0.05 probability level.

** Significant at the 0.005 probability level.

*** Significant at the 0.0005 probability level.

† The significance of the *F*-test is given for the line effect for each parameter estimate.

‡ The individual IL is listed above and the yield quantitative trait locus (QTL) below.

§ Plant height was measured from the soil surface to the collar of the topmost leaf.

¶ Based on images taken following the 17 Apr. 2013 and 31 May 2013 planting dates.

Table 3. Differences in least squares means between each introgression line (IL) of rice and the recurrent parent, Jefferson (ILs minus Jefferson), for agronomic traits and yield components measured at maturity (the R9 growth stage). The data for each planting date are shown in Supplemental Fig. S1.

Trait	IL / QTL¶	yld1_A / *yld 1.2*	yld2_A / *yld 2.1*	yld2_B / *yld 2.1*	yld3_A / *yld 3.2*	yld6_A / *yld 6.1*	yld6_B / *yld 6.1*	yld8_A / *yld 8.1*	yld9_A / *yld 9.1*
Days to heading†		2.96*	6.21***	5.14***	0.63	−0.94	−0.92	0.45	4.96***
R9 plant height (cm)‡		−5.45	4.31	−1.85	−0.71	0.92	1.49	7.85*	0.14
R9 tiller number		0.37	−3.54	−0.83	5.81*	−1.14	−0.83	5.21*	−2.49
R9 panicle number		1.01	0.82	1.88	3.29*	0.24	0.48	4.19*	0.02
Flag leaf length (cm)		10.70***	14.13***	14.68***	6.36*	2.42	2.84	9.90**	9.64**
Flag leaf width (cm)		−0.08	−0.18	-0.28**	−0.15	−0.10	−0.21*	−0.26*	0.03
Panicle length (cm)		2.13*	4.56***	4.21***	4.18***	0.88	2.13*	4.65***	4.03***
Primary branch number		−0.63	−0.02	−0.35	−0.84	1.17*	1.47*	0.14	1.62*
Total floret number		2.76	53.56**	66.37**	3.53	87.68***	81.93***	45.43*	66.49**
Aboveground biomass§ (cm^2)		335.96*	429.46**	308.67*	215.35	246.61	182.25	1064.12***	404.45**
Seed number		−5.32	44.31*	50.32*	−3.20	73.16**	72.82**	45.15*	31.17
1000-seed weight per main panicle (g)		4.37	1.72	−0.54	5.59*	−0.59	0.22	1.07	−1.09
1000-seed weight per plant (g)		1.60	−1.82	−0.42	0.09	−3.39	−2.58	−5.47*	1.54
Seed weight per plant (g)		9.65	28.12*	28.32*	6.09	11.58	12.57	8.79	19.52

* Significant at the 0.05 probability level.

** Significant at the 0.005 probability level.

*** Significant at the 0.0005 probability level.

† Measured at the R4 growth stage.

‡ Plant height was measured from the soil surface to the tip of the main panicle.

§ Aboveground biomass measurement was taken at or near the R9 growth stage (Counce et al. 2000), which had the maximum biomass.

¶ QTL, quantitative trait locus.

Relative Differences in Yield Component Traits

Plants thrived throughout the growing season for each of the planting dates except near maturity for the 18 Sept. 2014 planting. The overcast winter conditions beginning in late November and problems with the greenhouse heating system resulted in very poor seed set and thus the data taken at maturity (final plant height to the panicle tip, flag leaf length and width, and panicle yield components) for this planting date were not included in the final analysis. The distributions of all trait measurements for each IL can be seen as quantile box-and-whisker plots in Supplemental Fig. 1 grouped by planting date. In the linear model using the combined planting date data, the planting date and line effects were significant for all traits ($p < 0.05$), except for seed weights, which were measured only for a single planting date (data not shown). Significant line × plant date interactions ($p < 0.05$) were found for all traits except days to heading and flag leaf width (data not shown); however, the main effects explained over 50% of the variation. Significant differences in agronomic traits and yield components were detected for each of the ILs when contrasted with the Jefferson recurrent parent (Table 3). In each case, the significant difference was in a positive direction (the trait increased relative to the recurrent parent) except for flag leaf width and the 1000-seed weight per plant for yld8_A. Days to heading significantly increased in yld1_A, yld2_A, yld2_B, and yld9_A. Plant height significantly increased in yld8_A. Tiller number and panicle number increased in yld3_A and yld8_A. Panicle length was significantly increased in every IL except yld6_A. Panicle branch number was increased in *yld 6.1* lines (yld6_A, yld6_B) and yld9_A. Floret number significantly increased in all ILs except yld1_A and yld3_A. Flag leaf length significantly increased in all lines except yld6_A and yld6_B, whereas flag leaf width significantly decreased in yld2_B, yld6_B, and yld8_A compared with Jefferson. As seen in the growth curves, a significant increase in aboveground biomass compared with Jefferson was noted in yld1_A, yld2_A, yld2_B, yld9_A, and especially yld8_A.

Evaluation of seed production revealed that *yld 2.1* (yld2_A, yld2_B), *yld 6.1* (yld6_A, yld6_B) and *yld 8.1* produced more seed on the main panicle than Jefferson. Compared with Jefferson, the 1000-seed weight for the main panicle was significantly heavier for yld3_A and 1000-seed weight per plant was lighter for yld8_A. The seed weight per plant was greater for all the ILs than for the Jefferson parent and was significant for the *yld 2.1* introgression (yld2_A and yld2_B), confirming the yield advantage of these ILs.

Correlation coefficients among only the ILs (not Jefferson) (Table 4) revealed that days to heading had significant negative correlations with plant height, tiller number, flag leaf length and width, panicle length, primary branch number, and total number of seeds and florets, suggesting that among the ILs, those that shifted from the vegetative to the reproductive phase earlier were associated with greater source (height, tillers, flag leaf size) and greater

Table 4. Pearson's correlation coefficients for the agronomic and yield component traits of rice taken at maturity (R9 growth stage). The mean of each introgression line (IL) at each of four planting dates was used in this analysis.

	Days to heading (R4)	PHT	T_n	P_n	FLL	FLW	PL	PB_n	TF_n	UF_n	ABG	S_n	TSW_{pan}	TSW_{pl}
PHT‡	−0.80***	–	–	–	–	–	–	–	–	–	–	–	–	–
T_n	−0.66***	0.32	–	–	–	–	–	–	–	–	–	–	–	–
P_n.	0.11	−0.30	0.54**	–	–	–	–	–	–	–	–	–	–	–
FLL	−0.50**	0.45*	0.65***	0.23	–	–	–	–	–	–	–	–	–	–
FLW	−0.83***	0.68***	0.68***	−0.04	0.71***	–	–	–	–	–	–	–	–	–
PL	−0.56**	0.54**	0.66***	0.15	0.84***	0.66***	–	–	–	–	–	–	–	–
PB_n	−0.83***	0.75***	0.54**	−0.09	0.56**	0.87***	0.53**	–	–	–	–	–	–	–
TF_n	−0.74***	0.77***	0.24	−0.38*	0.43*	0.70***	0.47*	0.79***	–	–	–	–	–	–
UF_nn	−0.26	0.18	0.29	−0.10	0.41*	0.55**	0.38*	0.45*	0.46*	–	–	–	–	–
ABG†	−0.62**	0.63**	0.68***	0.89***	0.60**	0.61***	0.46*	0.70***	0.28	0.12	–	–	–	–
S_n	−0.74***	0.79***	0.17	−0.39*	0.35	0.59***	0.40*	0.73***	0.96***	0.19	0.24	–	–	–
TSW_{pan}	0.71***	−0.74***	−0.38	0.52*	−0.63**	−0.73***	−0.64**	−0.78***	−0.80***	−0.35	0.41	−0.78***	–	–
TSW_{pl}	0.80*	0.00	−0.69	0.16	0.72*	−0.09	0.08	0.17	0.26	0.19	−0.16	0.21	−0.05	
SW_{pl}	0.43	−0.77*	−0.36	−0.42	0.30	0.40	0.39	0.01	−0.42	0.45	−0.59	−0.52	0.54	0.17

* Significant at the 0.05 probability level.

** Significant at the 0.005 probability level.

*** Significant at the 0.0005 probability level.

† The maximum aboveground biomass means were calculated from the largest biomass calculation, which was at or near the R9 growth stage.

‡ PHT, plant height, T_n, tiller number, P_n, panicle number; FLL, flag leaf length; FLW, flag leaf width; PL, panicle length; PB_n, primary branch number; TF_n, total floret number; UF_n, unfilled floret number; AGB, aboveground biomass; S_n, seed number, TSW_{pan}, 1000-seed weight per panicle; TSW_{pl}, 1000-seed weight per plant; SW_{pl}, total seed weight per plant

sink traits (panicle length, panicle branches, total florets, and total number of seed) but lower seed weight. The 1000-seed weight for the main panicle was positively correlated with days to heading but negatively correlated with plant height, flag leaf size, panicle length and primary branch number, and number of florets and seed. An increase in flag leaf length and width was associated with an increase in the overall size of the panicle (length, number of florets, primary branches), tiller number, and aboveground biomass but a decrease in 1000-seed weight per panicle. However, the flag length was positively associated with 1000-seed weight per plant. In addition, among the ILs, plants that flowered later were vegetatively smaller and had a smaller sink size with fewer seed but greater seed weight. Increased panicle length was associated with more total florets, more panicle branching, and more seed but also with more unfilled florets and lower main panicle seed weight. Lastly, aboveground biomass had a significant positive correlation with plant height, number of tillers and panicles, flag leaf size, and panicle length and branching.

DISCUSSION

The purposes of this greenhouse study were (i) to confirm previously demonstrated transgressive variation for yield enhancement relative to Jefferson observed in multilocation field trials (Imai et al., 2013) and attributed to *O. rufipogon* introgressions at six loci in eight ILs and (ii) to characterize the phenological, agronomic and yield component traits more fully to elucidate the mechanisms by which the yield gains are achieved. The *O. rufipogon* parent was not included in this study because it is photoperiod-sensitive, unlike Jefferson and its offspring, which are day-neutral. Considering the dramatic difference in plant architecture between Jefferson and the *O. rufipogon* parent (Fig. 1), these BC_3 ILs are relatively similar to each other and to the recurrent parent, having a commercially acceptable phenotype. Of the 14 yield components assayed, significant differences were detected between at least one IL and the recurrent parent for every trait (Table 3). Many of the yield components that were associated with a particular QTL initially reported by Thomson et al. (2003) were validated in this study (Supplemental Table S1); in addition, some new associations with yield related traits were discovered.

Validation of Yield Introgressions in Comparison to the Recurrent Parent

Thomson et al. (2003) reported that a Jefferson allele near the *yld 1.2* QTL was associated with increased grains (seed) per panicle and spikelets (florets) per panicle. In this study, we observed a significant increase compared with Jefferson in days to heading and panicle length corresponding to the *O. rufipogon* allele of the QTLs *dth1.2* and *pl1.1* (Thomson et al., 2003), respectively, along with a significant increase in flag leaf length and aboveground biomass (Table 2; Table 3). Thus, the yld1_A IL flowers and moves through the reproductive stages more slowly (Table 1), has a slower plant height growth rate, and later panicle development on the basis of the inflection point (Table 2).

The *yld 2.1* ILs (yld2_A and yld2_B) were observed to have significantly slower phenological development in the late vegetative and reproductive stages (Table 1), increased days to heading, longer flag leaf length, longer panicles, increased biomass, more florets and seed per panicle, and more yield per plant (Table 2; Table 3). Similarly, Thomson et al. (2003) reported an increased number of spikelets (*spp2.1*) and grains (*gpp2.1*) per panicle for this yield QTL. The panicle length QTL, *pl12.1* (Thomson et al. 2003; Supplemental Table S1), is in the same region as the background introgression on chromosome 12 for these ILs (Imai et al., 2013) and the grain weight QTL, *gw5.1*, is in the same region as the background introgression on chromosome 5 in the yld2_A IL. These background introgressions may explain the significant increase in panicle length and seed weight per plant attributed to the *O. rufipogon* parent. A large negative tiller number inflection point was determined for yld2_A IL, suggesting earlier maximum tiller development. The positive panicle number inflection point for both ILs suggests later panicle development than in Jefferson (Fig. 2; Table 2).

The *O. rufipogon yld 3.2* introgression on chromosome 3 was reported to be associated with more florets and seed per panicle (Thomson et al., 2003). These specific traits were not confirmed in this study; however, significant increases in flag leaf length, panicle length, tiller number, panicle number, seed weight of the main panicle, and biomass growth rate were observed (Table 2; Table 3). The panicle length QTL, *pl9.1*, attributed to the *O. rufipogon* allele (Thomson et al. 2003; Supplemental Table S1), is in the same region as the background introgression on chromosome 9 (Imai et al., 2013). Colocated with *yld 3.2,* is the *OsTB1* gene, which is a homolog of the maize (*Zea mays* L.) *TEOSINTE BRANCHED 1* gene. In rice, it has been shown to negatively regulate lateral branching and affect tiller number (Takeda et al., 2003). *Oryza rufipogon* is known to have a wide tiller angle that is very different from the erect tiller angle found in Jefferson. (Note that in Fig. 1., the *O. rufipogon* tillers are tied together and a stake is keeping the plant erect.) Although this plant architecture was not reported by Thomson et al. (2003) as being associated with the *yld 3.2* introgression, we observed this plant architecture with the two ILs (yld_3A and yld9_A) with introgressions on chromosomes 3 and 9 (Fig. 1).

For the *yld 6.1* introgression on chromosome 6, Thomson et al. (2003) reported an association with an increased percent seed set. Similarly, we observed significant increases in the number of florets, seeds, and primary panicle branches, along with a faster growth rate for plant height (Table 2; Table 3). Overall, the *yld 6.1* ILs moved through the phenological growth stages more quickly than Jefferson, especially the R2 early reproductive stage (Table 1).

The increases in plant height and grains per panicle observed by Thomson et al. (2003) for the *yld 8.1* QTL were confirmed in this study along with increases in tiller number, panicle number, flag leaf length, panicle length, number of florets, and aboveground biomass but a lower 1000-seed weight per plant was observed (Table 3). The yld8_A IL also had a higher asymptote for plant height, tiller number, panicle number, and biomass than Jefferson (Table 2; Fig. 2). The panicles per plant QTLs, *ppl3.1* and *ppl7.1* (Thomson et al., 2003; Supplemental Table S1), were located in the same region as the background introgressions for yld8_A on chromosomes 3 and 8 (Imai et al., 2013) and thus may contribute to the increased number of tillers and panicles.

The *yld 9.1* introgression associated with the yld9_A IL was observed to have several significantly delayed phenological growth stages at the later vegetative and reproductive stages (Table 1). The greater inflection point for panicle number and the higher asymptote for biomass indicates that this IL had delayed maximum panicle production and greater total biomass relative to Jefferson (Table 2). In addition, it had increased days to heading, flag leaf length, panicle length, primary panicle branching, florets per panicle, and biomass (Table 3). The increases in panicle length and total floret number correspond to the field-based QTL for panicle length (*pl9.1*) and spikelets per panicle (*spp9.1*) observed by Thomson et al. (2003). The field-based days to heading QTL, *dth3.4*, was in the same region as the nontargeted introgression on chromosome 3 (Imai et al., 2013). Thomson et al. (2003) also reported that the *yld 9.1* QTL was associated with an open tiller type associated with the *O. rufipogon* allele. This is supported in Fig. 1, which demonstrates that yld9_A has a very wide tiller angle compared with Jefferson. The *TILLER ANGLE CONTROL-1* gene, which has been cloned, resides in this introgressed region (Wang and Li, 2008; Yu et al., 2007).

Similarities and Differences among the Yield QTL Introgressions

Although the targeted QTLs reside on six different chromosomes, some similarities are seen in their impact on phenological, agronomic and yield component traits. A general trend that was observed among the ILs to have delayed (more positive value) phenological growth relative to Jefferson (Table 1). The panicle primordia are developed some 10 d prior to panicle initiation, which occurs 25 to 30 d prior to heading (Yoshida, 1981). Thus, the potential number of florets and panicle branches are established shortly after the early tillering stage. Our results suggest that a slowed growth rate of some of the ILs relative to Jefferson may allow greater accumulation of photosynthates at this early critical stage of panicle initiation. In addition, four of the ILs associated with *yld 1.2*, *yld 2.1*, and *yld 9.1* introgressions were associated with significantly more days to heading than Jefferson (Table 3). Samonte et al. (2006) demonstrated that heading date was highly correlated (r = 0.86) with main culm node number, which is an important biomass component, as each culm node is associated with a leaf. However, among just the ILs, lines with earlier heading were associated with increases in several yield component traits (Table 4). This suggests that there is a sensitive balance between source and sink plant components to optimize yield. Jefferson has a short (24.3 cm) and broad (1.6 cm) flag leaf compared with other commercial varieties. For all but the *yld 6.1* introgression lines, flag leaf length was significantly greater than Jefferson (Table 3). Among the ILs, flag leaf length was positively associated with several panicle yield components (Table 4). In contrast, for the most part, the ILs had a narrower flag leaf than Jefferson (Table 3) but among the ILs, the flag leaf width was positively correlated with panicle yield components (Table 4). Although all of the leaves are important sites of photosynthesis throughout the lifecycle of the plant, it is the uppermost leaves that are responsible for providing assimilates to the developing panicles and the flag leaf has the longest life span (Yoshida, 1981). Yue et al. (2006) determined that flag leaf length was correlated with florets per panicle ($r > 0.50$), whereas flag leaf width was less important. Wang et al. (2012) showed that flag leaf dimensions are positively correlated with panicle branching and panicle weight. Our results suggest that these ILs have more photosynthetic capacity than Jefferson primarily because of large differences in flag leaf length and, to a lesser extent, as seen among just the ILs, because of flag leaf width. These same six ILs with longer flag/leaves also had significantly longer panicles than Jefferson (Table 3), indicating greater sink capacity, which is supported by the positive correlations between panicle length and panicle yield components (Table 4).

Aboveground biomass, determined using image analysis, was significantly correlated among the ILs with days to heading, plant height, tiller number, panicle number, flag leaf size, panicle length, and branching but not with the seed traits (Table 4). The ILs had higher aboveground biomass than Jefferson and this was significant for all but *yld 3.2* and *yld 6.1* (Table 3). The aboveground biomass estimate for Jefferson was 2062 cm^2 and that of the ILs ranged from 2244 to 3126 cm^2. Sheehy et al. (2004) presented a biphasic growth pattern in rice that demonstrated the shift in the resources accumulated during the vegetative phase through to development of the panicles. Vegetative development is key to establishing the photosynthetic potential of the crop; however, photosynthesis that occurs during the grain-filling phase accounts for over half of the carbohydrate translocated to the grain (Yoshida, 1981). Our results suggest that the *O. rufipogon* introgressions impacted both source capacity (slower phenological development and longer flag leaves) and sink capacity (panicle length, number of florets, number of seed, and seed weight), along with more effective use of photosynthetic resources (greater number of panicle-bearing tillers).

The set of traits that explain the yield increases compared with Jefferson vary among the individual ILs, suggesting that the yield gains are occur through different mechanisms (Table 3). Lines with introgressions at *yld 2.1*, *yld 6.1*, *yld 8.1*, and *yld 9.1* have longer panicles with a greater number of florets, leading to increased seed number. The ILs with introgressions at *yld 3.2* and *yld 8.1* have an increased tiller number, leading to an increased number of panicles; they also have longer panicles. An increase in heading date associated with *yld 1.2*, *yld 2.1*, and *yld 9.1* may also partially explain a yield increase (Samonte et al., 2006). Of note, a field study of three durum wheat (*Triticum durum* L. var. *durum*) lines with *Thinopyrum ponticum* (Podp.) Barkworth & D.R. Dewey introgressions for the long arm of chromosome 7A examined many of the same traits reported here and revealed a significant increase in tiller number, biomass and flag leaf dimensions, resulting in increased grain yield (Kuzmanovic et al., 2016). Greater increases in yield may be achieved by pyramiding introgressions at multiple loci into a single line. Yield components may also help to predict which combinations of loci will have the greatest impact on yield. As part of the breeding process to increase yield in elite breeding lines, it will be important to select against any undesirable traits associated with some of the introgressions. For example, increased tiller angle is an undesirable trait that is present in the yld3_A, yld8_A and yld9_A ILs (Fig. 1). Selection against the undesirable alleles could be successful through elimination of background introgressions or by breaking linkage drag. If the undesirable traits are a pleiotropic effect of the yield-enhancing genes themselves, then selecting parents with compensating traits and making new crosses may reduce or eliminate the associated undesirable trait(s) in the progeny.

There is the potential for more than one locus in the ILs to be responsible for the observed phenotypic differences because the ILs contain some additional background introgressions (Fig. 1; Imai et al., 2013). In addition, it is possible that the target introgressions span multiple loci-conferring phenotypes and thus the targeted yield introgressions may resolve into multiple linked loci through fine mapping, as demonstrated with the days to heading QTL, *dth1.1* (Thomson et al., 2006). Moreover, the linkage drag that is often associated with the yield loci introduced from progenitor species may have additional agronomic effects. Imai et al. (2013) observed that additional backcrossing to remove background introgressions had no positive effect and, in some cases, reduced yields. Future work will focus on additional backcrosses to eliminate background introgressions and screening for recombination within the target introgressions to fine-map the yield loci. There remains the possibility that the phenotypic differences observed in the ILs are caused by epigenetic effects rather than DNA sequence differences in the introgressions. In addition, chromosome segment substitution line populations may be used to reduce background introgressions (Ali et al., 2010; Tung et al., 2010), increase mapping resolution, and identify epigenetic effects.

Nondestructive Yield Component Measurements Captured Throughout the Lifecycle

Measurements of plant growth over time may capture yield components that are not apparent from measurements taken only at one point in time. Plant height, tiller number, panicle number, and aboveground biomass measurements taken over the lifecycle of the plant revealed differences in nonlinear growth model parameters (Table 2). The significant differences in plant height growth rate for *yld 1.2* and *yld 6.1*; tiller number inflection point in yld2_A; and panicle number inflection point for *yld 2.3*, *yld 2.1*, and *yld 9.1* (Table 2) were not reflected in the measurements at maturity (Table 3). The significant difference in the asymptotes of tiller number and panicle number in yld3_A and yld8_A (Table 2) coincides with a significant difference in tiller number and panicle number at maturity (Table 3), suggesting that the asymptote parameter, representing the plateau of the curve, determined final harvest measurements for these traits. The results of the repeated measurements were not as consistent in the ILs sharing the same yield QTLs as they were in the results from single harvest measurements. This may be because these growth curves capture more subtle genotypic responses throughout the lifecycle of the plant than is realized by measurement at a single time point. A novel image analysis procedure was developed and tested as a nondestructive method for estimating aboveground biomass (see methods). The method was effective in identifying positive estimates of growth rate in all ILs when contrasted with the recurrent parent (Table 2). Use of automated image phenotyping systems might improve the ability to measure these growth parameters through the use of large numbers of individuals and more frequent measurements.

The substantial overlap between yield component differences detected in the greenhouse and known QTLs for yield components measured in the field (Fig. 2; Thomson et al., 2003) suggests that greenhouse findings will translate to trait differences in a field environment and that phenotypes observed in plants grown in greenhouse pots are comparable to plants grown in an unconfined field soil environment. The advantage of greenhouse phenotyping includes better environmental control and reduced labor in making repeated measures and images over the growth cycle of the plant. Further research is needed to determine if all of the yield component effects and their relative magnitudes are representative of the yield response in multiple field environments. In addition, it is not known whether the yield components that are detected first in a greenhouse assay will translate to substantial yield gains in field plots; future research will address this question.

CONCLUSIONS

In this study, we have shown that transgressive variation for yield and yield components discovered through AB-QTL field studies (Thomson et al., 2003; McCouch et al., 2007) can be more fully characterized through detailed phenology and yield component studies that can be conducted in the greenhouse. This supports an additive model of transgressive variation where introgressions from the agronomically inferior wild donor resulted in a delayed growth rate, particularly during the transition from the late vegetative to the early reproductive phases and possibly increased source capacity along with an increased sink demand, as shown by more total florets and greater seed size. The ability to accurately measure phenological changes over time and yield components in a stable greenhouse environment will accelerate breeding efforts that make use of wild, underused germplasm and lead to a better understanding of the molecular mechanisms that underlie transgressive variation. This will enable future research aimed at fine mapping and ultimately positional cloning of yield loci. Fine mapping of the loci will provide tightly linked molecular markers to be used by plant breeders for marker-assisted selection, whereas positional cloning will provide insights into the genetic mechanisms controlling yield.

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