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ORIGINAL ARTICLE

Mapping QTLs for yield and photosynthesis-related traits in three consecutive backcross populations of *Oryza sativa* cultivar Cottondora Sannalu (MTU1010) and *Oryza rufipogon*

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Abstract

Main conclusion **Identification of trait enhancing QTLs for yield and photosynthesis-related traits in rice using interspecific mapping population and chromosome segment substitution lines derived from a cross between** *Oryza sativa* **and** *Oryza rufipogon***.**

Abstract Wild rice contains novel genes which can help in improving rice yield. Common wild rice *Oryza rufipogon* is a known source for enhanced photosynthesis and yield-related traits. We developed $BC_2F_{2:3:4}$ mapping populations using *O. rufipogon* IC309814 with high photosynthetic rate as donor, and elite cultivar MTU1010 as recurrent parent. Evaluation of 238 BC_2F_2 families for 13 yield-related traits and 208 BC_2F_2 families for seven photosynthesis-related physiological traits resulted in identification of significantly different lines which performed better than MTU1010 for various yield contributing traits. 49 QTLs were identified for 13 yield traits and 7 QTLs for photosynthesis-related traits in BC_2F_2. In addition, 34 QTLs in BC_2F_3 and 26 QTLs in BC_2F_4 were also detected for yield traits.11 common QTLs were identified in three consecutive generations and their trait-increasing alleles were derived from *O. rufipogon*. Significantly, one major effect common QTL *qTGW3.1* for thousand grain weight with average phenotypic variance 8.1% and one novel QTL *qBM7.1* for biomass were identified. Photosynthesis-related QTLs *qP*$_N$*9.1*, *qP*$_N$*12.1*, *qP*$_N$*12.2 qSPAD1.1* and *qSPAD6.1* showed additive effect from *O. rufipogon*. A set of 145 CSSLs were identified in BC_2F_2 which together represented 87% of *O. rufipogon* genome. In addition, 87 of the 145 CSSLs were significantly different than MTU1010 for at least one trait. The major effect QTLs can be fine mapped for gene discovery. CSSLs developed in this study are a good source of novel alleles from *O. rufipogon* in the background of Cottondora Sannalu for rapid improvement of any trait in rice.

Keywords Wild rice · *Oryza rufipogon* · Yield · Photosynthesis · BILs · QTLs · CSSLs

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Abbreviations

DFF	Days to 50% flowering
PH	Plant height
NT	Number of tillers
NPT	Number of productive tillers
SPY	Single plant yield
BM	Biomass
TDM	Total dry matter
HI	Harvest index
TGW	Thousand grain weight
PDP	Per day productivity
FLL	Flag leaf length
FLW	Flag leaf width
SPAD	Soil and plant analysis development
P_N	Net photosynthesis
g_s	Stomatal conductance
C_i	Intercellular CO_2 concentration
E	Transpiration rate
WUE	Water use efficiency
*i*WUE	Intrinsic water use efficiency
CE	Carboxylation efficiency
PV	Phenotype variance
QTL	Quantitative trait loci
CSSLs	Chromosome segment substitution lines

Introduction

Rice (*Oryza sativa*) is one of the most valuable crops in the world and contributes to human food supply as a staple crop for over half of mankind. With the increase of population and decrease of cultivated land, food production is facing huge challenges. Hence, developing high-yielding improved varieties remains one of the main goals in rice breeding. In the last decades, rice grain yield has not significantly improved and appeared to reach a plateau (Zhu et al. 2017).

The wild crop species have long been used in plant breeding (Harlan 1976). Several valuable genes accumulated in wild rice were lost during domestication process and are absent in cultivated rice. The advanced backcross QTL (AB-QTL) strategy was proposed to identify and transfer agronomically useful QTLs from wild relatives to enhance the genetic base of crops available to breeders (Tanksley and Nelson 1996). Wild rice accessions have been used previously in mapping studies. QTLs/genes for conferring resistance to different biotic and abiotic stresses have been mapped and also for improvement of yield using wild species (Brar and Khush 1997; Brar and Singh 2011; Singh et al. 2016). Potential alleles for seedling vigour and related traits also identified from wild accessions (Addanki et al. 2018). Several studies have helped to identify and simultaneously introgress trait-enhancing alleles from wild species of rice into high-yielding elite cultivars. *O. rufipogon*, a perennial and diverse wild progenitor of Asian cultivated rice, was most commonly used in yield QTL mapping studies and was found to contribute more favourable alleles than other AA genome wild species. In addition, the *O. rufipogon* derived QTLs are highly congruent and detected on the same chromosomal locations, and this clearly shows the consistency and accuracy of these QTLs (Swamy and Sarla 2008). The first such study employed a cross between an accession of *O. rufipogon* (IRGC105491) with a CMS line female parent (Xiao et al. 1998). Although the *O. rufipogon* accession was phenotypically inferior for all 12 traits studied, transgressive segregation was observed for all traits, and 51% of the QTLs detected had beneficial alleles from *O. rufipogon* (Xiao et al. 1998). Subsequently, there have been several such studies to map trait-enhancing QTLs from *O. rufipogon* (Marri et al. 2005; Qiao et al. 2016; Qi et al. 2017; Singh et al. 2018; Qin et al 2018; Zou et al. 2020; Yuan et al. 2020; Hu et al. 2021) and genes that increase yield have been identified from *O. rufipogon* (Thalapati et al. 2012). A high yielding stress tolerant rice variety Gosaba 6 (Chinsurah Nona2) derived from KMR3/*O. rufipogon* and released in 2016 for coastal saline areas in West Bengal has become quite popular and also in flood prone areas with 5–5.6t/ha yield (Bhowmick et al.2014). It is evident that introgression of genomic regions from *O. rufipogon* can help in improving rice for several traits (McCouch et al. 2007; Neelam et al. 2018; Yang et al. 2020; Gaikwad et al. 2021).

Increasing rice yield is a main challenge for improving global food security (Khush 2013) and could also be achieved by increasing the rate of net CO_2 assimilation rate (P_N) in individual leaves (Long et al. 2006; Murchie et al. 2009). Identifying QTLs controlling photosynthesis parameters is a fundamental step in yield improvement (Gu et al. 2012). Increase in rate of leaf photosynthesis is important for the increase of yield potential of rice, since the rate of photosynthesis of the individual leaves affects dry matter production via photosynthesis within the canopy (Long et al. 2006; Murchie et al. 2009; Adachi et al. 2011; 2019). The use of natural genetic variation in photosynthesis within species can be an effective strategy for crop improvement (Flood et al. 2011). Wide variation in P_N has been reported among rice cultivars and in wild species (Yeo et al. 1994; Masumoto et al. 2004; Kanemura et al. 2007; Jahn et al. 2011; Kiran et al 2013; Kondamudi et al 2016) however, most of the natural genetic resources have yet to be tapped. Several advanced populations, including backcross introgression lines (BIL) and chromosome segment substitution lines (CSSLs), have been developed to facilitate the genetic analysis of rice (Balakrishnan et al. 2020). Improvements in the quantification of photosynthesis have reduced measurement times while maintaining accuracy in the field (Long and Bernacchi, 2003). This helps to facilitate the identification of quantitative trait loci (QTLs) and isolation of

underlying genes. In recent studies, several QTLs for P_N have been identified in rice (Teng et al. 2004; Hu et al. 2009; Takai et al. 2010; Gu et al. 2012; Ramchander et al. 2016; Adachi et al. 2019). However, a very limited number of genes (e.g., GREEN FOR PHOTOSYNTHESIS, Carbon assimilation rate 8) responsible for photosynthesis has been identified (Takai et al. 2013; Adachi et al. 2017). Rao et al. 2018a, b analysed introgression lines (ILs) using *O. nivara* and found two BILs 166S and 248S with high and consistent net photosynthesis compared to recurrent parent Swarna. Rao et al. 2019 measured flag leaf photosynthesis pigments in MTU1010 x *O. rufipogon* BILs at BC_2F_1 and found three lines BIL198-15, BIL198-16 and BIL198-29 showing higher chlorophyll than MTU1010. Likewise, ILs derived from cross between KMR3, a restorer line and *O. rufipogon* have been reported with enhanced P_N. Thirty-seven out of 40 ILs showed higher P_N than KMR3 and 20 ILs showed higher P_N than even *O. rufipogon,* the higher P_N parent (Haritha et al. 2017). Such ILs are a potential source for developing rice varieties and hybrids with higher biological yield.

Chromosome segment substitution lines (CSSLs) are useful in the genetic dissection of complex traits (Ali et al. 2010; Balakrishnan et al. 2019). CSSLs could eliminate most background noise from wild and related donor species due to advanced backcrossing and help in exploring novel QTLs for complex traits. CSSLs have been developed using *O. rufipogon* (Furuta et al. 2014; Subudhi et al. 2015; Qiao et al. 2016; Ogawa et al. 2016; Qin et al. 2018), *O. nivara* (Ma et al. 2016; Malathi et al. 2017), *O. minuta* (Guo et al.2013), *O. meridionalis* (Arbelaez et al. 2015; He et al. 2017) and *O. longistaminata* (Ramos et al. 2016). Based on these examples this study was undertaken to exploit the naturally occurring alleles from *O. rufipogon* by QTL mapping. *O. rufipogon* IC309814 is a wild rice accession collected from Odisha in Eastern India and was used for developing mapping population in the genetic background of the popular cultivated rice mega variety Cottondora Sannalu (MTU1010). This mid early duration high yielding mega rice variety MTU1010 has been used in several rice breeding programmes/research studies. Karwa et al. 2020 reported that MTU1010 showed more tolerance to heat stress than PR-113 at flowering stage due to its high spikelet fertility, lesser reduction in rate of photosynthesis and induced antioxidant system. BC_2F_6 MTU1010 lines possessing *Pup1* performed well under low P soil with high productive tillers, better root system architecture and higher yield compared to MTU1010 (Anila et al.2018). Improved MTU1010 introgression lines were developed through marker-assisted backcross breeding for biotic (Arunakumari et al., 2016) and abiotic stress (Vikram et al. 2011; Das et al. 2018; Anila et al. 2018 and Karwa et al 2020) resistance. However, wild introgression lines in MTU1010 background have not been used in QTL mapping or crop improvement previously. The main objectives of this study were to map QTL for photosynthesis and yield-related traits, to know the proportion of trait-enhancing alleles that can be obtained from this *O. rufipogon* accession and to identify high-yielding stable MTU1010 introgression lines.

Materials and methods

Plant material

Cottondora Sannalu here after referred as MTU1010, a mega rice variety released in 2000 by Andhra Pradesh Rice Research Institute (APRRI, Maruteru, India), was used as recurrent parent. MTU1010 is mid early duration variety, has a long slender grain type and gives yield of 6–6.5 t/ha during dry season. Wild accession *O. rufipogon* IC309814 with high net photosynthesis (Kiran et al. 2013 and Kondamudi et al. 2016) was used as male parent (Fig. 1).

Population development

F_1 plants were generated by crossing MTU1010 as female parent with *O. rufipogon* as male parent and were grown in greenhouse at Indian Institute of Rice Research (IIRR), Hyderabad. F_1s were confirmed as true interspecific hybrids using polymorphic SSR markers. True F_1 plants were backcrossed with recurrent parent MTU1010 and 64 BC_1F_1 seeds were obtained and sown but only 34 BC_1F_1 plants survived (Rao et al. 2018a, b). Each of the 34 plants were backcrossed to MTU1010 and approximately 900 BC_2F_1 seeds were collected and sown in nursery beds and later transplanted to the field under irrigated conditions. Maximum seed production was ensured during dry season 2015 and BC_2F_2 families were produced by selfing each plant. 238 BC_2F_2, BC_2F_3 and BC_2F_4 families were evaluated under field conditions in 2015, 2016 and 2018 respectively and the weather parameters are given in Supplementary Table1.

Phenotypic evaluation

Yield traits

MTU1010 and 238 BC_2F_2 families were grown in wet season 2015, BC_2F_3 families in wet season 2016 and BC_2F_4 in dry season 2018 at Research Farm, Indian Institute of Rice Research (IIRR), Hyderabad. Experiments were conducted using Randomized Complete Block Design (RCBD) with three replications each. The following 13 yield and related traits DFF- Days to 50% flowering (number of days from sowing to the time that 50% of the plants showed flowering), PH- Plant height (cm) (length in centimetres from the soil surface to the tip of the highest panicle at the time of harvest),

Fig. 1 Variation in grain and plant traits among parental lines and the population generated. **a** Seeds of parentsMTU1010 and *O. rufipogon* IC309814, **b** variation in grain size and colour of MTU1010 BC_2F_2 BILs. **c** Field view of MTU1010/*O. rufipogon* BC_2F_2 population in wet season 2015, one family is late to flower and measuring leaf photosynthesis in field. **d** Nursery and field view. **e** Assessment of Photosynthesis parameters. **f** Plants of MTU1010 and high yielding BIL- 47. **g** Variation in flag leaf length (FLL) and width (FLW) of MTU1010 BILs in BC_2F_2

TN- Tiller number (number of tillers at the time of harvest), PTN- Plant productive tiller number(number of productive tillers at the time of harvest), BM- Biomass (g) (weight of well-dried harvested plants without grains), SPY- Single plant yield (g) (weight of the harvested seeds per plant), TDM- Total dry matter (g), HI- Harvest index (%), TGW- Thousand grain weight (g) (1000grain weight was determined using seeds that were fully oven dried at 50 °C for 1 week),. PDP- Per day productivity, FLL- flag leaf length (cm), FLW- Flag leaf width (cm) (measured on main tiller in three middle plants in three replicates at longest and widest parts of the flag leaf), SPAD-Soil and plant analysis development (soil and plant analysis development value of the fully extended flag leaf on the main stem was measured using SPAD-502, Konica-Minolta, Japan). Only 13 yield traits were phenotyped in BC_2F_3 and only 9 yield traits were assessed in BC_2F_4. 208 BC_2F_2 families were evaluated for 7 photosynthesis-related traits during wet season 2015. These were P_N-net photosynthesis (µmol CO_2 m^{-2} S^{-1}), gS-Stomatal conductance (mol H_2O m^{-2} S^{-1}), C_i -Intercellular CO_2 Concentration (µmol mol^{-1}), E-Transpiration (mmol H_2O m^{-2} S^{-1}), WUE_i -Intrinsic water use efficiency, *CE* -Carboxylation efficiency, WUE-Water use efficiency.

Photosynthesis-related traits

During wet season 2015, three fully expanded flag leaves on the main culms of three randomly selected BC_2F_2 plants in each replication were used for measurement of photosynthesis-related traits here after referred as photosynthesis traits during anthesis. The middle part of the flag leaves was screened from 10:00 am to 12:30 pm under bright sunlit condition (1000 µmol m^{-2} $s^{-1}$of photosynthetic active radiation, 30 °C of the leaf chamber temperature, and ambient CO_2 levels 387 ppm) using a portable Li-COR6400 Photosynthesis

System in the field. The four photosynthesis parameters measured were rate of photosynthesis (P_N) [µmol (CO_2) m^{-2} s^{-1}], stomatal conductance (g_s) [mol (H_2O) m^{-2} s^{-1}], transpiration rate (E) [mmol (H_2O) m^{-2} s^{-1}], internal CO_2 concentration (C_i) [µmol mol^{-1}]. From these data, three parameters were derived viz., Water use efficiency (WUE-P_N/E), intrinsic water use efficiency (WUE_i-P_N/g_s) and carboxylation efficiency (CE- P_N/C_i) and were used for analysis.

Statistical analysis

The field experiments were conducted in a randomized complete block design with three replications. Statistical analysis was carried out using Statistical Tool for Agricultural Research (STAR 2.0.1), Plant Breeding Tools (PBTools 1.4) (version 1.4, http://bbi.irri.org/products) for descriptive statistics and stability analysis. Multiple correlations among yield and photosynthesis-related traits were also estimated using Pearson's correlation coefficient method to assess the influence of photosynthesis traits on yield components.

Genotyping

Total genomic DNA was isolated from fresh leaf samples of 238 BC_2F_2 plants and the parents, following CTAB method (Doyle and Doyle 1987). In all, 582 SSR markers were tested to detect polymorphism between parents and 248 were polymorphic. Of these, only 161 polymorphic SSRs (Supplementary Table 2) distributed on all 12 chromosomes segregated clearly in population. PCR was carried out in thermal cycler (G-STORM, United States) with a final reaction volume of 10 µl containing 15 ng of genomic DNA, 1X assay buffer, 200 mM of dNTPs, 1.5 mM $MgCl_2$, 10 pmol of forward and reverse primer and 1 unit of Taq DNA polymerase (Thermo Scientific). PCR cycles were programmed as follows: initial denaturation at 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min and a final extension of 10 min at 72 °C. Amplified products were resolved in 3% agarose gel prepared in 0.5 X TBE buffer and electrophoresed at 120 V for 2 h in general. Gels were stained with ethidium bromide and documented using gel documentation system (Alpha Imager, United States).

Linkage mapping and QTL analysis

Linkage map was constructed based on genotypic data of 238 BILs using 161 polymorphic SSR markers on all chromosomes using MAP functionality (BC_2RIL) of QTL IciMapping v4.12 (Meng et al. 2015) using the Kosambi mapping function (Kosambi 1944). QTL detection was carried out by Composite Interval Mapping and Inclusive Composite Interval Mapping (ICIM). The threshold LOD score was determined by performing a 1000-permutation test at a significance level of $P < 0.05$.

Identification of CSSL set

Optimal number of chromosomal segment substitution lines that include the entire genome of *O. rufipogon* were identified based on genotypic data of 161 polymorphic loci in 238 BC_2F_2 BILs in the background of the recurrent parent MTU1010 using the software CSSL Finder 3 (http://mapdisto.free.fr/CSSLFinder/).

Results

Phenotype evaluation

A total of 238 BC_2F_2 families were evaluated for 13 yield-related traits. DFF varied from 80 to108 days with a mean of 94 days. 16 BC_2F_2 families showed significantly higher DFF and 34 showed lower DFF compared to MTU1010 (94 days). Tiller number ranged from 11 to 46 with a mean of 25, five lines had significantly higher and one-line had lower tiller number than MTU1010. Single plant yield ranged from 6.5 to 46.1 g with a mean of 25.56 g. Heritability values of yield-related traits ranged from 0.73(PTN) and 0.95 (PH) and for photosynthetic traits it was from 0.83(E) to 0.92(Ci) (Supplementary Table 3). 29 BC_2F_2 families gave at least 10% higher yield than MTU1010 and only 15 families showed significantly lower yield than MTU1010. Harvest index in one family (V-191) was significantly higher and in 29 families significantly lower than MTU1010. One family (V-113) showed higher PDP. Mean values of traits in parents and range in BC_2F_2, BC_2F_3 and BC_2F_4 families are given in Table 1. 208 BC_2F_2 families were also evaluated for photosynthesis-related traits. P_N ranged from 8.39 to 46.22 µmol (CO_2) m^{-2} s^{-1} with a mean of 24.76 µmol (CO_2) m^{-2} s^{-1}, 10 families showed higher and 2 families lower P_N than MTU1010 and 2 families showed P_N higher than even *O. rufipogon*. Carboxylation efficiency ranged from 0.04 to 0.290 mol m^{-2} s^{-1} with a mean of 0.10 mol m^{-2} s–1, 7 families showed higher than MTU1010, 4 families showed lower CE than MTU1010 and 5 families higher than even *O. rufipogon*.

BC_2F_3 families were evaluated in wet season 2016, for 13 yield-related traits.73 families showed significantly higher PH and 2 families showed significantly more TDM. One family V-47 showed higher SPY (41 g) than MTU1010 (21.5 g). Likewise, BC_2F_4 families were evaluated in dry season 2018 for 9 yield-related traits and lines significantly different from MTU1010 for each trait were identified using pairwise mean comparison. 97 BC_2F_4 families showed significantly more and 3 significantly lesser DFF than MTU1010 (90 days). One family V-44 showed significantly higher values for PH, BM and TDM. V-61 showed

Table 1 Means and range of yield and physiology-related traits in BC_2F_2, BC_2F_3 and BC_2F_4 mapping population of MTU1010/*O. rufipogon*

S. No.	Trait	MTU1010	*O. rufipogon*	Range			Number of families showing > 15% increase over MTU1010			Stable lines high yielding lines		
				BC_2F_2	BC_2F_3	BC_2F_4	BC_2F_2	BC_2F_3	BC_2F_4	BC_2F_2		
1	DFF	94	–	80–108	92 – 131	83–108	0	82	9	95.5	96.0	97.0
2	PH	81.7	80.4	51 – 126.4	56.3 – 118.3	61–138.7	48	42	72	79.55	70.00	79.44
3	TN	26.2	45	11.8 – 46.6	7 – 35.7	7.3–23.7	49	184	3	32.11	35.00	23.45
4	PTN	25.1	38	10.9 – 42.8	7 – 34.3	6–21	46	182	4	31.00	33.78	22.45
5	BM	40.6	–	20.9 – 83.2	14.6 – 45.4	17.2–50.8	45	11	11	56.51	38.37	40.82
6	SPY	31.3	–	6.5 – 46.1	6 – 41.4	8–31.5	19	29	21	36.59	38.25	27.46
7	TDM	71.9	–	34.9 – 103.2	16.9 – 81.2	30.8–79.6	24	15	6	93.10	76.62	68.28
8	HI	43.5	–	15.3 – 60.2	12.7 – 56.5	20.9–51	12	37	12	38.50	49.66	40.22
9	TGW	20.4	–	15.1 – 25.0	8 – 23.8	16.1–27.1	8	0	2	19.76	19.23	20.09
10	PDP	25.2	–	4.8 – 38.6	4.1 – 32.87	–	25	20	–	29.16	30.35	21.64
11	FLL	25.5	17.5	16.1 – 42	12 – 33.7	–	59	52	–	31.57	24.13	26.97
12	FLW	1.6	1.45	1.07 – 2.0	0.8 – 1.65	–	2	11	–	1.50	1.53	1.50
13	SPAD	38.7	40.7	30.6 – 54.6	29.2 – 47.4	–	14	17	–	42.73	41.00	39.13
14	P_N	22.3	28.6	10.0 – 45.2	–	–	78	–	–	32.22	24.74	24.33
15	g_s	0.3	0.429	0.138 – 0.607	–	–	171	–	–	0.435	0.305	0.515
16	C_i	223.2	241	165 – 300	–	–	77	–	–	222.5	218.4	268.8
17	*E*	8.8	12.5	4.1 – 14.1	–	–	122	–	–	11.39	8.79	10.52
18	WUE_i	77.9	66.6	35.1 – 105.6	–	–	8	–	–	74.28	80.99	47.60
19	CE	0.1	0.12	0.041 – 0.269	–	–	47	–	–	0.145	0.115	0.090
20	WUE	2.5	2.30	1.67 – 4.12	–	–	13	–	–	2.84	2.82	2.31

DFF Days to 50% flowering, *PH* Plant height (cm), *TN* Tiller number, *PN* Panicle number, *BM* Biomass (g), *SPY* Single plant yield (g), *TDM* Total dry matter (g), *HI* Harvest index (%), *1000* Thousand grain weight (g), *FLL* flag leaf length (cm), *FLW* Flag leaf width (cm), *SPAD* Soil and plant analysis development, P_N Net photosynthesis (µmol (CO2) m^{-2} S^{-1}), g_S Stomatal conductance (mol$H_2O$$m^{-2}$ S^{-1}), c_i Intercellular CO2 Concentration (µmol mol^{-1}), *E* Transpiration (mmolH_2O m^{-2} S^{-1}), WUE_i Intrinsic water use efficiency, *CE* Carboxylation efficiency, *WUE* Water use efficiency, – data not available

significantly lower values for BM and TDM. For TGW, 7 families showed significantly higher and 13 families significantly lower values than MTU1010. The frequency distribution of yield and photosynthesis-related traits showed normal distribution (Supplementary Figs. 1, 2, 3). In the mapping population, transgressive segregants were observed for all yield and photosynthesis traits.

Correlation

Multiple correlation analysis among yield-related traits showed highly significant correlation between TN and PTN. SPY showed significant positive correlation with BM, TDM, TGW and HI. TGW was positively correlated with TN, PTN, SPY, TDM, PDP and HI (Tables 2, 3 and 4). Correlation analysis was performed between yield and photosynthesis-related traits in BC_2F_2. The rate of photosynthesis (P_N) was significantly correlated with BM, SPY and HI. P_N was significantly correlated with g_S, *E*, WUE_i, *CE* and WUE, but not with intercellular CO_2 concentration. SPY was significantly correlated with CE and WUE. PH and WUE showed highly significant correlation.

Stability analysis

Significantly better yielding lines compared to the parent MTU1010, showing consistent yield performance across the generations were detected. Top 50 among the 238 lines were subjected to stability analysis using AMMI and GGE biplot analysis and we found that five lines G37(V-47), G31(V-34), G41(V-6), G34(V-36) and G39 (V-54) were both stable and high yielding (Fig. 2). From which won where biplot, it was observed that G37 (V-47) and G31(V-34) are better suitable for environments E2 (Wet season 2016) and E3 (Dry season 2018) while G24(V-291) for E1 (Wet season 2015). The high yielding lines and their yield along with stability parameters are given in Supplementary Table 4.

Table 2 Correlations of yield and photosynthesis-related traits in MTU1010 x *O. rufipogon* BC_2F_2 mapping population in wet season 2015

	DFF	PH	TN	PTN	BM	SPY	TDM	HI	TGW	PDP	FLL	FLW	SPAD	Pn	gs	Ci	E	WUE_i	CE	WUE
DFF	1																			
PH	− 0.02	1																		
TN	− 0.07	− 0.11	1																	
PTN	− 0.09	− 0.11	0.93***	1																
BM	0.05	0.60***	0.36***	0.36***	1															
SPY	− 0.20**	− 0.24***	0.15*	0.17*	0.00	1														
TDM	− 0.09	0.32***	0.37***	0.38***	0.78***	0.62***	1													
HI	− 0.21**	− 0.49***	− 0.11	− 0.09	− 0.54***	0.82***	0.09	1												
TGW	− 0.26***	− 0.12	0.15*	0.16*	− 0.09	0.35***	0.15*	0.35***	1											
PDP	− 0.32***	− 0.23***	0.15*	0.17*	− 0.02	0.99***	0.60***	0.82***	0.37***	1										
FLL	0.12	0.26***	− 0.06	− 0.06	0.17*	− 0.16*	0.03	− 0.24***	− 0.14*	− 0.17*	1									
FLW	0.18**	− 0.17**	− 0.07	− 0.10	− 0.03	0.07	0.02	0.05	0.02	0.04	0.24**	1								
SPAD	0.04	− 0.39***	0.07	0.07	− 0.16*	0.08	− 0.08	0.13*	− 0.11	0.08	0.04	0.06	1							
P_N	− 0.14*	− 0.12	0.00	0.00	− 0.13*	0.20**	0.03	0.24***	0.07	0.21**	− 0.07	0.00	0.12	1						
gs	0.06	− 0.32***	0.13*	0.12	− 0.20**	0.08	− 0.11	0.18**	0.01	0.07	− 0.05	0.02	0.26***	0.41***	1					
Ci	0.17*	− 0.22**	0.13*	0.12	− 0.11	− 0.05	− 0.12	0.01	0.00	− 0.07	− 0.02	− 0.01	0.14*	− 0.42***	0.63***	1				
E	0.02	− 0.30***	0.04	0.06	− 0.18**	0.07	− 0.10	0.16*	− 0.01	0.07	− 0.08	0.06	0.30***	0.58***	0.70***	0.18**	1			
WUE_i	− 0.16*	0.27***	− 0.12	− 0.12	0.14*	0.04	0.14*	− 0.03	0.00	0.06	0.03	− 0.02	− 0.20**	0.30***	− 0.72***	− 0.98***	− 0.32***	1		
CE	− 0.18**	− 0.01	− 0.04	− 0.04	− 0.05	0.19**	0.08	0.19**	0.04	0.21**	− 0.05	− 0.01	0.04	0.92***	0.07	− 0.70***	0.34***	0.61***	1	
WUE	− 0.18**	0.17*	− 0.05	− 0.07	0.04	0.14*	0.12	0.10	0.08	0.16*	0.01	− 0.06	− 0.17*	0.55***	− 0.28***	− 0.70***	− 0.35***	0.72***	0.72***	1

*, ** and *** represent the significant level at 5%, 1% and 0. 01% only

DFF Days to 50% flowering, *PH* Plant height (cm), *TN* Tiller number, *PTN* Plant productive tiller number, *BM* Biomass (g), *SPY* Single plant yield (g), *TDM* Total dry matter (g), *HI* Harvest index (%), *TGW* Thousand grain weight (g), *PDP* Per day productivity(PDP), *FLL* flag leaf length (cm), *FLW* Flag leaf width (cm), *SPAD* Soil and plant analysis development, P_N Net photosynthesis (µmol CO2 m^{-2} S^{-1}), g_S Stomatal conductance (molH2O m^{-2} S^{-1}), c_i Intercellular CO_2 Concentration (µmol mol^{-1}), *E* Transpiration (mmolH2Om^{-2} S^{-1}), WUE_i Intrinsic water use efficiency, *CE* Carboxylation efficiency, *WUE* Water use efficiency

Table 3 Correlations of yield-related traits in MTU1010 x *O. rufipogon* BC_2F_3 mapping population in wet season 2016

	DFF	FLL	FLW	SPAD	PH	TN	PTN	SPY	BM	TDM	HI	TGW	PDP
DFF	1												
FLL	− 0.02	1											
FLW	0.15*	0.13*	1										
SPAD	0.12	− 0.08	0.10	1									
PH	− 0.03	0.26***	− 0.02	− 0.28***	1								
TN	− 0.07	0.07	− 0.08	− 0.11	0.00	1							
PTN	− 0.06	0.08	− 0.06	− 0.11	0.00	0.99***	1						
SPY	− 0.05	− 0.01	0.40***	0.01	0.12	0.38***	0.40***	1					
BM	− 0.02	0.21*	0.25***	− 0.11	0.41***	0.54***	0.55***	0.69***	1				
TDM	− 0.04	0.10	0.36***	− 0.06	0.28***	0.50***	0.52***	0.92***	0.91***	1			
HI	− 0.11	− 0.18**	0.27***	0.06	− 0.22**	0.06	0.07	0.71***	0.08	0.44***	1		
TGW	− 0.35***	− 0.07	− 0.09	− 0.04	− 0.17*	0.24***	0.25***	0.25***	0.11	0.20**	0.39***	1	
PDP	− 0.14*	− 0.01	0.39***	0.01	0.12	0.38***	0.40***	0.99***	0.69***	0.92***	0.71***	0.28***	1

*, ** and *** represent the significant level at 5%, 1% and 0. 01% only

DFF Days to 50% flowering, *PH* Plant height (cm), *TN* Tiller number, *PTN* Plant productive tiller number, *BM* Biomass (g), *SPY* Single plant yield (g), *TDM* Total dry matter (g), *HI* Harvest index (%), *TGW* Thousand grain weight (g), *PDP* Per day productivity(PDP), *FLL* flag leaf length (cm), *FLW* Flag leaf width (cm), *SPAD* Soil and plant analysis development

Table 4 Correlations of yield-related traits in MTU1010 x *O. rufipogon* BC_2F_4mapping population in dry season 2018

	DFF	PH	TN	PN	SPY	BM	TDM	HI	TGW
DFF	1								
PH	0.04	1							
TN	− 0.26***	− 0.07	1						
PTN	− 0.24***	− 0.08	0.98***	1					
SPY	0.18**	0.07	0.07	0.09	1				
BM	0.11	0.48***	0.26***	0.28***	0.24***	1			
TDM	0.18**	0.36***	0.22**	0.24***	0.77***	0.81***	1		
HI	0.10	− 0.20**	-0.09	− 0.09	0.79***	− 0.38***	0.23***	1	
TGW	− 0.28***	− 0.13*	0.10	0.09	− 0.10	− 0.14*	− 0.16*	− 0.01	1

*, ** and *** represent the significant level at 5%, 1% and 0. 01% only

PH Plant height (cm), *TN* Tiller number, *PTN* Plant productive tiller number, *BM* Biomass (g), *SPY* Single plant yield (g), *TDM* Total dry matter (g), *HI* Harvest index (%), *TGW* Thousand grain weight (g)

Genotyping of BC_2F_2

Considering all the 161 loci and 238 BILs, a total of 38,318 alleles were detected covering all 12 chromosomes. Segregation data showed that 70.3% alleles were homozygous for MTU1010, 13.9% homozygous for *O. rufipogon* and15.2% were heterozygous. In all, 5% of SSRs showed appearance of non-parental novel bands on chromosomes 2, 6, 7, 8, 11 and 12. The *O. rufipogon* introgressions among BILs ranged from 2.5 to 39.8%, with a mean of 13.9%. Number of heterozygotes at any locus ranged from 1.2 to 35.4. RM231 and RM60 on chromosome 3 showed the highest number of heterozygotes. IL V-331 had highest number of 57 heterozygous loci (35.4% of all loci) and IL V-249 had lowest number (only two) of heterozygous loci.

QTL mapping

QTLs were identified for all 13 yield-related traits in this study. In all, 49 QTLs were identified for yield-related traits and 7 QTLs for photosynthesis-related traits in BC_2F_2, 34 QTLs in BC_2F_3 and 26 QTLs in BC_2F_4 for yield-related traits (Fig. 3). In all, 31% of these QTLs in three generations showed a trait-increasing effect from *O. rufipogon*.

QTLs identified in BC_2F_2 for yield and photosynthesis-related traits

In all, 49 QTLs were identified for all 13 traits. Of these, 11 (22%) QTLs had a trait increasing effect from *O. rufipogon*. 13 QTLs were identified for HI and only one

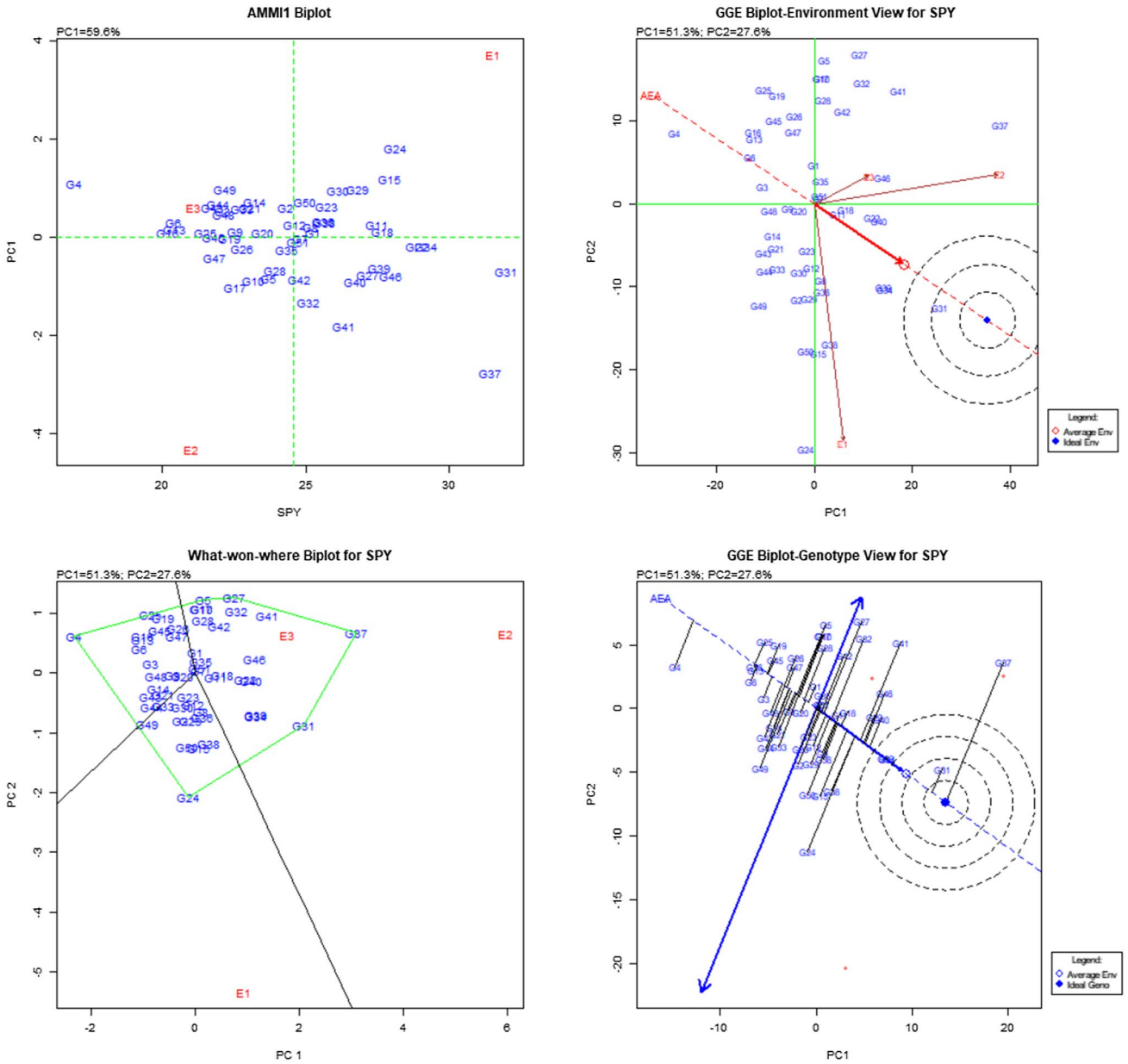

Fig. 2 AMMI and GGE biplots showing the stability of 50 high yielding lines among the MTU1010/*O. rufipogon* population used in this study

each for FLL and BM. Three QTLs $qP_N9.1$, $qP_N12.1$, $qP_N12.2$ showed PV from 2.8 to 6.9% and increasing effect of these QTLs was from *O. rufipogon*. A total of six FLW QTLs were identified on chromosomes 1, 3, 5 and 10 with PV ranging from 3.3 to 9.3. Of these, two were common in BC_2F_2 and BC_2F_3. In all the three QTLs *qSPY3.1, qSPY3.2* and *qSPY6.1* MTU1010 allele was trait-enhancing with LOD 2.7, 4.0 and 4.1 and PV 3.5, 4.3 and 8.6% respectively. Of five QTLs detected for SPAD, two major QTLs *qSPAD1.1* and *qSPAD6.1* with PV 7.4 and 8.2% respectively were derived from *O. rufipogon*. A total of 7 QTLs were identified for photosynthesis-related traits. Three QTLs each were identified for intercellular CO_2 concentration (*Ci*) and Intrinsic water use efficiency (WUE_i). Major effect QTLs, $qWUE_i6.1$ and *qCi7.1* showed PV of 10 and12.7% respectively. One QTL *qE1.1*for transpiration rate (E) was identified on chromosome 1 at RM7594-RM3738 with 3.1 LOD and 2.5% PV. Trait enhancing alleles in 4 QTLs *qCi6.1, qCi7.1, qE1.1* and $qWUE_i9.1$ were from MTU1010 and in 3 QTLs *qCi9.1,* $qWUE_i3.1$, $qWUE_i6.1$from *O. rufipogon*. Co-localisation of QTLs for yield and photosynthesis traits was

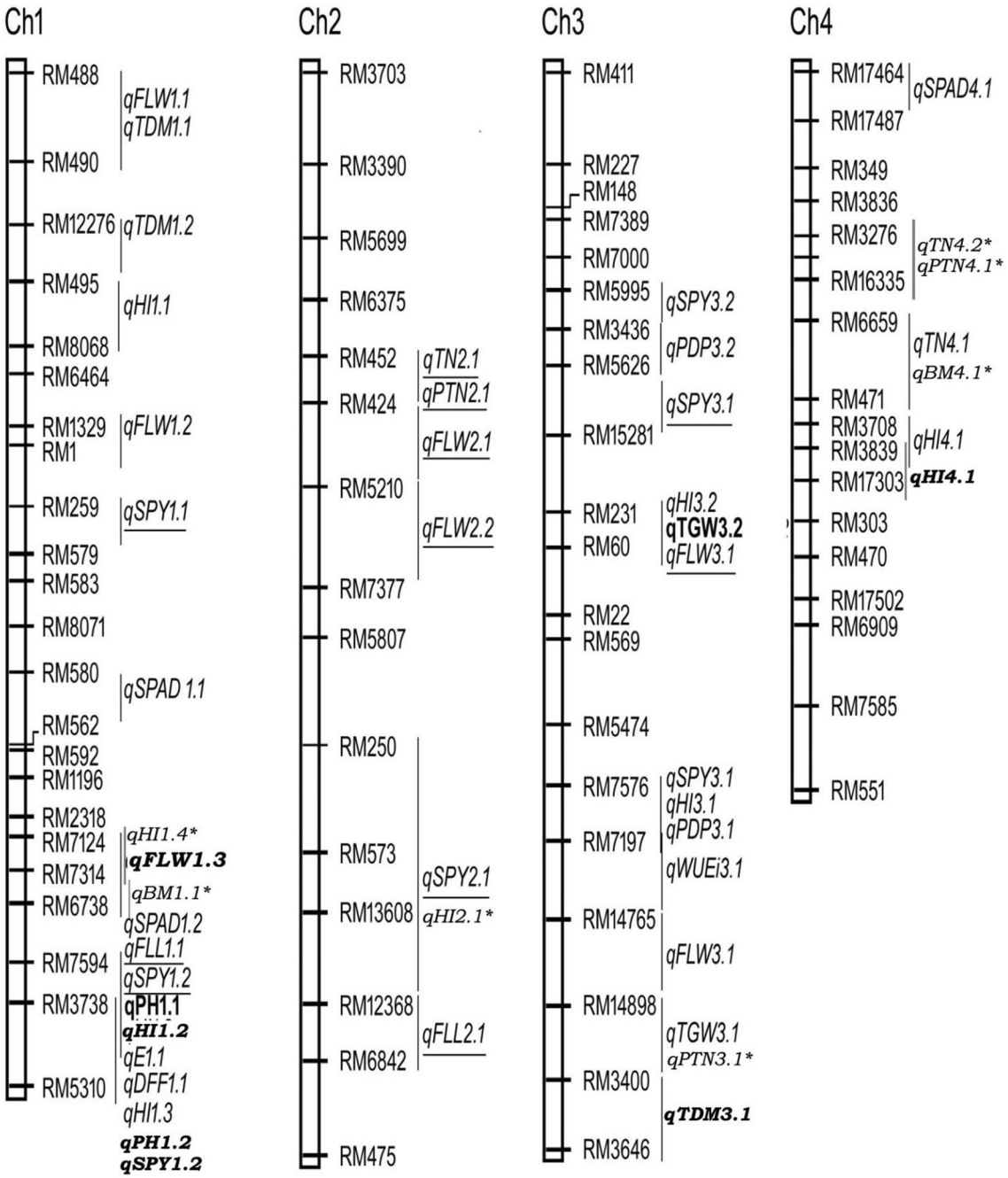

Fig. 3 QTLs identified in MTU1010 x *O. rufipogon* BC_2F_2, BC_2F_3 and BC_2F_4 mapping population. QTLs identified in more than one generation are shown in bold, QTLs identified in BC_2F_3 are underlined, QTLS identified in BC_2F_4 are given star symbol

observed at four chromosomal regions. On chromosome 1, QTLs *qPH1.1*, *qSPY1.1*, *qHI1.1*, *qSPAD1.1*, *qFLL1.1* at RM7594-RM3738 were co-localised with *qE1.1* for transpiration rate. On chromosome 3 at RM7197, QTLs *qSPY3.1*, *qHI3.1*, *qPDP3.1* were co-localised with *qWUEi*. At RM402-RM3183 three QTLs *qFLW6.1*, *qDFF6.1* and *qWUEi6.1* were co-localised on chromosome 6. On chromosome 7 at RM336-RM6389, three QTLs *qBM7.1*, *qTDM7.1* and *qCi7.1* were co-localised.

QTLs identified in BC_2F_3 for yield traits

In all, 34 QTLs were identified for 12 traits. No QTLs were identified for SPAD. Of 34 QTLs, 9 (26%) QTLs

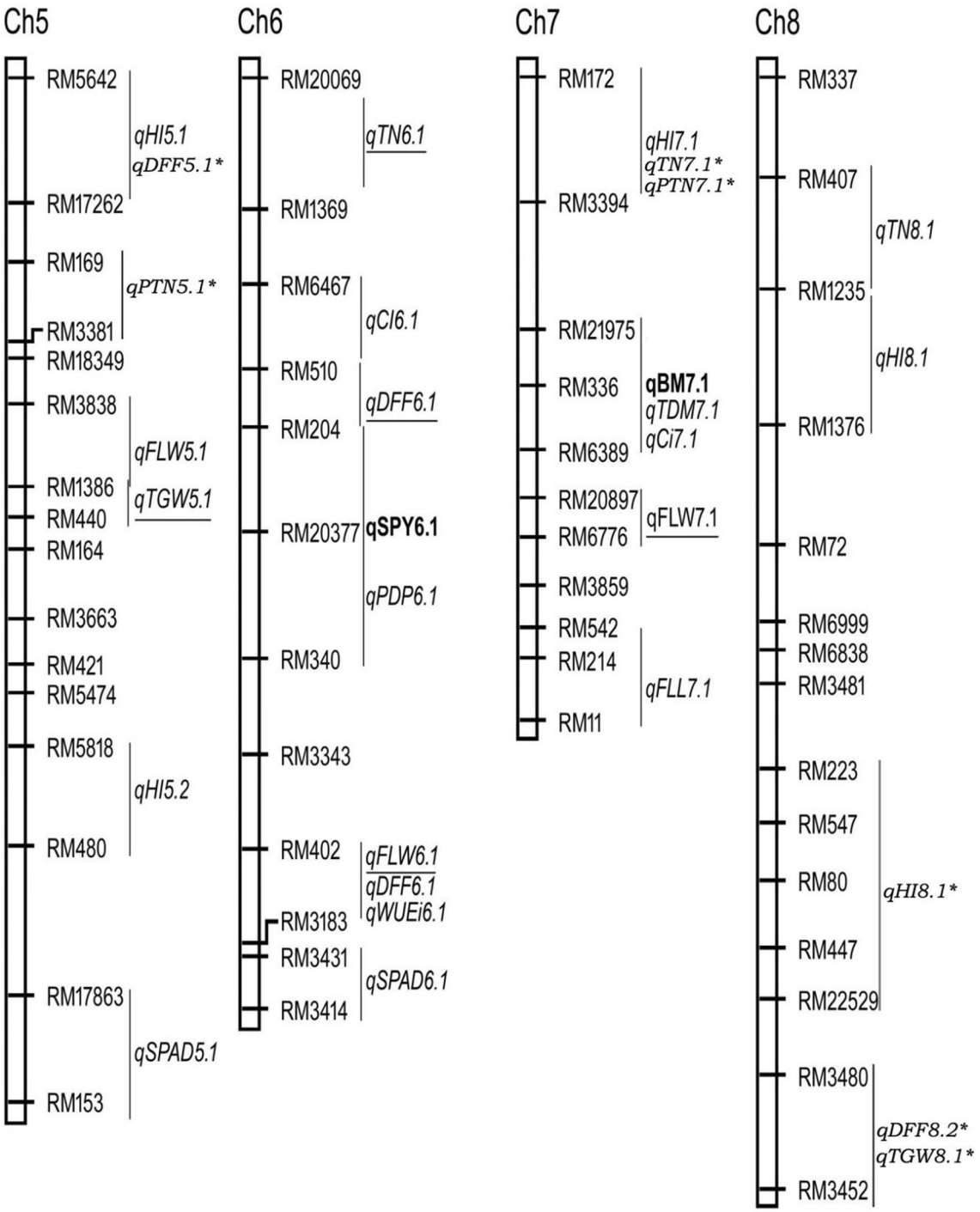

Fig. 3 (continued)

were derived from *O. rufipogon*. 11QTLs were identified for FLW. Six QTLs *qSPY1.1, qSPY1.2, qSPY2.1, qSPY3.1, qSPY6.1* and *qSPY9.1* were detected for SPY and only *qSPY2.1*, at RM250-RM12368 showed increasing effect from *O. rufipogon.* QTL *qTGW5.1* was identified at RM1368-RM440 with LOD 5.1 and PV 1.2%. One QTL each for DFF, PH, P_N and HI were identified. QTL $qP_N2.1$ explained PV of 6.3% with an additive effect of − 3.3. MTU1010 contributed to increase in biomass at *qBM7.1, qBM9.1* and *qBM9.2* with an average PV of 4.3%.

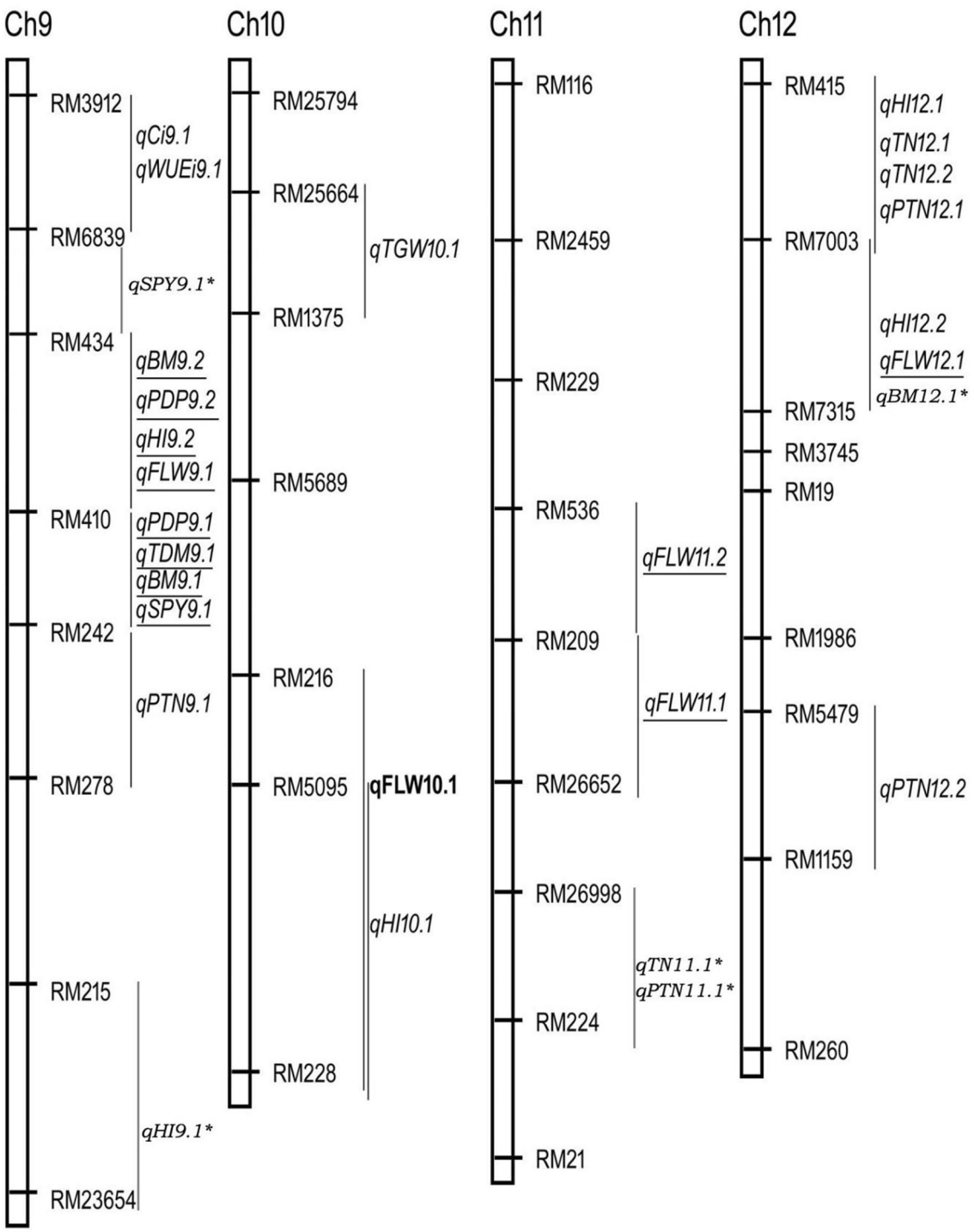

Fig. 3 (continued)

Table 5 Yield and photosynthesis-related QTLs detected in MTU1010/*Oryza rufipogon* BC_2F_2, BC_2F_3 and BC_2F_4

Trait	Chr	Marker interval	CIM			Generation
			LOD	PV (%)	Additive	
Days to 50% flowering (DFF)						
qDFF1.1	1	RM3738 – RM5310	8.1	4.5	2.8	BC_2F_2
qDFF5.1	5	RM5642 – RM17962	5.0	2.7	−2.1	BC_2F_4
qDFF6.1	6	RM402 – RM3183	11.4	13.5	1.9	BC_2F_2
qDFF6.1	6	RM510 – RM204	4.2	4.1	−2.4	BC_2F_3
qDFF8.1	8	RM1376 – RM3480	14.4	9.6	−6.5	BC_2F_4
qDFF8.2	8	RM3480 – RM3452	12.3	9.3	−6.5	BC_2F_4
Plant height (PH)						
qPH1.1	**1**	**RM7594 – RM3738**	**22.6**	**4.8**	**−13.4**	**BC_2F_2**
qPH1.1	**1**	**RM7594 – RM3738**	**12.0**	**5.8**	**−11.2**	**BC_2F_3**
qPH1.1	**1**	**RM7594 – RM3738**	**13.1**	**2.8**	**−16.4**	**BC_2F_4**
qPH1.2	**1**	**RM3738 – RM5310**	**30.6**	**5.2**	**−15.4**	**BC_2F_2**
qPH1.2	**1**	**RM3738 – RM5310**	**16.6**	**3.0**	**−16.3**	**BC_2F_4**
Number of tillers per plant (TN)						
qTN2.1	2	RM452 – RM424	3.0	7.5	−3.5	BC_2F_3
qTN4.1	4	RM471 – RM6659	3.7	2.6	−1.2	BC_2F_2
qTN4.2	4	RM16335 – RM3276	3.2	9.3	−1.7	BC_2F_4
qTN6.1	6	RM20069 – RM1369	2.6	3.8	1.5	BC_2F_3
qTN6.2	7	RM172 – RM3394	3.5	4.1	−0.9	BC_2F_4
qTN8.1	8	RM407 – RM1235	3.6	1.0	−1.7	BC_2F_2
qTN11.1	11	RM224 – RM26998	4.4	5.8	1.2	BC_2F_4
qTN12.1	12	RM415 – RM7003	6.8	3.8	0.3	BC_2F_2
qTN12.2	12	RM415 – RM7003	7.1	3.2	−6.9	BC_2F_2
Number of productive tillers per plant (PTN)						
qPTN2.1	2	RM452 – RM424	2.7	6.3	−3.3	BC_2F_3
qPTN3.1	3	RM3400 – RM14898	2.6	6.4	0.3	BC_2F_4
qPTN4.1	4	RM16335 – RM3276	3.3	9.0	−1.6	BC_2F_4
qPTN5.1	5	RM169 – RM3381	3.2	3.5	−1.0	BC_2F_4
qPTN7.1	7	RM172 – RM3394	3.2	3.8	−1.0	BC_2F_4
qPTN9.1	9	RM278 – RM242	2.9	2.9	−1.2	BC_2F_2
qPTN11.1	11	RM224 – RM26998	4.2	5.1	1.1	BC_2F_4
qPTN12.1	12	RM415 – RM7003	5.9	7.0	−5.5	BC_2F_2
qPTN12.2	12	RM5479 – RM1159	3.7	5.6	−0.9	BC_2F_2
Biomass (BM)						
qBM1.1	1	RM7341 – RM6738	4.6	3.9	−0.2	BC_2F_4
qBM4.1	4	RM471 – RM6659	4.5	7.7	1.9	BC_2F_4
qBM7.1	**7**	**RM336 – RM6389**	**7.9**	**2.7**	**1.7**	**BC_2F_2**
qBM7.1	**7**	**RM21975 – RM336**	**3.5**	**5.0**	**0.3**	**BC_2F_3**
qBM9.1	9	RM242 – RM410	3.7	3.4	2.0	BC_2F_3
qBM9.2	9	RM410 – RM434	3.5	4.5	2.5	BC_2F_3
qBM12.1	12	RM7003 – RM7315	5.7	12.8	−4.8	BC_2F_4
Single plant yield (SPY)						
qSPY1.1	1	RM259 – RM579	2.7	3.4	2.2	BC_2F_3
qSPY1.2	**1**	**RM7594 – RM3738**	**3.7**	**4.1**	**3.9**	**BC_2F_3**
qSPY1.2	**1**	**RM3738 – RM5310**	**3.9**	**9.8**	**1.9**	**BC_2F_4**
qSPY2.1	2	RM250 – RM12368	2.9	7.7	−4.6	BC_2F_3
qSPY3.1	3	RM7197 – RM7576	4.0	4.3	1.9	BC_2F_2
qSPY3.2	3	RM3436 – RM5995	4.1	3.5	3.6	BC_2F_2
qSPY3.1	3	RM15281 – RM5626	2.7	4.8	4.5	BC_2F_3

Table 5 (continued)

Trait	Chr	Marker interval	CIM			Generation
			LOD	PV (%)	Additive	
qSPY6.1	**6**	**RM20377 – RM340**	**2.7**	**8.6**	**1.1**	**BC_2F_2**
qSPY6.1	**6**	**RM204 – RM20377**	**3.0**	**4.8**	**1.6**	**BC_2F_3**
qSPY9.1	9	RM242 – RM410	5.4	6.8	3.2	BC_2F_3
qSPY9.2	9	RM434 – RM6839	4.1	7.5	0.2	BC_2F_4
Total dry matter (TDM)						
qTDM1.1	1	RM488 – RM490	3.5	5.9	2.7	BC_2F_2
qTDM1.2	1	RM12276 – RM495	3.3	2.8	0.1	BC_2F_2
qTDM3.1	**3**	**RM3646 – RM3400**	**2.9**	**3.5**	**4.1**	**BC_2F_3**
qTDM3.1	**3**	**RM3646 – RM3400**	**3.6**	**4.7**	**1.7**	**BC_2F_4**
qTDM7.1	7	RM336 – RM6389	4.3	2.2	3.6	BC_2F_2
qTDM9.1	9	RM242 – RM410	6.9	12.5	5.7	BC_2F_3
Harvest index (HI)						
qHI1.1	1	RM495 – RM8068	2.6	1.0	3.2	BC_2F_2
qHI1.2	**1**	**RM7594 – RM3738**	**3.4**	**1.4**	**4.7**	**BC_2F_2**
qHI1.2	**1**	**RM7594 – RM3738**	**9.0**	**11.7**	**5.1**	**BC_2F_4**
qHI1.3	1	RM3738 – RM5310	3.5	2.6	6.5	BC_2F_2
qHI1.4	1	RM7124 – RM7341	5.2	5.6	2.2	BC_2F_4
qHI2.1	2	RM250 – RM12368	4.4	4.2	−1.9	BC_2F_4
qHI3.1	3	RM7197 – RM7576	3.1	3.4	1.3	BC_2F_2
qHI3.2	3	RM60 – RM231	3.9	1.7	3.9	BC_2F_2
qHI4.1	**4**	**RM3839 – RM3708**	**4.5**	**1.4**	**4.9**	**BC_2F_2**
qHI4.1	**4**	**RM17303 – RM3839**	**4.8**	**6.4**	**4.1**	**BC_2F_4**
qHI5.1	5	RM5642 – RM17962	2.7	1.5	2.1	BC_2F_2
qHI5.2	5	RM5818 – RM480	3.2	1.1	4.0	BC_2F_2
qHI7.1	7	RM172 – RM3394	3.7	2.6	3.6	BC_2F_2
qHI8.1	8	RM1235 – RM1376	2.8	5.3	0.8	BC_2F_2
qHI8.1	8	RM22529 – RM223	3.2	9.7	2.0	BC_2F_4
qHI9.1	9	RM410 – RM434	8.8	1.6	11.0	BC_2F_3
qHI9.1	9	RM23654 – RM215	5.1	4.7	3.6	BC_2F_4
qHI10.1	10	RM228 – RM5095	2.9	3.4	5.8	BC_2F_2
qHI12.1	12	RM415 – RM7003	3.4	3.7	4.7	BC_2F_2
qHI12.2	12	RM7003 – RM7315	2.6	3.8	6.7	BC_2F_2
Thousand grain weight (TGW)						
qTGW3.1	3	RM3400 – RM14898	3.6	9.5	0.8	BC_2F_2
qTGW3.2	**3**	**RM60 – RM231**	**9.5**	**15.2**	**0.9**	**BC_2F_2**
qTGW3.1	**3**	**RM60 – RM231**	**3.0**	**1.1**	**1.0**	**BC_2F_3**
qTGW5.1	5	RM1386 – RM440	4.2	1.2	0.6	BC_2F_3
qTGW8.1	8	RM3480 – RM3452	9.3	16.8	1.2	BC_2F_4
qTGW10.1	10	RM1375 – RM25664	3.0	6.1	0.5	BC_2F_2
Per day productivity (PDP)						
qPDP3.1	3	RM7197 – RM7576	3.3	3.6	1.4	BC_2F_2
qPDP3.2	3	RM5626 – RM3436	3.2	3.1	2.8	BC_2F_2
qPDP6.1	6	RM20377 – RM340	3.6	11.2	1.2	BC_2F_2
qPDP9.1	9	RM242 – RM410	7.3	7.5	2.9	BC_2F_3
qPDP9.2	9	RM410 – RM434	8.7	10.6	3.8	BC_2F_3
Flag leaf length (FLL)						
qFLL1.1	1	RM7594 – RM3738	12.2	18.5	−3.5	BC_2F_3
qFLL2.1	2	RM12368 – RM6842	3.8	6.4	−1.5	BC_2F_3
qFLL7.1	7	RM542 – RM11	4.8	7.9	−2.0	BC_2F_2

Table 5 (continued)

Trait	Chr	Marker interval	CIM			Generation
			LOD	PV (%)	Additive	
Flag leaf width (FLW)						
qFLW1.1	1	RM488 – RM490	3.7	4.1	0.1	BC_2F_2
qFLW1.2	1	RM1329 – RM1	4.4	3.6	0.01	BC_2F_2
qFLW1.3	**1**	**RM7124 – RM7341**	**6.3**	**6.2**	**0.1**	**BC_2F_2**
qFLW1.1	**1**	**RM7341 – RM6738**	**4.3**	**1.7**	**0.1**	**BC_2F_3**
qFLW2.1	2	RM424 – RM5210	5.6	5.3	0.01	BC_2F_3
qFLW2.2	2	RM5210 – RM7337	5.6	5.5	0.01	BC_2F_3
qFLW3.1	3	RM14898 – RM14765	4.1	9.4	0.01	BC_2F_2
qFLW3.1	3	RM60 – RM231	4.8	1.8	0.1	BC_2F_3
qFLW5.1	5	RM3838 – RM1386	3.0	3.4	0.1	BC_2F_2
qFLW6.1	6	RM402 – RM3183	5.5	6.0	−0.1	BC_2F_3
qFLW7.1	7	RM20897 – RM6776	2.5	3.5	0.01	BC_2F_3
qFLW9.1	9	RM410 – RM434	3.6	1.3	0.01	BC_2F_3
qFLW10.1	**10**	**RM5095 – RM216**	**3.6**	**4.6**	**0.01**	**BC_2F_2**
qFLW10.1	**10**	**RM228 – RM5095**	**2.8**	**4.2**	**−0.1**	**BC_2F_3**
qFLW11.1	11	RM26652 – RM209	2.5	3.4	0.01	BC_2F_3
qFLW11.2	11	RM209 – RM536	3.7	4.4	0.01	BC_2F_3
qFLW12.1	12	RM7003 – RM7315	2.9	4.8	0.1	BC_2F_3
SPAD (SPAD)						
qSPAD1.1	1	RM580 – RM562	3.3	7.4	−2.0	BC_2F_2
qSPAD1.2	1	RM7594 – RM3738	4.8	6.5	1.5	BC_2F_2
qSPAD4.1	4	RM17487 – RM17464	4.3	5.1	1.1	BC_2F_2
qSPAD5.1	5	RM17863 – RM153	2.9	5.2	1.0	BC_2F_2
qSPAD6.1	6	RM3431 – RM3414	6.0	8.3	−1.9	BC_2F_2
Intercellular CO_2 concentration (Ci)						
qCi6.1	6	RM6467 – RM510	3.7	4.8	9.2	BC_2F_2
qCi7.1	7	RM336 – RM6389	4.0	12.7	16.2	BC_2F_2
qCi9.1	9	RM3912 – RM6839	4.3	4.2	−8.6	BC_2F_2
Transpiration rate (E)						
qE1.1	1	RM7594 – RM3738	3.1	2.5	0.7	BC_2F_2
Intrinsic water use efficiency (WUEi)						
$qWUE_i3.1$	3	RM7197 – RM14765	3.2	4.0	−5.4	BC_2F_2
$qWUE_i6.1$	6	RM402 – RM3183	3.3	10.4	−8.8	BC_2F_2
$qWUE_i9.1$	9	RM3912 – RM6839	3.5	3.0	5.0	BC_2F_2

QTLs identified in more than one generation are shown in bold

DFF Days to 50% flowering, *PH* Plant height (cm), *TN* Tiller number, *PTN* Plant productive tiller number, *BM* Biomass (g), *SPY* Single plant yield (g), *TDM* Total dry matter (g), *HI* Harvest index (%), *TGW* Thousand grain weight (g), *PDP* Per day productivity(PDP), *FLL* flag leaf length (cm), *FLW* Flag leaf width (cm), *SPAD* Soil and plant analysis development, C_i Intercellular CO_2 Concentration (µmol mol^{-1}), *E* Transpiration (mmolH2Om^{-2} S^{-1}), WUE_i Intrinsic water use efficiency, *PV* Phenotypic variance and Additive-additive effect

QTLs identified in BC_2F_4 for yield traits

In all, 26 QTLs were identified for 9 traits. Of 26 QTLs, 13 (50%) QTLs were derived from *O. rufipogon.* Six QTLs were identified for HI, two for PH and 5 for PTN. Three QTLs *qPTN4.1, qPTN5.1,* and *qPTN7.1* showed increasing effect from *O. rufipogon.* Two QTLs *qSPY1.1, qSPY9.1* were identified for single plant yield and both were derived from MTU1010. One major QTL *qTGW8.1* was identified at RM3480 – RM3452 with LOD 9.3 and PV 16.8% with increasing effect from MTU1010.

Common QTLs identified in BC_2F_2, $BC_2F_3$and BC_2F_4

There were six QTLs common in BC_2F_2 and BC_2F_3; 3 QTLs in BC_2F_2 and BC_2F_4 and 2 QTLs in BC_2F_3 and BC_2F_4 (Table 5). Only one QTL *qPH1.1* for plant height was identified in BC_2F_2, BC_2F_3 and BC_2F_4 generations with LOD ranging from 12 to 22.6 and PV from 2.8%to 5.8%. *O. rufipogon* allele increased plant height by 13.6 cm.

QTLs identified in both BC_2F_2 and BC_2F_3

Six QTLs *qPH1.1, qFLW1.1, qFLW10.1, qSPY6.1, qTGW3.1* and *qBM7.1*were identified in BC_2F_2 and BC_2F_3. QTL *qSPY6.1* showed an average LOD of 2.9 and PV of 6.7%.QTL *qTGW3.1*was identified at RM60 – RM231 with an average LOD 6.2 and PV 8.1%. *qBM7.1* was identified at RM336 – RM6389 with an average LOD 5.7 and PV 4%. The trait enhancing allele at *qFLW1.1*, *qSPY6.1, qTGW3.1* and *qBM7.1* was from MTU1010 in BC_2F_2 and BC_2F_3 generations.

QTLs identified in both BC_2F_2 and BC_2F_4

Three QTLs *qPH1.2, qHI1.2* and *qHI4.1* were identified in BC_2F_2 and BC_2F_4. *qPH1.2* was identified at RM3738 – RM5310 with an average LOD 23.6 and average PV of 4.1%, with plant height increasing effect from *O. rufipogon.* For harvest index two QTLs were identified in BC_2F_2 andBC_2F_4 generations, one QTL *qHI1.2* was identified at RM7594 – RM3738 with an average LOD of 6.2 and PV 6.5%. Another QTL *qHI4.*1 was detected at RM3839 with an average LOD of 4.6 and PV 4%.

QTLs identified in both BC_2F_3 and BC_2F_4

Two QTLs *qSPY1.2* and *qTDM3.1* were identified in BC_2F_3 and BC_2F_4. *qSPY1.2* was identified at RM3738 with an average LOD 3.8 and PV7.0%. *qTDM3.1* was identified between RM3646 and RM3400 with an average LOD 3.2 and PV4.1%. The increasing effect of these QTLs was from MTU1010. Only one common QTL *qPH1.1* was identified in all three generations. At *qPH1.1*, increase in additive effect was observed from 13.4 to 16.4 cm and decrease in LOD (22.6 to 13.1) and PV (4.8 to 2.8%) from BC_2F_2 to BC_2F_4. The QTLs with highest LOD score are plant height QTLs *qPH1.1* RM7594-RM3738 which is about 1.4 Mb and *qPH1.2* RM3738-RM5310. Which is about 4.2 Mb. *qPH1.1* identified at RM7594-RM3738 region and are close to the well-known semi- dwarf locus *sd-1*, the green revolution gene. This QTL region was scanned for the presence of putative candidate genes and found GH3 family genes, such as *OsGH3.1*, *OsGH3-2*, *GA2ox3*, *OsCesA4* and *d10/dwarf10,* which play critical roles in growth and development regulations. The alleles in this region is from *O. rufipogon* that increase height and suitable for further sequencing and functional analysis. Decrease in PV from BC_2F_2 to BC_2F_3 was observed at common QTLs *qTGW3.2*, *qSPY6.1, qFLW1.1* and *qFLW10.1*. At the two common QTL *qSPY1.2*, *qTDM3.1* identified in BC_2F_3 and BC_2F_4, increase in LOD and PV and decrease in additive effect was observed. Interestingly, increase in LOD, PV and additive

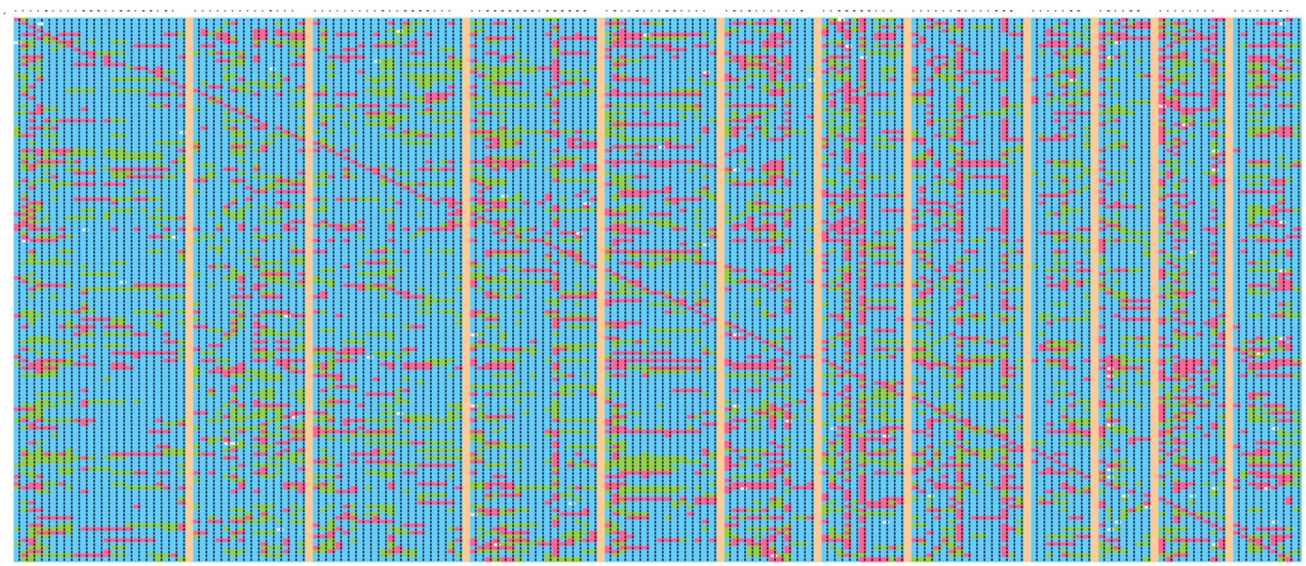

Fig. 4 Graphical genotypes of 145 MTU1010 chromosomal segment substitution lines with *O. rufipogon* segments using 161 SSRs in BC_2F_2. Blue- MTU1010, Pink-*O. rufipogon* segments. Green- heterozygotes. Each column flanked by yellow lines represents a chromosome from 1 to 12 left to right

effect was observed at common QTL *qHI1.2* and *qHI4.1* in BC_2F_2 and BC_2F_4. As per our knowledge this is the first-time phenotyping was conducted in 3 subsequent generations after back crossing for QTL mapping.

Identification of CSSLs in BC_2F_2 MTU1010/O. rufipogon

Genotypic data of 161 polymorphic loci in 238 BC_2F_2 MTU1010/*O. rufipogon* lines was imported to CSSL Finder software to shortlist an optimal CSSL set. A total of 145 CSSLs out of 238 BC_2F_2 were identified with homozygous chromosome segments from *O. rufipogon* substituting MTU1010 segments (Fig. 4). High number of CSSLs (19) were obtained for chromosomes 1 and 3; 15 CSSLs for chromosome 4 and 15 CSSLs for chromosomes 2, 5 and 8. The number of substituted segments in each CSSL ranged from 4 to 36, with an average of 27.5. Complete coverage of overlapping *O. rufipogon* segments was seen on chromosomes 3, 5, 9, 11 and 12. However, small regions on chromosomes 1, 2, 4, 6, 7, 8 and 10 were not represented in the 145 CSSL set.

Discussion

In the present study, we identified a total of 116 QTLs for yield and photosynthesis traits in BC_2F_2, BC_2F_3 and BC_2F_4 generations. It is significant that 31% of the trait-enhancing alleles were from *O. rufipogon*. These 3 generations were phenotyped for at least 9 common traits DFF, PH, TN, PTN, SPY, BM, TDM, HI and TGW. Among these only one QTL *qPH1.1* appeared in each of the three generations; 5 QTLs across BC_2F_2 and BC_2F_3; 3 QTLs across BC_2F_2 and BC_2F_4 and 2 QTLs appeared consistently in BC_2F_3 and BC_2F_4. It was observed that LOD and PV reduced in advanced generations as there was a general reduction in phenotypic variability with exceptions in case of SPY, HI, TDM and BM. This may be due to the reduction of heterozygosity in the advanced selfed generations which in turn led to reduction in phenotypic variability (Balakrishnan et al. 2020).

O. rufipogon allele increased PH in one major effect QTL *qPH1.1* at RM7594-RM3738 which was identified in all three generations. QTL *PH1.1* is located close (~2 Mb) to the well-known semi-dwarf locus *sd-1* gene. Increase in plant height from *O. rufipogon* alleles was also reported previously (Moncada et al. 2001; Thomson et al. 2003; Septiningsih et al. 2003; Marri et al. 2005; Qiao et al. 2016). The same flanking markers were linked to plant height in other mapping populations derived from only cultivated species. In IR64/Koshihikari CSSLs, CRATs (chromosome regions affecting a trait) were detected for plant height at heading at RM7594 region (Ujiie et al. 2016) and *qph1* in RIL population of Nanyangzhan/Chuan7 at RM3738 (Bai et al. 2011), the two markers flanking *qPH1.1* in our study. In mapping populations derived from crosses between *O. rufipogon* IRGC105491 as male and diverse cultivars as female, a total of 26 QTLs for grain weight were detected (Noroozi and Sattari 2015). At least 167 QTLs associated with TGW and about 13 genes associated with grain shape and weight have been isolated by map-based cloning strategies (Huang et al. 2013). In our study, we identified QTL *qTGW3.1* at RM60-RM231 in BC_2F_2 and BC_2F_3 with 15.2% PV in BC_2F_2 and 1.1% PV in BC_2F_3. One QTL *qTGW5.1* at RM1386-RM440 was identified with 1.2% PV only in BC_2F_3. At RM60 locus, *qTGW3.1* was identified previously in SSSL population of HJX74/*O. meridionalis* in two seasons (He et al. 2017), in backcross population derived from cross Milyang 23/*O. glaberrima* (Kang et al. 2013). RM231 locus was reported to be significantly associated with plant paddy weight under stressful and non-stressful conditions (Tabkhkar et al. 2018).

One major QTL for grain weight *OsqGW5.1* was identified using SSR and SNP markers-based QTL-seq analysis and differential expression profiling (Daware et al. 2016). Hu et al. (2018) identified novel QTL *qTGW3.1* in NILs derived from CW23 and PA64, which encodes OsSK41, a member of the GLYCOGEN SYNTHASE KINASE 3/SHAGGY-like family. NILs carrying the loss-of-function allele of OsSK41 increased grain length and weight. *qGW3.1* was fine mapped in the peri-centromeric region on chromosome 3 in F_2 and F_3 generations of BC_4 and BC_5 of Jefferson/*O. rufipogon* IRGC105491 populations (Li et al. 2004). Another QTL *qGW3* was fine mapped between sequence tagged site markers WGW16 and WGW19 in F_2, F_3, BC_2F_2 populations from Baodali/Zhonghua11 (Huang et al. 2013). Major effect QTL *qTGW3.1* identified between RM60- RM231 in our study is located ~ 17 kb from *qGW3.1* mapped earlier (Li et al 2004).

The QTL *qSPY6.1* identified at RM20377-RM340-RM204 showed PV of 6.7%, and increased yield by 1.35 g. Previously, RM204 was tagged for several yield-related QTLs, such as *qpss6.1*, *qgw6*, q*spp6*, *qns6* in meta-QTL *MQTL*6.1 (Swamy and Sarla, 2011) and *qCu6.1*for copper in ILs derived from Teqing/*O. rufipogon* (Garcia-Oliveira et al. 2009). The gene *DEP3*-dense and erect panicle 3, which confers high grain yield in rice is reported in the vicinity of RM340 (Bian et al.2010). Likewise, RM336 flanking *qBM7.1* in our study was previously reported to be associated with plant height, culm length, spikelet number and sheath blight resistance (Marathi et al. 2012; Mohammadi et al. 2013; Yadav et al. 2015; Kim et al. 2017).

Flag leaf photosynthesis contributes more than half of all the carbohydrates in rice seed (Li et al. 1998). More than 200 QTLs for flag leaf traits such as FLL and FLW have been reported in different mapping populations (Jiang et al. 2010; Bian et al. 2014; Zhang et al. 2015; Liu et al. 2015 and Tang et al. 2018). One QTL *qFLL7.1* in BC_2F_2 and two QTLs *qFLL1.1* and *qFLL2.1* in BC_2F_3 were identified for flag leaf

length and trait- increasing alleles were from *O. rufipogon*. 17 QTLs were identified for FLW, of which two were QTLs *qFLW1.1* and *qFLW10.1*. QTL *qFLW1.1* at RM7124 was also reported by Bian et al. (2014) in the Sasanishiki/Habataki CSSL population in three environments and thus appears to be a stable QTL. QTL *qFLW10.1* identified at RM5095-RM216-RM228 showed an average PV of 4.4%. Chen et al. (2012) identified *qFLW10* at the same locus RM5095 with PV 5.3% in RIL population derived from a cross between *javanica* D50 and *indica* HB277. They also fine mapped major QTL *qFLW4* near *NAL1* (Narrow angle leaf 1) gene. In our study, no major effect QTLs were identified for flag leaf traits on chromosome 4.

Two major QTLs *qSPY1.2* at RM3738, *qTDM3.1*at RM3646 – RM3400 were identified in BC_2F_3 and BC_2F_4. *qSPY1.2* at RM3738 was earlier reported to be linked to black-streaked dwarf virus (Zhang et al. 2016) and plant height (Bai et al.2011). RM3400 was reported to be associated with spikelet length and spikelet length width ratio (Deborah et al. 2017) and kilo grain weight (Mao et al. 2011). Three major QTLs *qPH1.2* (RM3738-RM5310), *qHI1.2* (RM7594-RM3738), *qHI4.1* (RM3839) were identified in BC_2F_2 and BC_2F_4. These loci were reported to be linked to flag leaf photosynthesis (Adachi et al.2019); early senescence 4 (*es4*) (Wang et al.2019); chlorophyll content (Zhang et al. 2016) and mesocotyl elongation (Lee et al. 2012). QTLs identified in BC_2F_2 were also detected in BC_2F_4 but not in BC_2F_3. Discrepancy in QTL expression among generations could be attributed to biological and experimental reasons. Interactions between QTL and the genetic background, genotype-by-environment interaction might affect the apparent appearance of a QTL in across generations (Chaib et al. 2006).

Major QTLs *qSPY2.1*, *qPTN9.1, qBM9.1, qSPY9.1* and *qPDP9.1* were identified at RM250 on chromosome 2 and RM242 on chromosome 9. These QTL-dense regions were reported to harbour QTLs *qgw2.1*, *qyld2.1*, *qsnp2.1*, *qsn2.1*, *qgn2.1*, *qgw9.1*, *qyldp9.*1, *qpl9.1* and *qph9.1* in populations derived from *O. rufipogon* (Thomson et al. 2003; Septiningsih et al. 2003; Marri et al. 2005 and Keong et al. 2012). Likewise, RM415 linked with *qTN12.1*, *qTN12.2*, *qPTN12.1* for tiller number was reported as linked to *qFe12.1* for iron and *qPL12.1* or panicle length in different mapping populations (Talukdar et al. 2017; Swamy et al. 2018). RM5479 linked to *qPTN12.2* in BC_2F_2 was also reported for calcium, magnesium and phosphorus accumulation in Teqing/*O. rufipogon* population (Garcia-Oliveira et al. 2009). QTL *qGL12.2* was fine mapped in secondary F_2 and F_3 CSSL41/9311 populations (Qi et al. 2018) and it is interesting that this CSSL was developed from 9311/*O. rufipogon* population (Qi et al.2017).

QTLs for photosynthesis-related traits

Wild species *O. rufipogon* and *O. nivara* are potential sources of enhanced P_N for introgression into cultivated rice (Zhao et al. 2010; Kiran et al. 2013; Kondamudi et al. 2016 and Haritha et al. 2017, 2019). The *O. rufipogon* accession IC309814 with high photosynthesis rate was chosen as a donor in our study. Ten families showed higher P_N than MTU1010 and 2 families V-111and V-109 showed higher P_N than even donor *O. rufipogon* accession. A set of 40 KMR3 x *O. rufipogon* WR120 ILs were characterised for P_N net photosynthetic rate (Haritha et al. 2017). Significant positive association between photosynthetic traits and total dry matter was observed. 37 ILs showed higher P_N than KMR3 and 20 ILs than even *O. rufipogon* the higher P_N parent indicating that ILs with higher P_N than the wild rice can be obtained. Likewise, leaf photosynthetic traits were evaluated in Swarna/*O. nivara* BILs (Haritha et al 2019). Correlation analysis showed P_N was positively correlated with BM, SPY and HI; SPY with CE and WUE; PH with WUE. Significant positive association between photosynthetic traits and yield traits was observed (Haritha et al. 2019; Rao et al. 2018a, b). P_N showed significant association with gs and CE. Previous reports also showed significant correlation of P_N with gs and CE (Zhao et al. 2010; Kiran et al. 2013; Ramchander et al. 2016).

Seven QTLs *qCi6.1, qCi7.1, qCi9.1, qE1.1, qWUE3.1, qWUE6.1* and *qWUE9.1* were identified for photosynthesis-related traits. QTLs for photosynthetic rate (P_N), Intercellular CO_2 partial pressure (Ci), stomatal conductance for CO_2 (gs), transpiration rate (*Tr*) were reported previously in different chromosomal locations and mapping populations (Teng et al. 2004; Takai et al.2010; Adachi et al. 2011; 2014; Gu et al. 2012; Ramchander et al. 2016; Adachi et al. 2019). QTLs for leaf net photosynthetic rate were reported in reciprocal BILs and CSSLs derived from Takanari and Koshihikari (Adachi et al. 2019). In a RIL population of Dongnong 425 and Changbai10, a total of 23 QTLs for seven photosynthesis-related traits were identified in control, salt-alkali stress conditions (Sun et al. 2019). Using chromosome introgression lines of Shennong265/Haogelao, QTLs identified near RM410 on chromosome 9 showed a significant multiple effects on photosynthesis rate, *gs*, *Tr*, quantum yield of PSII, across development stages and treatments (Gu et al. 2012). In our study, QTLs *qCi9.1*, *qWUEi9.1* were identified at RM3912-RM6839 on chromosome 9, while at RM410 a cluster of 8 yield and related QTLs *qBM9.1, qBM9.2, qSPY9.1, qTDM9.1, qHI9.1, qPDP9.1, qPDP9.2* and *qFLW9.1* were detected indicating this locus was a significant region for enhancing yield and photosynthesis together. Likewise, four yield-related QTLs including one common QTL *qPH.1* were

clustered with QTL *qE1.1* for transpiration rate. Co-localisation of QTLs for grain yield and photosynthesis traits were identified in backcross introgression lines of Teqing and Lemont. *qGY10.1* for grain yield and $qP_N10.1$ for flag leaf net photosynthetic rate were identified at RM258-RM228 (Zhao et al. 2008). Co-localization of QTL for yield and photosynthesis-related traits indicates that the identified QTL region may be used in marker-assisted-breeding for simultaneous improvement of multiple yield traits to develop high-yielding rice varieties.

Non parental bands

An interesting observation from this study was 5% of SSRs showed appearance of non-parental novel bands on chromosomes 2, 6, 7, 8, 11 and 12. This occurrence of non-parental bands could be due to genomic interactions of cultivated and wild species or an activation of some transposable elements producing novel bands, genome re-patterning, and alteration in DNA methylation patterns (Aggarwal et al. 1997; Brar and Khush 2002; Wang et al. 2005). Non-parental bands were also detected in DH populations of *O. sativa*/*O. glaberrima*, introgression lines of *O. sativa*/*O. brachyantha* and *O. sativa*/*O. granulata* (Brar and Khush 2002; Aggarwal et al. 1997). Hashemi et al. (2009) reported appearance of non-parental bands in DNA profiling of rice hybrids and their parental lines and addressed that it might probably correspond to heteroduplex molecules formed by two allelic sequences. Similar observations were also reported in DH population of *Brassica juncea*, where 2.8% of SSRs showed non-parental novel bands and were termed as 'locus-specific variation'. These variations were predicted due to inherent instability of SSR and stress conditions faced during microspore culture (Dhaka et al. 2017). The SSR regions showing non-parental bands in the study were analysed *in-silico* and revealed their association with nuclear transcription factor proteins, homeo-box proteins and RNA polymerase sigma regions.

CSSLs

A set of 145 CSSLs with substituted chromosomal segments from *O. rufipogon* in the genetic background of MTU1010 were identified. 87% of the entire genome of the donor parent *O. rufipogon* was represented in overlapping chromosome segments in the CSSL set. In earlier reports, the genome coverage of different accessions of *O. rufipogon* ranged from 68 to 99% (Tian et al. 2006; Tan et al. 2007; Furuta et al. 2014; Arbelaez et al. 2015; Qiao et al. 2016; Qin et al. 2018). In addition, CSSLs derived from wild species *O. nivara*, *O. longistaminata*, *O. meridionalis*, *O. glumepatula*, and *O. glaberrima* have been constructed and introgressions of donor genome of these wild species ranged from 55 to 90% (Shim et al. 2010; Arbelaez et al. 2015; Ramos et al. 2016; Ma et al. 2016; He et al. 2017; Malathi et al. 2017). The uncovered/missing segments in this CSSL set might be due to factors, such as gametophytic genes, hybrid sterility, genetic divergence, and photosensitivity (Qiao et al. 2016). In the development of CSSLs, the number of polymorphic SSRs covering the entire genome plays a vital role. Higher the marker density, lower the number of introgression segments lost. 33 CSSLs were developed covering 90% of *O. rufipogon* W0106 in the background of Koshihikari (Furuta et al. 2014). Qiao et al. 2016 developed 198 CSSLs (BC_3 to BC_7) with 85% *O. rufipogon* introgression in the background of 9311, using 313 polymorphic SSRs. Subudhi et al. (2015) developed 74 CSSLs covering 99% of the weedy rice donor PSRR-1 genome in the background of cultivar Bengal. 77 lines were developed with 93.59% coverage of LSWR (*O. rufipogon*) in the background of 9311 (Qin et al. 2018). It is interesting to note that 39 of 145 CSSLs were significantly different than recurrent parent MTU1010 for 7 traits (PH, TN, PTN, TGW, BM, TDM and HI) in BC_2F_2, BC_2F_3 and BC_2F_4 in the positive or negative direction. These significantly different lines are very useful for fine mapping and gene discovery of the respective traits. In a parallel study, BC_2F_1 BILs were further backcrossed till BC_4F_2 to further genotype and develop CSSLs and now in BC_4F_5 and evaluation of these ILs is currently underway to identify novel alleles from *O. rufipogon*.

Conclusion

QTL mapping of MTU1010/*O. rufipogon* BC_2F_2, BC_2F_3 and BC_2F_4 populations resulted in identification of 49, 34 and 26 yield-related QTLs respectively and a total of 7 QTLs for photosynthesis-related traits. 31% of the trait-increasing QTL alleles were derived from *O. rufipogon*. Major effect QTLs *qTGW3.1* and novel QTL *qBM7.1* can be further validated and fine mapped. A set of 145 CSSLs, including 75 lines which were significantly different from MTU1010 were identified for at least one trait and these CSSLs can be used as genetic sources to map respective traits for marker assisted transfer of newer alleles. Additionally, all CSSLs with known substituted segments from *O. rufipogon* can also be screened for other useful traits, such as resistance to biotic and abiotic stresses.

Author contribution statement SN conceptualized, designed and supervised experiments along with DB and SV. VY performed the experiments. KA, MS, SM, KB and SD

supported conducting the field experiments. DB and SD analysed the data. VY wrote the manuscript with contributions from DB and MS. SN and DB contributed to the final revision of the manuscript. Funding acquisition and Resources is by SN and DB.

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Data availability All data generated or analysed during this study are included in this published article and its supplementary information flies.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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