

Supplemental materials for Biofertilizer with *Bacillus pumilus* TUAT1 spores improves growth, productivity, and lodging resistance in forage rice.

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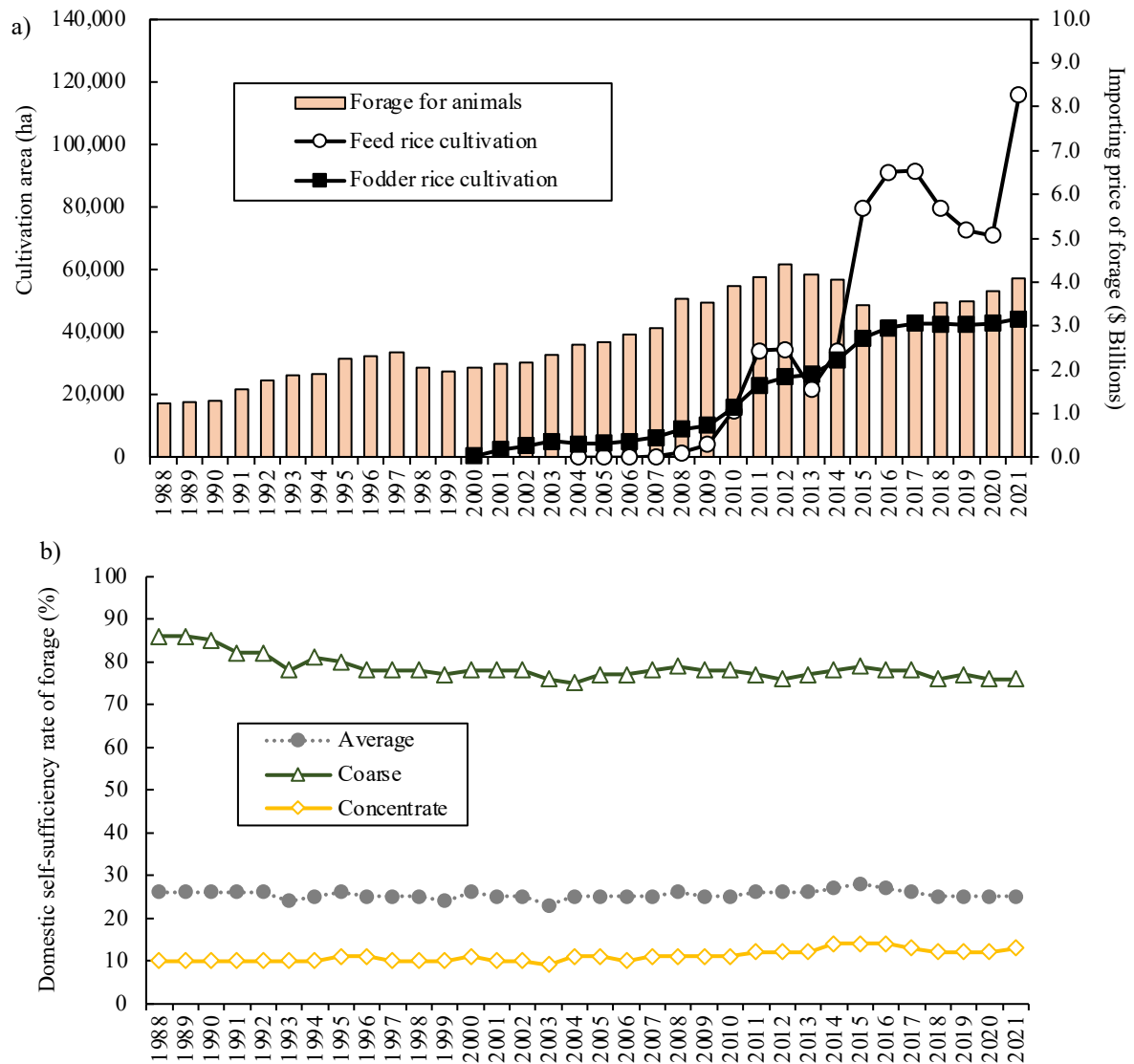


Figure S1. Circumstances surrounding animal forage in Japan. a) Area of the forage rice cultivation (feeds rice and fodder rice) according to the reports of the Ministry of Agriculture, Forestry and Fishers (MAFF), and the importing price of animal forage according to the Trade Statistics of Japan. The prices were converted to USD by the calendar based yearly average exchange rates reported by BOJ Time-Series Data Search. b) Domestic self-sufficiency rate of forage reported by MAFF.

Table S1. Analyzed soil characters of the paddy field.

Categories	Unit	Ave.	±	SE
pH (H ₂ O)	-	6.3	±	0.0
Cation exchange capacity (CEC)	cmol _c kg ⁻¹	13	±	0.3
NH ₃ -N	mg kg ⁻¹	13	±	0.5
Available N	mg kg ⁻¹	73	±	1.5
Absorption coefficient of P	-	683	±	14.4
Total P (P ₂ O ₅)	mg kg ⁻¹	370	±	14.4
Exchangeable K	mg kg ⁻¹	320	±	21.8
Exchangeable Ca	mg kg ⁻¹	273	±	27.2
Exchangeable Mg	mg kg ⁻¹	530	±	17.8
Humus	%	4.0	±	0.1
Available Si	mg kg ⁻¹	160	±	2.72
Free iron oxides	%	1.1	±	0.1
Ca/Mg	-	3.7	±	0.0
Mg/K	-	3.9	±	0.2
P ₂ O ₅ /Mg	-	0.7	±	0.0
Lime saturation factor (LSF)	%	71	±	2.6
Base saturation (BS)	%	94	±	2.6

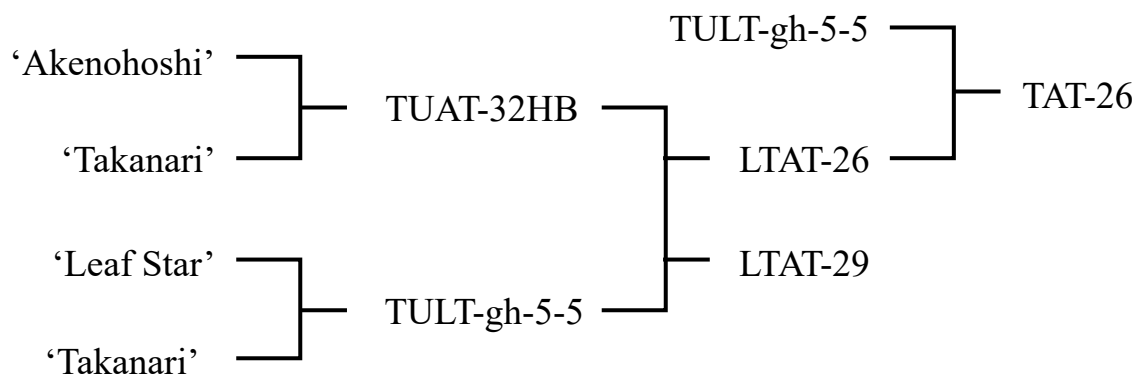


Figure S2. Pedigree chart of the analyzed new forage rice lines (LTAT-29 and TAT-26)

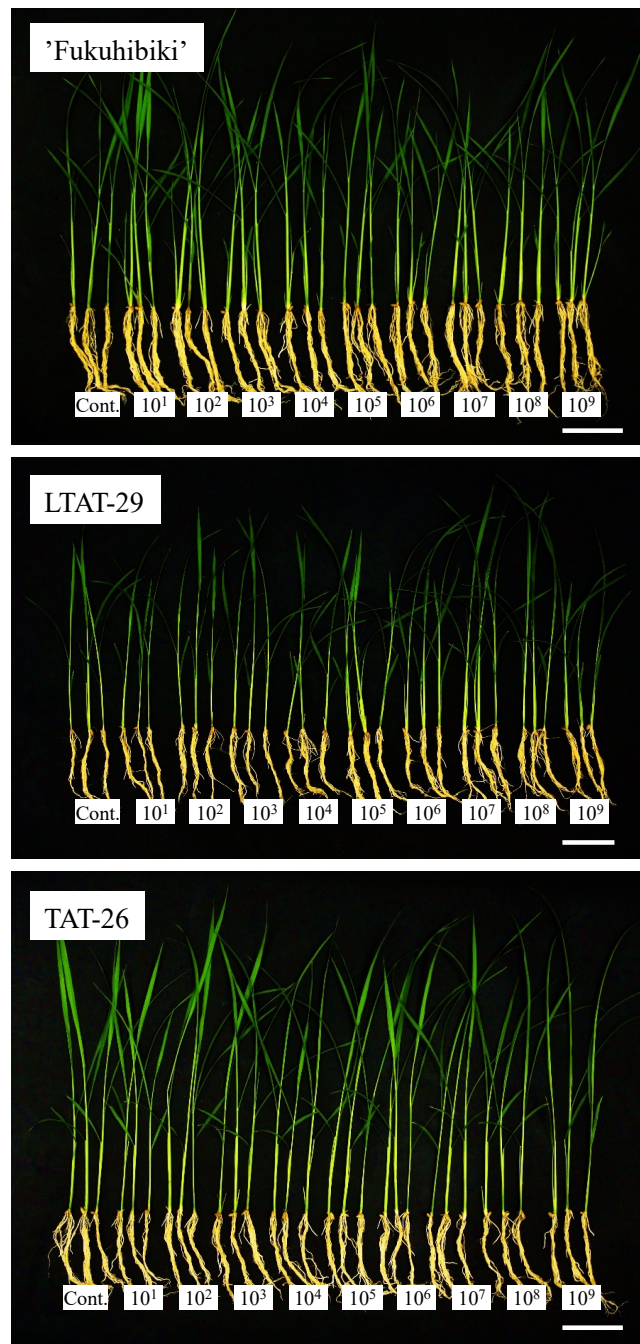


Figure S3. Seedlings on the plant box.

Cont.: Control (non-inoculated).

Table S2. Results for ANOVA on the resuspended culture inoculation test (n=3).

ANOVA (<i>p</i> value)	Shoot	Root
Cultivar	0.066	0.005
Concentration	0.155	0.000
Cultivar * Concentration	0.050	0.243

Table S3. Results for Dunnett's test (two-sided) on the resuspended culture inoculation test (n=3).

Concentration (<i>p</i> value)	Shoot	Root
10 ¹ CFU ml ⁻¹	0.472	1.000
10 ² CFU ml ⁻¹	0.774	0.929
10 ³ CFU ml ⁻¹	0.999	0.725
10 ⁴ CFU ml ⁻¹	0.970	0.987
10 ⁵ CFU ml ⁻¹	0.955	0.037
10 ⁶ CFU ml ⁻¹	0.961	0.862
10 ⁷ CFU ml ⁻¹	0.967	0.000
10 ⁸ CFU ml ⁻¹	0.953	0.616
10 ⁹ CFU ml ⁻¹	1.000	0.671

Table S4. Summary of the seedlings inoculated with Kikuichi / Yume-bio on the nursery box at transplanting stage.

Variety	Application	Number of leaves	Shoot length (cm)	Fresh weight (mg)		RSWL (mg cm ⁻¹)
				Root	Shoot	
'Fukuhibiki'	Control	3.3 ± 0.1 **	12.7 ± 0.5	61.5 ± 7.5 *	74.4 ± 6.3	5.82 ± 0.28
	BF	3.0 ± 0.0	18.5 ± 1.0 **	49.7 ± 2.4	105.1 ± 11.2 *	5.66 ± 0.31
	(BF/Control)	(0.90)	(1.45)	(0.81)	(1.41)	(0.97)
LTAT-29	Control	2.5 ± 0.2 *	13.7 ± 0.2	66.5 ± 9.7	78.9 ± 6.5	5.75 ± 0.39 *
	BF	2.1 ± 0.1	18.8 ± 1.7 *	65.7 ± 4.9	92.5 ± 5.4 *	4.94 ± 0.18
	(BF/Control)	(0.81)	(1.37)	(0.99)	(1.17)	(0.86)
TAT-26	Control	2.6 ± 0.1 **	14.6 ± 0.8	77.4 ± 9.4	81.8 ± 7.5	5.58 ± 0.27 **
	BF	2.0 ± 0.1	20.5 ± 0.6 **	71.7 ± 10.1	91.0 ± 9.0	4.42 ± 0.30
	(BF/Control)	(0.79)	(1.40)	(0.93)	(1.11)	(0.79)

'Fukuhibiki' was harvested at 21 days after sowing. LTAT-29 and TAT-26 were harvested at 14 days after sowing. BF: Biofertilizer, RSWL: Ratio of shoot fresh weight to the shoot length. * and ** indicate significant differences at $p \leq 0.05$ and 0.01 levels between control and biofertilizer application in each treatment, respectively. Standard deviation (SD) was shown (*t*-test, two-sided). n = 3.

Table S5. Colonized numbers of *B. pumilus* TUAT1 at basement of the root at the transplanting period.

Number of Bacteria (CFU g ⁻¹)	'Fukuhibiki'	LTAT-29	TAT-26
Total	5.40×10^5	3.10×10^5	1.70×10^5
Spores	2.80×10^4	1.10×10^4	4.60×10^4
Vegetative cells	5.12×10^5	2.99×10^5	1.24×10^5

CFU: Colony forming unit.

Table S6. Summary plant growth surveyed after 8 weeks transplanting.

Variety	Distance	Fertilization	TN (m ⁻²)			Height (cm)			SPAD		
			Control	BF	(BF/Control)	Control	BF	(BF/Control)	Control	BF	(BF/Control)
'Fukuhibiki'	15 × 30	N2	259.7	301.9	(1.16)	56.9	62.0	(1.09)	40.1	42.3	(1.06)
'Fukuhibiki'	15 × 30	N4	261.2	324.1 *	(1.24)	59.0	59.2	(1.00)	42.7	42.5	(1.00)
'Fukuhibiki'	30 × 30	N2	181.7	197.2	(1.09)	60.3	61.9	(1.03)	42.0	42.3	(1.01)
'Fukuhibiki'	30 × 30	N4	193.1	173.9	(0.90)	62.9 *	57.4	(0.91)	42.4	42.0	(0.99)
LTAT-29	15 × 30	N2	196.8	252.3	(1.28)	63.9	67.0	(1.05)	40.1	42.2	(1.05)
LTAT-29	15 × 30	N4	241.2	219.0	(0.91)	65.0	64.2	(0.99)	41.4	43.9	(1.06)
LTAT-29	30 × 30	N2	138.0	139.5	(1.01)	63.0	68.9	(1.09)	40.0	43.2 *	(1.08)
LTAT-29	30 × 30	N4	133.6	105.1	(0.79)	64.1	62.0	(0.97)	43.1	44.1	(1.02)
TAT-26	15 × 30	N2	136.9	125.8	(0.92)	75.0	82.2	(1.10)	45.3	44.5	(0.98)
TAT-26	15 × 30	N4	145.0	142.8	(0.98)	72.5	75.6	(1.04)	46.3	46.7	(1.01)
TAT-26	30 × 30	N2	97.7	96.6	(0.99)	78.7	81.1	(1.03)	47.3	46.5	(0.98)
TAT-26	30 × 30	N4	93.2	99.2	(1.06)	77.1	73.6	(0.95)	46.7	47.7	(1.02)
ANOVA (<i>p</i> value)											
Biofertilizer			n.s.			n.s.			0.008		
Variety			< 0.000			< 0.000			< 0.000		
Distance			< 0.000			n.s.			0.024		
Fertilization			n.s.			0.002			0.001		
Biofertilizer * Variety			0.027			n.s.			0.019		
Biofertilizer * Distance			0.006			0.028			n.s.		
Biofertilizer * Fertilization			0.049			< 0.000			n.s.		

TN: Tillers number. BF: Biofertilizer. n.s.: No significance. * and ** indicate significant differences at $p \leq 0.05$ and 0.01 levels between control and biofertilizer application in each treatment, respectively (t-test, two-sided). n = 3.

Table S7. Summary plant growth surveyed after 13 weeks transplanting.

Variety	Distance	Fertilization	TN (m ⁻²)			Height (cm)			SPAD		
			Control	BF	(BF/Control)	Control	BF	(BF/Control)	Control	BF	(BF/Control)
'Fukuhibiki'	15 × 30	N2	244.2	290.1	(1.19)	90.1	94.7	(1.05)	34.4	35.3	(1.02)
'Fukuhibiki'	15 × 30	N4	250.9	304.1 **	(1.21)	94.1	96.5 *	(1.03)	36.7	38.3	(1.04)
'Fukuhibiki'	30 × 30	N2	176.9	190.9	(1.08)	94.1	97.2 *	(1.03)	39.0	42.1 **	(1.08)
'Fukuhibiki'	30 × 30	N4	196.5	196.5	(1.00)	98.3 *	93.5	(0.95)	41.6	42.6 *	(1.02)
LTAT-29	15 × 30	N2	177.6	216.8 *	(1.22)	96.3	102.5 *	(1.06)	30.1	32.0 **	(1.06)
LTAT-29	15 × 30	N4	216.8	239.0	(1.10)	99.2	98.7	(1.00)	32.1	36.1 *	(1.13)
LTAT-29	30 × 30	N2	145.0	154.7	(1.07)	99.1	101.6	(1.02)	33.5	34.2	(1.02)
LTAT-29	30 × 30	N4	162.1	177.2	(1.09)	98.5	97.3	(0.99)	34.5	39.4 **	(1.14)
TAT-26	15 × 30	N2	117.7	127.3	(1.08)	124.5	127.6	(1.03)	38.5	39.2	(1.02)
TAT-26	15 × 30	N4	131.0	133.9	(1.02)	122.9	125.2	(1.02)	41.3	39.4	(0.95)
TAT-26	30 × 30	N2	91.0	101.0	(1.11)	130.2	130.5	(1.00)	42.8	42.1	(0.98)
TAT-26	30 × 30	N4	106.9	103.6	(0.97)	127.3	124.9	(0.98)	46.2	44.1	(0.95)
ANOVA (<i>p</i> value)											
Biofertilizer			< 0.000			0.024			0.003		
Variety			< 0.000			< 0.000			< 0.000		
Distance			< 0.000			0.004			< 0.000		
Fertilization			< 0.000			n.s.			< 0.000		
Biofertilizer * Variety			n.s.			n.s.			< 0.000		
Biofertilizer * Distance			0.010			0.003			n.s.		
Biofertilizer * Fertilization			n.s.			0.001			n.s.		

TN: Tillers number. BF: Biofertilizer. n.s.: No significance. * and ** indicate significant differences at $p \leq 0.05$ and 0.01 levels between control and biofertilizer application in each treatment, respectively (t-test, two-sided). n = 3.

Table S8. Summary of the slope of the approximation straight lines, regression analysis (R^2) and Pearson correlation test (two-sided) for yield components of ‘Fukuhibiki’ and LTAT-29.

	NSP		PRR		WBR		TPN		GBRY	
	F	L	F	L	F	L	F	L	F	L
Slope	0.11	1.13	0.21	1.51	-0.89	2.36	1.59	0.93	1.12	1.75
R^2	0.03	0.91	0.03	0.97	0.24	0.66	0.93	0.85	0.70	0.73
Pearson (P value)	0.83	0.04	0.84	0.01	0.51	0.19	0.03	0.08	0.16	0.15

NSP: Number of spikelets in a panicle, PRR: Percentage of ripening rice seeds to total numbers, WBR: weights of one thousand gross brown rice, TPN: Total panicle numbers in a square meter, GBRY: Gross brown rice yield in 100 square meters. F: ‘Fukuhibiki’, L: LTAT-29.

Table S9. The quality as WCS, line TAT-26.

Distance	Fertilization	Biofertilizer	CP	CFA	CFI	CA	NFE	NFC	ADF	NDF	OCC	OCW	Oa	Ob
15 × 30	N2	Control	51.8	16.4 *	229	125	578	366	275	456	500	500 *	83.6	417
15 × 30	N2	BF	50.8	14.3	222	120	593	388	276	443	522 *	478	75.8	402
		(BF/Control)	(0.98)	(0.88)	(0.97)	(0.96)	(1.03)	(1.06)	(1.00)	(0.97)	(1.04)	(0.96)	(0.90)	(0.96)
15 × 30	N4	Control	58.7	15.3 **	250	120	557	314	311	509 *	464	536 *	105 *	431
15 × 30	N4	BF	59.9	13.7	243	125 *	558	325	303	492	488 *	512	84.0	428
		(BF/Control)	(1.02)	(0.93)	(0.97)	(1.04)	(1.00)	(1.04)	(0.97)	(0.97)	(1.05)	(0.96)	(0.80)	(0.99)
30 × 30	N2	Control	49.9	12.7	238	122	577	356	299	475	491	509	81.1	428
30 × 30	N2	BF	45.5	13.3	240	120	581	358	291	478	491	509	77.1	432
		(BF/Control)	(0.92)	(1.00)	(1.01)	(0.98)	(1.01)	(1.01)	(0.973)	(1.01)	(1.00)	(1.00)	(0.92)	(1.01)
30 × 30	N4	Control	61.9	13.7	248	131	545	315	308 *	495	480	520	87.6	432
30 × 30	N4	BF	61.2	14.0	248	146	531	297	301	498	478	522	86.6	435
		(BF/Control)	(0.98)	(1.00)	(1.00)	(1.11)	(0.97)	(0.94)	(0.98)	(1.01)	(1.00)	(1.00)	(0.99)	(1.01)
ANOVA (<i>p</i> value)														
Biofertilizer			n.s.	0.050	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.046	n.s.
Distance			n.s.	0.001	0.016	n.s.	0.006	0.009	n.s.	0.042	n.s.	n.s.	n.s.	n.s.
Fertilization			< 0.000	n.s.	< 0.000	0.037	< 0.000	< 0.000	< 0.000	< 0.000	0.001	0.001	0.010	n.s.
Biofertilizer * Distance			n.s.	0.004	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.040	0.040	n.s.	n.s.
Biofertilizer * Fertilization			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

The unit of each value is milligram per gram (mg g⁻¹). CP: Crude protein, CFA: Crude fat, CFI: Crude fiber, CA: Crude ash, NFE: Nitrogen free extract, NFC: Non-fiber carbohydrate, ADF: Acid detergent fiber, NDF: Neutral detergent fiber, OCC: Organic cellular content, OCW: Organic cell wall, Oa: Organic a fraction in OCW (hydrolyzed rapidly by cellulase), Ob: Organic b fraction in OCW (resistant to cellulase digestion). BF: Biofertilizer. n.s.: No significance. * and ** indicate significant differences at $p \leq 0.05$ and 0.01 levels between control and biofertilizer application in each treatment, respectively (t-test, two-sided). n = 3.

Table S10. Summary of effects of BF inoculation on the lodging factors of rice.

Variety	Distance	Fertilization	Biofertilizer	Plant height (cm)	Shoot FW (kg)	Culm number (hill ⁻¹)	Pushing resistance (N hill ⁻¹)	Pushing resistance (N culm ⁻¹)	Bending moment (kg · cm)	Lodging index (%)
'Fukuhibiki'	15 × 30	N2	Control	90.5	0.175	14 *	8.68	0.60	15.9	133.6 *
'Fukuhibiki'	15 × 30	N2	BF	96.6 **	0.169	12	10.46	0.89 **	16.3	103.2
			(BF/Control)	(1.07)	(0.97)	(0.83)	(1.21)	(1.49)	(1.03)	(0.77)
'Fukuhibiki'	15 × 30	N4	Control	90.2	0.171	12	9.61	0.80	15.4	105.0
'Fukuhibiki'	15 × 30	N4	BF	92.8	0.162	14 **	10.17	0.74	15.1	102.7
			(BF/Control)	(1.03)	(0.947)	(1.16)	(1.06)	(0.92)	(0.98)	(0.98)
'Fukuhibiki'	30 × 30	N2	Control	93.3	0.253	18	13.64	0.76	23.6	114.9
'Fukuhibiki'	30 × 30	N2	BF	101.5 **	0.337 **	20	18.94 **	0.94 **	34.2 **	122.1
			(BF/Control)	(1.09)	(1.33)	(1.13)	(1.39)	(1.23)	(1.45)	(1.06)
'Fukuhibiki'	30 × 30	N4	Control	104.6 *	0.207	19	16.39	0.86	21.7	86.4
'Fukuhibiki'	30 × 30	N4	BF	93.8	0.228	17	15.71	0.92	21.4	98.7
			(BF/Control)	(0.90)	(1.10)	(0.91)	(0.96)	(1.07)	(0.99)	(1.14)
LTAT-29	15 × 30	N2	Control	132.7	0.146	6	9.22	1.54	19.4	140.8
LTAT-29	15 × 30	N2	BF	143.7 **	0.194 **	7 *	10.61	1.48	28.0 **	183.5 *
			(BF/Control)	(1.08)	(1.33)	(1.21)	(1.15)	(0.96)	(1.45)	(1.30)
LTAT-29	15 × 30	N4	Control	142.8	0.179	8	7.78	0.98	25.5	226.7 **
LTAT-29	15 × 30	N4	BF	146.2 *	0.187	9	12.38 **	1.39 **	27.3	148.6
			(BF/Control)	(1.02)	(1.04)	(1.13)	(1.59)	(1.42)	(1.07)	(0.66)
LTAT-29	30 × 30	N2	Control	147.2	0.287	12	19.32	1.69 **	42.3	145.6
LTAT-29	30 × 30	N2	BF	150.9 *	0.330	14	19.42	1.37	50.2	178.6 **
			(BF/Control)	(1.03)	(1.15)	(1.20)	(1.01)	(0.81)	(1.19)	(1.23)
LTAT-29	30 × 30	N4	Control	150.2 **	0.269	12	14.87	1.26	40.4	182.8 **
LTAT-29	30 × 30	N4	BF	143.5	0.284	13	20.66 **	1.56 **	40.7	132.5
			(BF/Control)	(0.96)	(1.06)	(1.12)	(1.39)	(1.24)	(1.01)	(0.73)
TAT-26	15 × 30	N2	Control	148.4 *	0.214	6	9.56	1.55	31.7	247.0 *
TAT-26	15 × 30	N2	BF	144.3	0.224	7	13.39 **	2.10 *	32.3	164.7
			(BF/Control)	(0.97)	(1.05)	(1.05)	(1.40)	(1.36)	(1.02)	(0.67)
TAT-26	15 × 30	N4	Control	151.4	0.203	6	11.09	1.75	30.7	191.6
TAT-26	15 × 30	N4	BF	150.6	0.245 **	7 *	14.27 *	1.93	36.8 **	181.8
			(BF/Control)	(1.00)	(1.20)	(1.19)	(1.29)	(1.10)	(1.20)	(0.95)
TAT-26	30 × 30	N2	Control	156.7 **	0.342	8	18.29	2.29	53.6	195.0
TAT-26	30 × 30	N2	BF	151.5	0.374	10 *	21.17	2.10	56.6	181.9
			(BF/Control)	(0.97)	(1.10)	(1.28)	(1.16)	(0.92)	(1.06)	(0.93)
TAT-26	30 × 30	N4	Control	154.7	0.308	9	20.15	2.35	48.1	159.6
TAT-26	30 × 30	N4	BF	158.4	0.355 *	10	20.44	2.23	56.3 *	204.8
			(BF/Control)	(1.02)	(1.15)	(1.12)	(1.01)	(0.95)	(1.17)	(1.28)
ANOVA (<i>p</i> value)										
Biofertilizer				0.050	< 0.000	0.002	< 0.000	0.046	< 0.000	0.024
Variety				< 0.000	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000
Distance				< 0.000	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000	n.s.
Fertilization				0.035	0.001	n.s.	n.s.	n.s.	0.029	n.s.
Biofertilizer * Variety				0.010	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Biofertilizer * Distance				n.s.	n.s.	n.s.	n.s.	0.027	n.s.	0.007
Biofertilizer * Fertilization				0.003	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

FW: Fresh weight. BF: Biofertilizer. n.s.: No significance. * and ** indicate significant differences at $p \leq 0.05$ and 0.01 levels between control and biofertilizer application in each treatment, respectively (t-test, two-sided). n = 10.