

Supplementary Materials for

Genome-Wide Association Study for Leaf Photosynthesis Using High-Throughput Gas Exchange System in Rice

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Table S1 Two-way ANOVA for the effect of variety, year, and their interaction. ***, significant ($P < 0.001$); n.s., not significant ($P \geq 0.05$)

Factor	Net CO ₂ assimilation rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		Leaf width (cm)	
	F	P	F	P
Variety	3.16	< 0.001***	36.64	< 0.001***
Year	381.76	< 0.001***	2501.97	< 0.001***
Interaction	0.94	0.760 ^{n.s.}	2.95	< 0.001***

Table S2 Detected peaks by GWAS of photosynthesis-related traits. Physical positions and $-\log_{10}(P)$ values of the leading SNPs in each QTL region are shown.

Target trait	QTL name	Year	Chr.	Position (bp)	$-\log_{10}(P)$
Net CO ₂ assimilation rate	<i>qCA1-1</i>	2018	1	23,525,820	5.24
		2019	1	22,352,813	4.57
	<i>qCA1-2</i>	2019	1	34,473,669	4.68
		GLM	1	34,709,284	4.82
	<i>qCA3</i>	2018	3	23,173,905	5.31
	<i>qCA4</i>	2018	4	6,766,390	4.14
		2019	4	6,969,945	4.19
		2020	4	6,947,055	4.79
		GLM	4	6,960,038	5.65
	<i>qCA6</i>	2019	6	2,386,833	4.97
		GLM	6	2,386,833	4.54
	<i>qCA7</i>	2019	7	6,433,570	4.71
	<i>qCA8</i>	2018	8	21,691,767	5.68
		2020	8	27,430,783	4.61
		GLM	8	26,172,877	4.84
	<i>qCA11-1</i>	2020	11	24,988,806	4.34
	<i>qCA11-2</i>	2020	11	27,113,457	4.77

Continued

Table S2 Detected peaks by GWAS of photosynthesis-related traits. Physical positions and $-\log_{10}(P)$ values of the leading SNPs in each QTL region are shown.

Target trait	QTL name	Year	Chr.	Position (bp)	$-\log_{10}(P)$
Leaf width	<i>qLW1</i>	2020	1	9,843,577	4.40
		GLM	1	10,043,653	4.67
	<i>qLW2</i>	2018	2	26,011,764	4.60
	<i>qLW3</i>	2019	3	34,149,588	5.04
	<i>qLW4-1</i>	2018	4	30,970,501	5.06
		2019	4	31,232,811	9.12
		2020	4	31,224,809	7.23
	<i>qLW4-2</i>	GLM	4	31,225,520	5.13
		2019	4	35,104,300	5.62
		2020	4	35,104,300	7.06
	<i>qLW5</i>	GLM	4	35,104,300	6.16
		2018	5	29,489,856	4.10
		2019	5	29,489,856	4.67
	<i>qLW6</i>	2020	5	29,567,728	4.54
		GLM	5	29,567,728	5.07
		2020	6	24,234,595	4.53
	<i>qLW8</i>	GLM	6	24,235,585	5.10
		2018	8	17,969,746	6.45
		2019	8	17,969,746	4.24
	<i>qLW10</i>	2020	8	17,969,746	5.54
		GLM	8	17,969,746	5.03
		2018	10	14,422,270	4.47
	<i>qLW12</i>	2020	10	14,422,270	6.21
		GLM	10	14,313,502	6.96
		2018	12	24,435,872	4.29
		2019	12	24,733,440	4.49

Continued

Table S2 Detected peaks by GWAS of photosynthesis-related traits. Physical positions and $-\log_{10}(P)$ values of the leading SNPs in each QTL region are shown.

Target trait	QTL name	Year	Chr.	Position (bp)	$-\log_{10}(P)$
Leaf area	<i>qLA3</i>	2020	3	27,536,074	5.22
	<i>qLA4-1</i>	2020	4	31,232,808	5.73
	<i>qLA4-2</i>	2020	4	35,137,572	6.87
	<i>qLA6</i>	2020	6	23,992,379	5.08
	<i>qLA7</i>	2020	7	3,874,854	4.40
	<i>qLA8-1</i>	2020	8	3,152,051	4.22
	<i>qLA8-2</i>	2020	8	28,246,508	4.79
	<i>qLA9</i>	2020	9	15,043,392	5.67
	<i>qLA11</i>	2020	11	25,216,824	4.48
Leaf mass per area	<i>qLMA4</i>	2020	4	30,775,442	5.39
SPAD value	<i>qSPAD1</i>	2020	1	4,899,870	4.50
	<i>qSPAD2</i>	2020	2	2,027,122	5.06
	<i>qSPAD4</i>	2020	4	31,381,928	4.44



Fig. S1 Scatter plots for the pair of the observed traits: net CO₂ assimilation rate (A), leaf width (LW), leaf area (LA), leaf mass per area (LMA), and SPAD value measured in 2018, 2019, and 2020. Black points indicate mean values in each variety (168 varieties with 6 biological replicates) and red lines indicate the linear regression lines. Pearson's correlation coefficients and significances are shown in Fig. 1

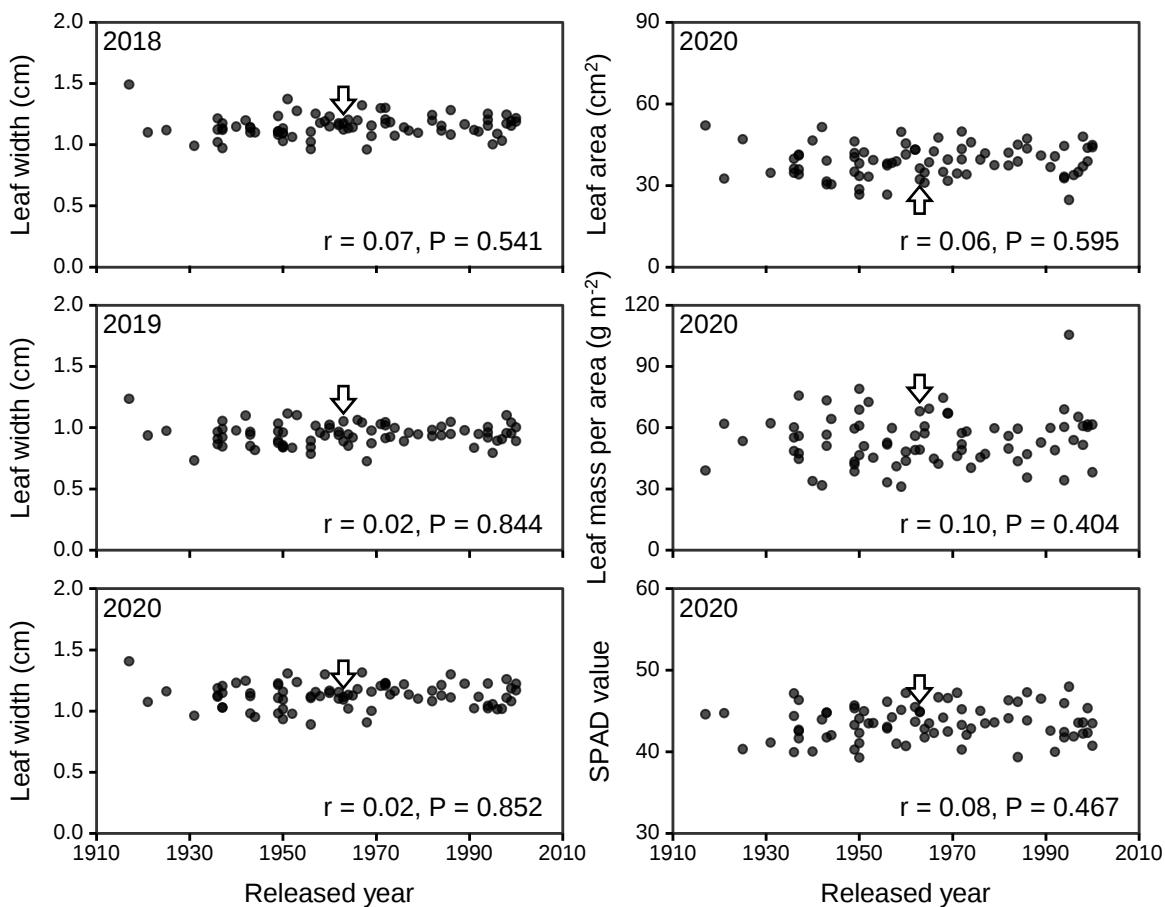


Fig. S2 Relationships between leaf width, leaf area, leaf mass per area, and SPAD value and the released year among the modern varieties. The numbers of upper left in each box indicate experimental year. Open arrows indicate the data of 'Nipponbare', a reference variety. r , Pearson's correlation coefficient

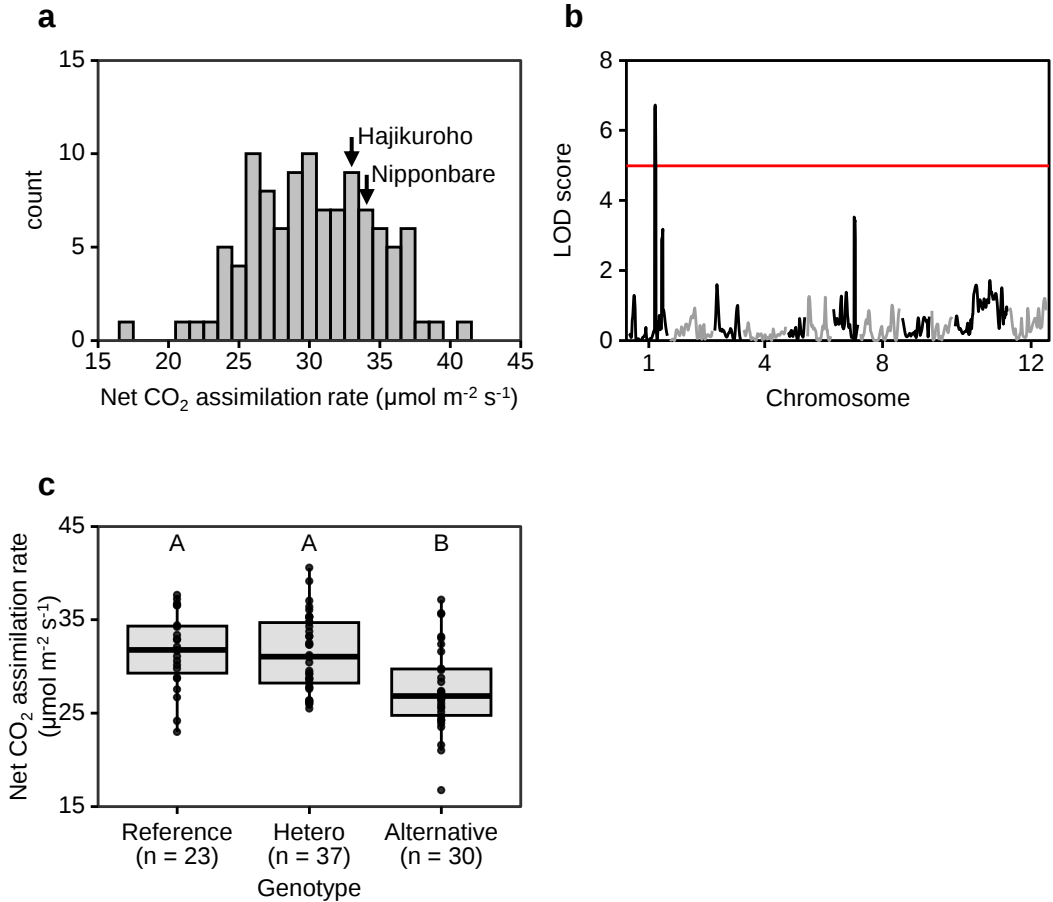


Fig. S3 Summary of the bi-parental QTL analysis. (a) Histogram of net CO₂ assimilation rate in the F2 population derived from ‘Nipponbare’ and ‘Hajikuroho’. Arrows indicate the bins including parental varieties. (b) Logarithm of the odds (LOD) score. Red line indicates the significance level of $P = 0.05$. (c) Boxplot of net CO₂ assimilation rate to compare the difference between the genotype of the significant peak. Different character means significant differences ($P = 0.05$, Turkey-Kramer multiple test). Boxplots: central line, median; boxes, interquartile range (IQR); whiskers, $1.5 \times \text{IQR}$; points, actual data

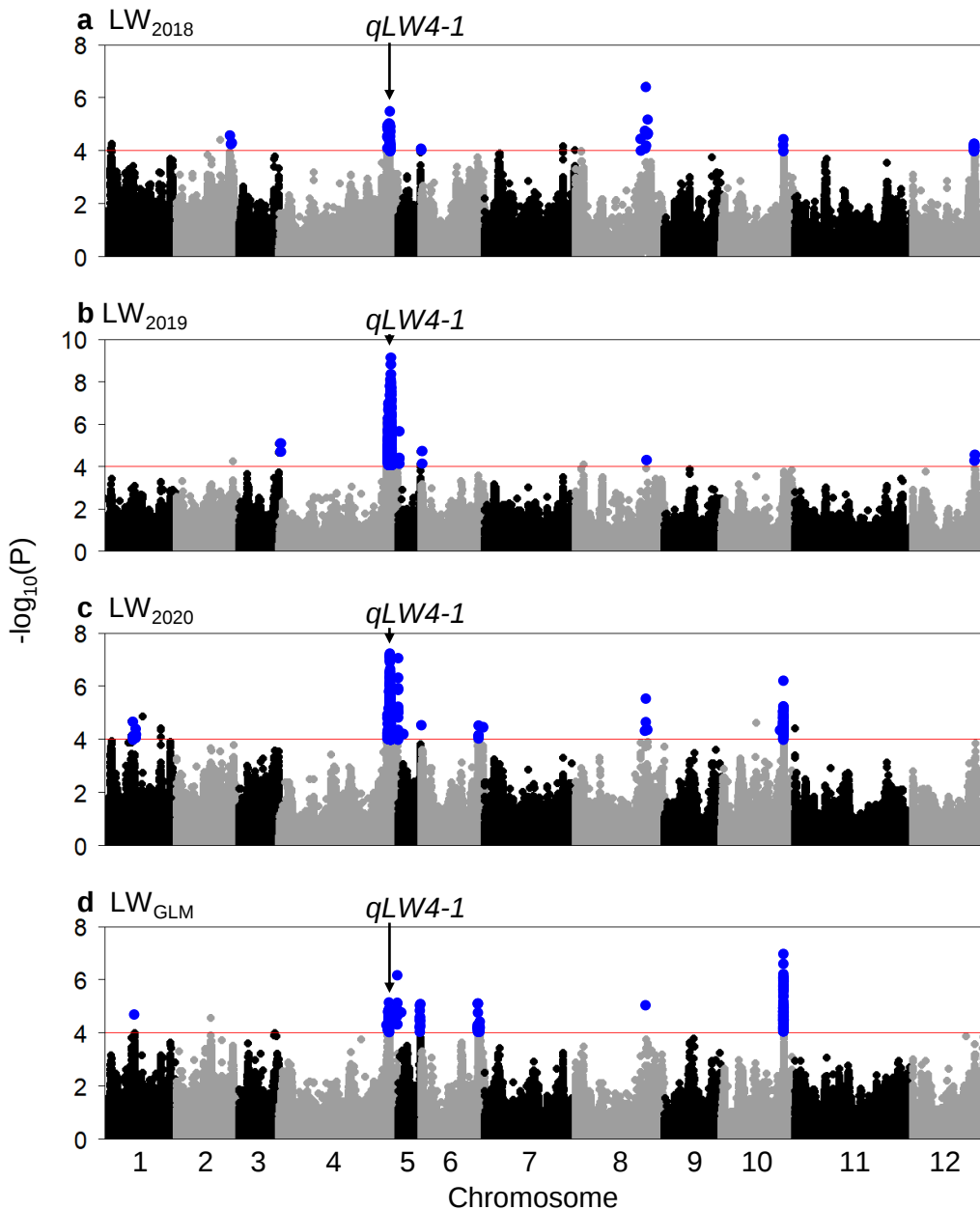


Fig. S4 Manhattan plots for GWAS of leaf width (a-d), leaf area (e), leaf mass per area (f), and SPAD value (g) in 2018 (a), 2019 (b), 2020 (c, e-g), and genetic score in the generalized linear regression model (d, see *materials and methods*). Red line indicates the threshold level ($-\log_{10}(P) = 4$). Blue points indicate the identified QTLs. $qLW4-1$, $qLA4-1$, $qLMA4$, and $qSPAD4$ are repeatedly identified QTLs harboring *NAL1* gene. All detected peaks are listed in Table S2

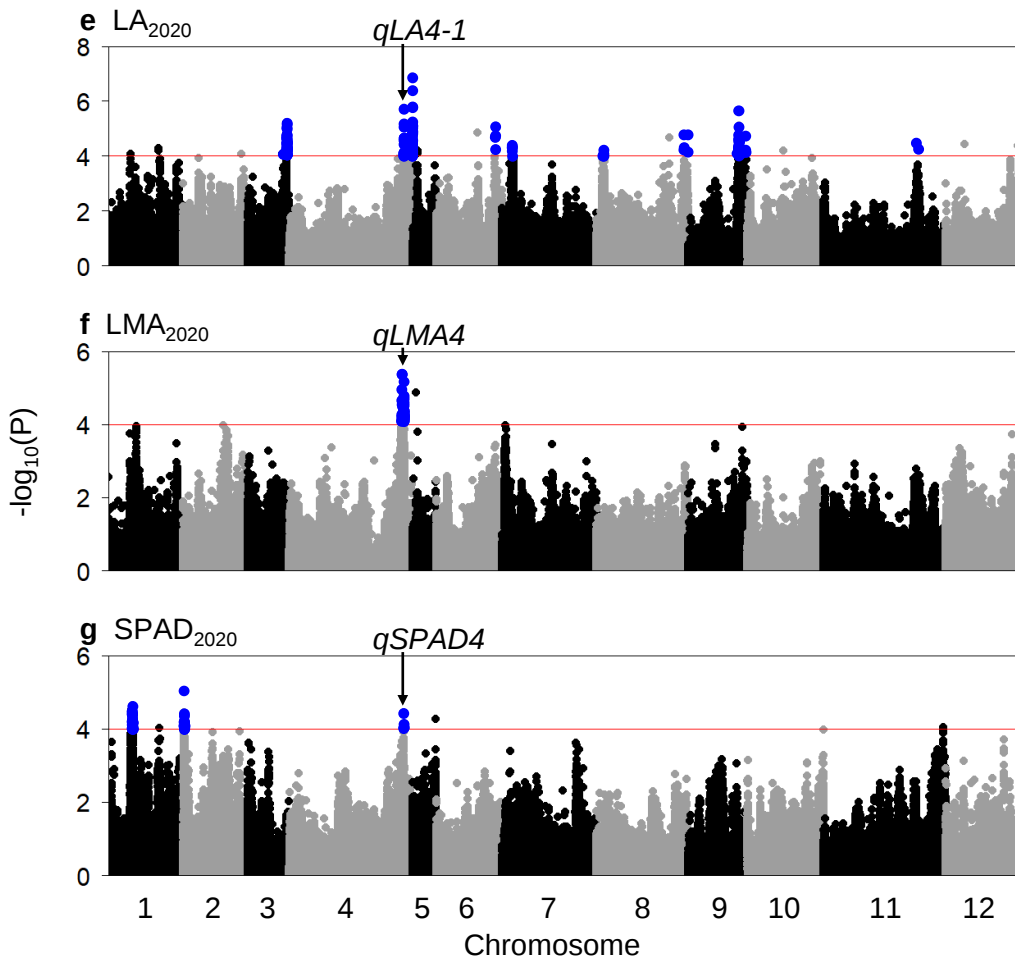


Fig. S4 Manhattan plots for GWAS of leaf width (a-d), leaf area (e), leaf mass per area (f), and SPAD value (g) in 2018 (a), 2019 (b), 2020 (c, e-g), and genetic component of the generalized linear regression model (d, see *materials and methods*). Red line indicates the threshold level ($-\log_{10}(P) = 4$). Blue points indicate the identified QTLs. *qLW4-1*, *qLA4-1*, *qLMA4*, and *qSPAD4* are repeatedly identified QTLs harboring *NAL1* gene. All detected peaks are listed in Supplementary Table S2