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Growth Rate, Weed Competitiveness, and Deepwater Avoidance of a New Type of Rice Line, Monster Rice 2

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Abstract

Organic farming is a cultivation practice with a lower environmental burden than conventional farming. The low yield and the difficulty of weed control in organic farming can be resolved by developing new suitable cultivars. This study investigated the adaptability of Monster Rice 2 (MR2), a new rice line with extra-long culms and high fertilizer use efficiency, to the three aspects of organic farming: growth under green manure (GM) application, weed competitiveness, and deepwater avoidance by comparing it with a semidwarf cultivar, Takanari. In GM application, the above-ground biomass of MR2 at the heading stage was marginally higher than that of Takanari due to a higher net assimilation rate. Although MR2 did not have higher weed competitiveness, it had a better survival rate and growth than Takanari under 20 cm of water in the early growth stage, when most weeds are typically exterminated. These MR2 properties are partially attributable to *SD1*, an allele that promotes shoot elongation, based on the results that a near-isogenic line of *SD1* showed slightly higher growth in GM application and significantly higher deepwater avoidance than Takanari. Our results suggest that long-culm rice has notable future utility and that MR2 could serve as a prototype breeding material in organic farming.

Discipline: Crop Science
Additional key words: deepwater management, late watergrass, long-culm, organic farming, *SD1*

Introduction

Organic farming is a cultivation practice that does not use chemical fertilizers (CF) and agrochemicals, including pesticides and herbicides (Lammerts van Bueren et al. 2002, Reganold & Wachter 2016, Wittwer et al. 2021). Benefits expected in organic farming are a low environmental burden, high biodiversity, and low consumption of natural resources, all of which contribute to sustainable food production (Mäder et al. 2002, Crowder et al. 2010, Tuomisto et al. 2012, Tuck et al. 2014). The unsolved problems of organic farming include low yield and instability due to the slow nutrient release from organic substances, as well as the effects of weeds, pests, and diseases (Ryan et al. 2004, Trewavas 2004, Murphy et al. 2007). In contrast, conventional farming uses substantial CF and agrochemicals to enhance yield and stability, but their excessive use is responsible for environmental and ecological disturbances (Lu & Tian 2017). In addition, conventional farming depends strongly on modern cultivars that demonstrate higher yields with high CF input (Khush 1999). These cultivars do not always show a stable high yield under organic farming (Foulkes et al. 1998, Murphy et al. 2007). Nevertheless, more than 95% of the area farmed organically employs modern cultivars (Lammerts van Bueren et al. 2011). Hence, developing new cultivars adapted to organic farming promises to increase yields (Lammerts van Bueren et al. 2011).

On organic farms, weeds are usually removed by hand or weeders instead of using chemical herbicides. This places a significant burden on farmers and takes more than twice as long as conventional rice farming in Japanese organic rice production (MAFF 2023).

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Developing weed-competitive cultivars could decrease these efforts (Murphy et al. 2008, Lammerts van Bueren et al. 2011). It has been reported that long-culm rice cultivars have a greater ability to suppress weeds than short-culm cultivars due to their inclined, longer leaves, which limit light penetration into the bottom of the canopy (Tachibana 2017). The Green Revolution in rice began in the 1960s, when semidwarf cultivars carrying the *semidwarf 1* (*sd1*) gene were developed. These contributed to boosting rice yield under the use of CF. However, the *sd1* gene increases the leaf inclination angle, making it more erect, relative to the *SD1* allele, decreases mutual shading of the canopy, and increases light penetration (San et al. 2020). This can increase biomass production in conventional farming but may reduce weed competitiveness in organic farming (Lammerts van Bueren et al. 2011).

Deepwater (DW) management, in which a water depth of 10-20 cm is maintained for several weeks immediately after transplanting, is another option to inhibit weed growth in organic farming (Arai & Sakai 2005). It has been reported that growth in 15 cm deep water for 30 days suppressed more than 90% of survivals in late watergrass (*Echinochloa phyllopogon* (=*E. oryzicola*)) and *Cyperus microiria* by oxygen deficiency and carbohydrate starvation (Kasahara & Kinoshita 1953, Arai & Miyahara 1956). However, DW management also inhibits rice seedling growth for the same reason (Hanada & Kagawa 1985). The emergence of tiller buds is strongly inhibited in rice during DW management and reduces tiller number and biomass production (Ohe et al. 1994, 2010; Watanabe et al. 2006; Iwasa et al. 2023, 2024). Under these conditions, traditional Japanese farmers have used large (over 18 cm), mature seedlings after a longer raising period that can avoid growth suppression under DW management (Matsuo 1957). Therefore, taller seedlings at the initial growth stage is appropriate for DW conditions immediately after transplanting. We hypothesize that the growth of long-culm rice would benefit more under DW conditions than semidwarf cultivars. For weed suppression, we considered high biomass production under 10-20 cm of water to be high DW avoidance.

We recently developed a new rice line, Monster Rice 2 (MR2). MR2 was bred from a cross between a sibling of Monster Rice 1 (MR1) (Nomura et al. 2019) with a long culm and Takanari with a short culm and high biomass production (Xu et al. 1997). Since MR2 was selected under reduced CF (3 kgN per 10 a) as a rice line with high biomass production, we believe that MR2 will show high growth in organic farming. MR2 is a larger plant than semidwarf cultivars due to having a non-DW rice *SD1* haplotype and shows high weed competitiveness and DW avoidance. In addition, it has thicker, stronger culms than Takanari, which may avoid yield loss due to lodging, which is often problematic among long-culm cultivars. We believe that these properties in MR2 are beneficial for organic farming.

The current study investigated three aspects of rice cultivars required in organic farming: (1) vigorous growth in no-fertilizer (NF) and green manure (GM) environments, (2) weed competitiveness in a GM environment, and (3) avoidance of DW treatment for three weeks after transplanting. We evaluated them by comparing the growth of MR2 and Takanari. We also used a near-isogenic line NIL-SD1 that carries the Koshihikari *SD1* allele substituted in the genetic background of Takanari to investigate (4) the role of the *SD1* allele on these properties and validate the results of DW avoidance for two years. From our results, we discuss the utility of long-culm rice cultivars with the *SD1* allele in organic farming and the direction of future breeding programs.

Materials and methods

1. Plant materials

Three rice (*Oryza sativa* L.) lines (MR2, NIL-SD1, and Takanari) were used. MR2 was bred from a cross between a sibling of MR1 and a line crossed between Leaf Star and Takanari (Fig. 1a-c; Nomura et al. 2019). NIL-SD1 is a near-isogenic line selected to introduce the *SD1* allele of Koshihikari to the genetic background of Takanari (Fig. A1a; San et al. 2020). Takanari is a high-yield, semidwarf *indica* cultivar bred in Japan. MR2 sequencing was performed by the Hokkaido System Science Co., Ltd. (Sapporo, Japan). The combined DNA fragments were end-repaired and ligated with a sequence adaptor using Illumina Hiseq X. The sequence of the *SD1* region of MR2 is identical to those of Koshihikari and the parental lines Akenohoshi and Leaf Star; however, they are different from that of Takanari with an *sd1* mutation originating from IR8 (Fig. 1d). Furthermore, the *SD1* haplotype of MR2 was confirmed to be the same as that of NIL-SD1 due to the identity of the sequence between MR2 and Koshihikari. Late watergrass (= *E. phyllopogon*, distributed by the Japan Association for the Advancement of Phyto-Regulators) was used as the paddy weed to evaluate weed competitiveness.

2. Experiment 1: Evaluating growth under different fertilizations

The experiment was conducted in 2019. The rice seeds of the three cultivars were sown in nursery boxes

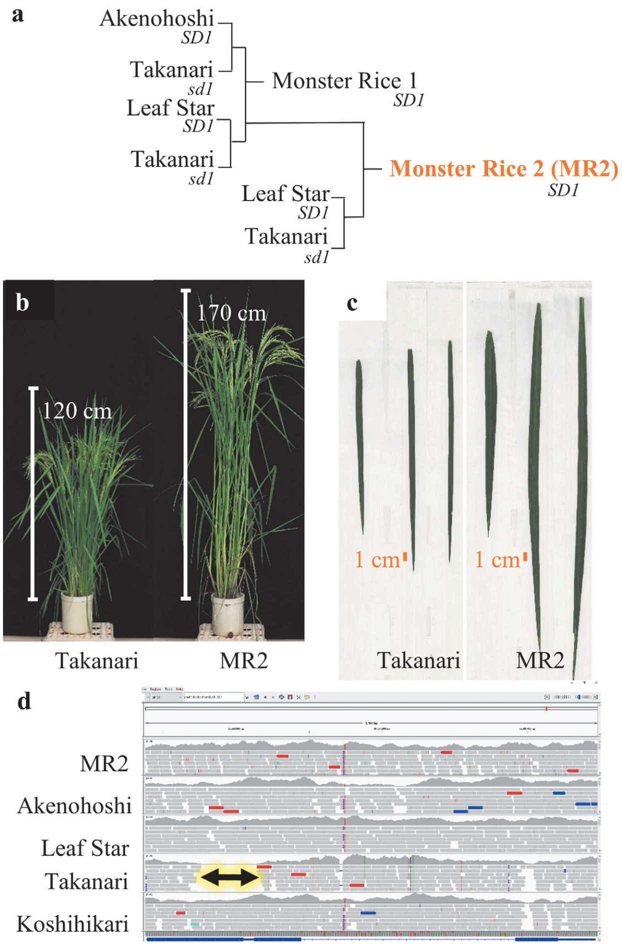

Fig. 1. Characteristics of the Monster Rice 2 (MR2) rice cultivar

(**a**) Pedigree of MR2, (**b**) gross morphological comparison of Takanari and MR2 at full heading, (**c**) top three expanded leaves from the flag leaves of Takanari and MR2, sorted from the left, (**d**) Integrative Genomics Viewer views of aligned reads of the *sd1* gene region in MR2, showing Akenohoshi, Leaf Star, Takanari, and Koshihikari. Alignments toward the Nipponbare IRGSP 1.0 (top row) are represented as gray polygons, and mismatched nucleotides appear as colored bars. The arrow indicates that deletion was found in Takanari between Exons 1 and 2.

on 24 April 2019. Seedlings at the fourth leaf stage were transplanted to a paddy field (an alluvial clay loam) of the Field Science Education and Research Center of the Faculty of Agriculture, Tokyo University of Agriculture and Technology (35° 39′ N, 139° 28′ E) with 22.2 hills m^{-2} (15 cm × 30 cm) and three plants per hill on 17 May 2019. The field was maintained under submerged conditions (~5 cm) through the growing season. Weed and insect control was conducted as required using chemical pesticides and herbicides following conventional practices.

Three fertilizer plots were provided: no fertilizer (NF), chemical fertilizer (CF), and green manure (GM). Each plot was about 60 m^2 (4.25 m × 14.40 m, with 1,332 hills) and had three replications in a randomized complete block design. All whole plant residues of the previous year were chopped up and mixed into the soil. Chemical fertilizer was applied to CF plots as the basal fertilizer with N (ammonium sulfate: LP50: LPS100 = 1:1:1), P_2O_5, and K_2O of 5, 6, and 6 kg/10 a, respectively. Topdressing was not done. In GM plots, hairy vetch (*Fabaceae*) and oats (*Poaceae*) were used as green manure (Asagi & Ueno 2009, Tarui et al. 2013). The hairy vetch and oats were sown on 19 November 2018 at 4 and 15 kg per 10 a, respectively. They were mixed into the soil on 24 April 2019. The GM in each plot was taken and ground into powder by a vibrating mill (Wonder Blender, Osaka Chemical, Co., Ltd.), and the nitrogen and carbon concentrations from each plant were analyzed by a CN-Corder (MT-700II, Yanaco Industry, Japan). The total amount of nitrogen input in GM plots, which was calculated by multiplying above-ground biomass by nitrogen concentration, was higher than that in CF plots (Table A1).

Three hills of Takanari and MR2 in each replicate were used in the growth survey. Plant length, tiller number, and leaf ages were measured every two weeks until the early ripening stage. The mean of three readings from the soil plant analysis development (SPAD) meter (SPAD-502, Konica Minolta Sensing Co., Ltd. Japan) was obtained near the midpoint of each leaf blade from five weeks after transplanting until the early ripening stage.

Above-ground biomass was examined on 17 May, 2 July, and 9 August 2019, which correspond to the transplanting (TP), maximum tillering (MT), and heading (HD) stages, respectively. Ten hills of Takanari, MR2, and NIL-SD1 in each replicate were harvested at the soil surface; two of the ten were separated into leaf blades, leaf sheaths plus stems, and panicles (if any); the other eight sample plants were bundled together. The total leaf area of the two hills was measured with an automatic leaf area meter (AAM-9, Hayashi Co., Ltd., Tokyo, Japan). All samples were then dried in a ventilated oven at 80°C for at least 72 hours. Crop growth rate (CGR), mean leaf area index (mean LAI), and net assimilation rate (NAR) of two adjacent sampling stages were calculated as follows,

$$\text{NAR (g m}^{-2}\text{ day}^{-1}) = \text{CGR (g m}^{-2}\text{ day}^{-1}) / \text{mean LAI (m}^2\text{ m}^{-2}). \quad (1)$$

3. Experiment 2: Evaluating weed competitiveness

The experiment was conducted in 2019. We grew three rice cultivars (Takanari, MR2, and NIL-SD1) and a weed species (late watergrass) in a paddy field. There were two types of plots: pure stands (where only rice was grown) and mixed stands (where rice and weeds were grown), with three replicates. The rice seedlings were grown and transplanted in the same manner as in Experiment 1. The seeds of late watergrass, immersed in distilled water, were evacuated with an aspirator and incubated in a petri dish with distilled water at room temperature. The seedlings of late watergrass were transplanted on 1 June 2019 to the rice paddy field. The seedlings were planted in the center of four adjacent rice plants, resulting in a planting density of 22.2 hills m^{-2} (15 cm × 30 cm), the same as for the rice according to Tachibana et al. (2015a). The fertilization was the same as the GM plot in Experiment 1.

The heights of rice and late watergrass in each replicate were measured approximately once every two weeks until the early ripening stage. The light intensities at the top of the rice canopy (I_r) and of the late watergrass canopy (I_o) were measured at three locations in each plot, approximately once every two weeks until heading. Measurements were taken between 09:00 and 12:00 under scattered light conditions using a custom-made light sensor with a silicon photodiode (S1133, Hamamatsu Photonics, Japan). The percentage of light intercepted by the late watergrass before reaching the rice canopy was calculated to be

$$\text{Light perception rate for late watergrass (\%)} = \{1 - (I_o/Ir)\} \times 100. \quad (2)$$

The plants from 10 to 12 hills of rice and late watergrass, respectively, were sampled on 2 August 2019 and dried in a ventilated oven before weighing.

4. Experiment 3: Evaluating DW avoidance

The experiments were conducted in 2019 and 2021. In 2019, we grew the seedlings of Takanari, MR2, and NIL-SD1 in the same way as in Experiment 1. In 2021, two cultivars, Takanari and NIL-SD1, were grown. Rice seeds were sown on 6 May, and the seedlings were transplanted to the paddy field on 19 May. Fertilization was the same as the CF plot in Experiment 1 in both years. We provided two water conditions, shallow water (SW) and deep water (DW). In 2019, we set the DW condition one week after transplanting by burying planters in a field (Fig. A4). In 2021, two adjoining fields were used for SW and DW testing. The water level was maintained at ~5 cm after transplantation. One week after transplanting, the water level was raised to 20 cm and maintained for three weeks in the DW condition, whereas the water level remained unchanged under SW conditions. The survival rate (i.e., the ratio of the number of plants before and after treatment) was calculated after the treatment. We sampled eight surviving plants in each plot on 7 June 2019 and 23 June 2021 and dried them in a ventilated oven at 80°C for at least 72 hours to measure the above-ground biomass.

5. Statistical analysis

Tukey's HSD test was performed to compare the phenotypic trait values in all the experiments among three rice cultivars and between late watergrass mixed in different rice cultivars in Experiment 2. The Student's t-test was performed to compare DW avoidance between Takanari and NIL-SD1 in 2021. All statistics were analyzed using JMP v.13 software (SAS Institute).

Results

1. Growth under different fertilizations

We examined the growth properties of MR2 under three fertilizer applications (i.e., NF, CF, and GM). The plant length of MR2 was greater than Takanari across the growing season, regardless of the fertilizer (Fig. 2a). The difference between the two cultivars gradually increased with the progress of the growth stage. MR2 grew to a maximum length of ~185 cm, which is about 60% longer than Takanari. Takanari had a higher tiller number than MR2 in all fertilizer plots (Fig. 2b). The tiller number of Takanari reached ~30 at the MT stage, which was three times that of MR2, and gradually decreased until the harvest, while that of MR2 remained unchanged after the maximum tillering stage. Leaf age remained similar regardless of cultivars and fertilizers (Fig. 2c). The MR2 SPAD value was kept higher than that of Takanari across growth stages in all fertilizer plots (Fig. 2d). In 2021, we found that the plant length of NIL-SD1 remained greater than of Takanari, especially in the later growth stage (Fig. A1).

Above-ground biomass was evaluated under different fertilizer applications (Table 1). Overall, there was little difference across the treatments and cultivars, except that NIL-SD1 had significantly higher above-ground biomass at the MT stage under CF plots than other cultivars. When comparing relative values against Takanari, we found that the above-ground biomass of MR2 at the HD stage was larger by 15% and 12% under CF and GM, respectively. We also found that the above-ground biomass of NIL-SD1 was approximately 10% higher than of Takanari at the HD stage under CF and GM application. These results indicate that the long-culm lines show vigorous growth with CF and GM, although they do not outperform that of the NF plot.

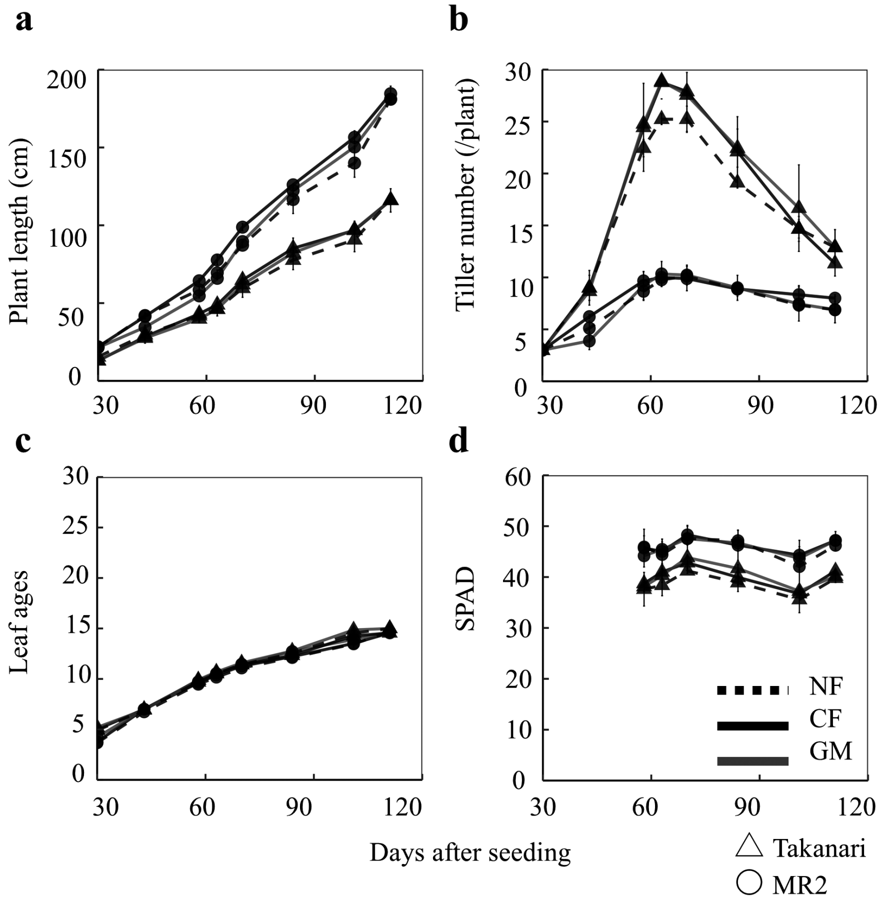

Fig. 2. Trait comparison between Takanari and Monstar Rice 2 (MR2) from transplantation to early ripening

Changes in **(a)** plant length, (**b**) tiller number, (**c**) leaf ages, and (**d**) SPAD value between Takanari (△) and MR2 (○) with no fertilizer (NF, dotted black line), chemical fertilizer (CF, solid black line), and green manure (GM, solid gray line) plots.

Table 1. Differences in above-ground biomass among Takanari, Monster Rice 2 (MR2), and NIL-SD1 for each growth stage under different fertilizations

		Biomass (g m^{-2})								
		NF			CF			GM		
TP	Takanari	0.2 ± 0.01	-	a	0.2 ± 0.01	-	a	0.2 ± 0.01	-	a
	MR2	0.2 ± 0.02	-	a	0.2 ± 0.02	-	a	0.2 ± 0.02	-	a
	NIL-SD1	0.2 ± 0.04	-	a	0.2 ± 0.04	-	a	0.2 ± 0.04	-	a
MT	Takanari	170.4 ± 40.1	(100)	a	223.2 ± 2.2	(100)	b	240.9 ± 21.3	(100)	a
	MR2	157.7 ± 37.8	(93)	a	211.6 ± 17.8	(95)	b	228.7 ± 16.9	(95)	a
	NIL-SD1	188.9 ± 22.7	(111)	a	276.2 ± 25.0	(124)	a	236.3 ± 27.9	(98)	a
HD	Takanari	1004.6 ± 124.2	(100)	a	1031.2 ± 103.2	(100)	a	933.6 ± 103.2	(100)	a
	MR2	985.4 ± 49.1	(98)	a	1185.1 ± 63.5	(115)	a	1041.2 ± 81.1	(112)	a
	NIL-SD1	975.8 ± 77.3	(97)	a	1123.6 ± 133.7	(109)	a	1069.6 ± 94.8	(115)	a

Values inside the brackets indicate the ratio to Takanari (100).

Calculation: [Biomass of rice line (g m^{-2})] / [Biomass of Takanari (g m^{-2})] × 100 (%)

Different lowercase letters (a or b) indicate the results of Tukey's HSD test within each treatment and growth stage ($p < 0.05$, n = 3).

TP: transplanting stage, MT: maximum tillering stage, HD: heading stage, NF: no fertilizer plot, CF: chemical fertilizer plot, GM: green manure plot

We conducted the growth analysis during the periods of TP to MT and MT to HD (Table 2). Although statistically significant differences were not detected between rice lines for any parameter, there were differences in NARs and mean LAIs. Under the NF plot, MR2 and NIL-SD1 showed a relatively higher mean LAI and lower NAR than Takanari. With CF, MR2 exhibited a lower mean LAI and higher NAR than Takanari during the MT-HD period. In contrast, NIL-SD1 had a higher mean LAI and lower NAR than Takanari during both the TP-MT and MT-HD periods. Under the GM plot, MR2 and NIL-SD1 had lower mean LAIs and higher NARs than Takanari in the MT-HD period. The higher NAR of these long-culm lines would cause an approximately 10% to 20% increase in above-ground biomass under the GM plot.

2. Weed competitiveness

We examined whether the greater plant length and the faster growth of the long-culm lines under the GM plot enhance weed competitiveness. Takanari was less tall than late watergrass across the growth period (Fig. 3). This allowed late watergrass to receive full sunlight (Fig. A2). MR2 showed significantly greater plant height than late watergrass throughout the growth period, and NIL-SD1's was higher several times during the growth period (Fig. 3). MR2 was able to interrupt the light perception of late watergrass by ~15% on average, and NIL-SD1 partially did so (Fig. A2). Nevertheless, late watergrass exhibited similar above-ground biomass regardless of which rice lines were planted together (Fig. A3). The relative value of above-ground biomass in rice in mixed to pure stands was also similar between the rice lines (71%-86%, Fig. A3).

3. DW avoidance in the early growth stage

To examine whether the large plant height in the early growth stage is advantageous under DW conditions, we compared the survival rate and above-ground biomass of the long-culm rice lines and Takanari at water depths of 20 cm in 2019. After three weeks of DW conditions, all plants in MR2 and NIL-SD1 survived, whereas 1/3 of the Takanari individuals died, a survival rate of 66.7% (Table 3). The above-ground biomass of the surviving DW MR2 and NIL-SD1 plants decreased by 55.5%-56.3% from that under SW conditions, while that of Takanari

Table 2. Differences in crop growth rate (CGR) among Takanari, Monster Rice 2 (MR2), and NIL-SD1 for each growth period

		NF						CF					
		CGR ($g\ m^{-2}\ day^{-1}$)		mean LAI ($m^{-2}\ m^{-2}$)		NAR ($g\ m^{-2}\ day^{-1}$)		CGR ($g\ m^{-2}\ day^{-1}$)		mean LAI ($m^{-2}\ m^{-2}$)		NAR ($g\ m^{-2}\ day^{-1}$)	
TP-MT	Takanari	3.70	(100)	0.85	(100)	4.38	(100)	4.85	(100)	1.00	(100)	4.85	(100)
	MR2	3.42	(92)	0.91	(107)	3.74	(85)	4.60	(95)	0.87	(87)	5.27	(109)
	NIL-SD1	4.10	(111)	0.92	(108)	4.48	(102)	5.99	(124)	1.16	(116)	5.19	(107)
MT-HD	Takanari	21.95	(100)	3.22	(100)	6.82	(100)	21.26	(100)	7.85	(100)	2.71	(100)
	MR2	20.71	(94)	4.61	(143)	4.49	(66)	25.62	(121)	4.60	(59)	5.57	(206)
	NIL-SD1	21.78	(99)	5.72	(178)	3.81	(56)	22.30	(105)	9.94	(127)	2.24	(83)

		GM					
		CGR ($g\ m^{-2}\ day^{-1}$)		mean LAI ($m^{-2}\ m^{-2}$)		NAR ($g\ m^{-2}\ day^{-1}$)	
TP-MT	Takanari	5.23	(100)	0.89	(100)	5.87	(100)
	MR2	4.97	(95)	0.73	(82)	6.82	(116)
	NIL-SD1	5.13	(98)	0.87	(98)	5.91	(101)
MT-HD	Takanari	18.23	(100)	7.70	(100)	2.37	(100)
	MR2	21.38	(117)	5.45	(71)	3.92	(165)
	NIL-SD1	21.93	(120)	5.82	(76)	3.77	(159)

Data are represented as the mean of three replications.
Values inside the brackets indicate the ratio to Takanari (100).
Calculation: [Data of rice line] / [Data of Takanari] × 100 (%)
Mean LAI: mean leaf area index, NAR: net assimilation rate. TP: transplanting stage, MT: maximum tillering stage, HD: heading stage. NF: no fertilizer plot, CF: chemical fertilizer plot, GM: green manure plot

decreased 88.2% (Table 3; Fig. A4). We also confirmed the higher above-ground DW biomass for NIL-SD1 than Takanari in 2021 (Fig. A5). These results indicate that the long-culm rice lines showed higher growth under 20 cm of water, whereas Takanari growth was severely inhibited.

Discussion

Organic farming is a promising solution for achieving sustainable agriculture as an alternative to conventional farming, but its low yield and instability reduce farmers' income and increase global food insecurity (Reganold & Wachter 2016). The difficulties in controlling weeds, insects, pests, and diseases also decrease the production efficiency of organic farming (Lammerts van Bueren et al. 2002, Matsuoka et al. 2022). Why these issues remain unsolved is likely that there have been few breeding programs to develop crop cultivars suitable for organic farming (Lammerts van Bueren et al. 2011). This study examined the adaptability of MR2, a newly developed rice line with an extra-long culm, relative to conventional rice cultivars. It was selected as a high biomass-producing rice line for low nitrogen fertilizer levels, about 3 kgN per 10 a, for the three aspects of organic farming systems: vigorous growth in NF and GM plots, weed competitiveness in the GM plot, and high survival and above-ground biomass under DW conditions in the early growth stage. We also tested if the high adaptability of MR2 was due to the elongation effect of the *SD1* haplotype using NIL-SD1 plants. All properties of MR2 and NIL-SD1 were compared with the high-yielding *sd1* cultivar Takanari.

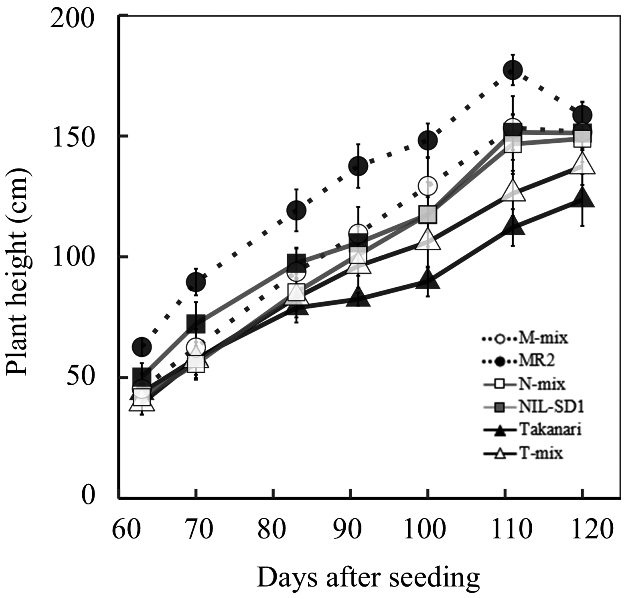

Fig. 3. Changes in plant height among Takanari, Monster Rice 2 (MR2), NIL-SD1, and late watergrass in mixed stands

M-mix (○, dotted black line), N-mix (□, solid gray line), and T-mix (△, solid black line) indicate that plant height of late watergrass mixed with MR2 (●, dotted black line), NIL-SD1 (■, solid gray line), and Takanari (▲, solid black line), respectively. Error bars indicate standard deviation (n = 3).

1. Growth under different fertilizations

Organic farmers use organic fertilizers, including grass, GM, and manures made from fish, soybeans, or animal dung instead of CF (Matsuoka et al. 2022). These organic fertilizers are typically mineralized more slowly than CF, causing low soil fertility in the early growth stages of organic farming (Asagi & Ueno 2009, Bhatt et al. 2019, Matsuoka et al. 2022). Therefore, rice cultivars for organic farming must have vigorous growth and larger roots for efficient absorption of soil nutrients, especially during early growth (Lammerts van Bueren et al. 2002). Based on the evidence that a greater plant height during early growth strongly correlates with higher biomass production (Niklas & Enquist 2001), we believe that MR2 provides vigorous growth under organic farming conditions such as NF and GM.

Table 3. Effect of water treatment on survival rate and above-ground biomass of Takanari, Monster Rice 2 (MR2), and NIL-SD1

	Survival rate (%)	Biomass (g m^{-2})				Decrement (%)
		SW		DW		
Takanari	66.7	99.3 ± 0.9	a	11.7 ± 0.2	b	88.2
MR2	100.0	102.8 ± 0.3	a	45.7 ± 0.5	a	55.5
NIL-SD1	100.0	112.2 ± 1.3	a	49.1 ± 0.3	a	56.3

SW and DW indicate shallow-water and deepwater plots, respectively. Different lowercase letters (a or b) indicate results of Tukey's HSD test ($P < 0.01$, n = 3).

With GM, the CGR of MR2 was approximately 1.2 times that of Takanari in the MT-HD stage (Table 2). The higher CGR of MR2 was attributable to the NAR, not the mean LAI, being higher than that of Takanari (Table 2). This suggests that MR2 has higher canopy photosynthesis than Takanari for the following reasons. We found that MR2 maintained a higher SPAD value, an indicator of estimated leaf chlorophyll content, than Takanari throughout the measurement period, as shown in Figure 2d (Watanabe et al. 1980, Chubachi et al. 1986). Since the SPAD value has a high positive correlation with the leaf photosynthesis rate (Huang & Peng 2004, Kato et al. 2004, Kumagai et al. 2009), MR2 may have higher leaf photosynthesis rates during the growth stages. Furthermore, MR2 was taller than Takanari (Fig. 2a). It has been reported that the greater height enhances the CO_2 diffusion inside stands and promotes the photosynthesis in lower leaves, as in the case of Tainung 67, a long-culm cultivar (Takeda et al. 1983, Kuroda et al. 1989). The smaller mean LAI of MR2 may even increase the CO_2 diffusion inside the canopy (Table 2). These assumptions should be confirmed in a future study. The ability of tillering of MR2 was only one-third of Takanari (Fig. 2b). This would be a reason for the smaller mean LAI in MR2. Introducing the tillering ability of Takanari to MR2 will lead to a further increase in above-ground biomass in GM application.

MR2 maintained a similar mean LAI in both NF and CF plots during the MT-HD stage, whereas Takanari had a lower mean LAI in the NF plot than in the CF plot. This may be caused by the ability to maintain the tiller number in NF plots. The number for NF MR2 was similar for CF and GM, while that for Takanari in the NF plot was lower than the CF and GM plots (Fig. 1b). It has been reported that low nitrogen content decreases LAI (Xue et al. 2004). Although MR2 has a small tiller number, its ability to expand leaf area without nitrogen fertilizer can be a valuable for breeding rice cultivars suitable for organic farming.

2. Weed competitiveness

MR2 plants were longer than late watergrass, while Takanari was shorter (Figs. 3, A2). However, there were no varietal differences in weed suppression ability between rice lines (Fig. A3). This indicates that the long-culm lines can interrupt the light perception of weeds but have limited ability of weed competitiveness for biomass production. The possible reasons for this are the varietal differences in tiller number and tiller spreading angle, which also affect light capture and weed suppression, including late watergrass and *Monochoria vaginalis* (Koarai & Morita 2003, Tachibana 2015b, Inagaki et al. 2021). Takanari has a short culm and spread tiller angle with a functional allele of *TAC1*, a gene locus on chromosome 9 that controls the tiller angle (Yu et al. 2007, Ogawa et al. 2021). On the other hand, MR2 has a long-culm, erect tiller angle with fewer tillers, as shown in Figure 1. This offset may be why there are no differences in biomass production among cultivars. Increasing the tiller number and spreading angle during the vegetative stage by accumulating advantageous alleles could enhance weed competitiveness of MR2.

3. DW avoidance during early growth for weed management

While DW management is an effective weed suppression technique in organic rice farming, it also suppresses the growth of the rice (Watanabe et al. 2006, Iwasa et al. 2023). The current study showed that MR2 had a higher survival rate and lower growth inhibition rate under DW conditions than Takanari (Table 3; Fig. A4). As seen in Figure 2a, the plant length of MR2 exceeded 20 cm at transplantation, whereas Takanari was ~17 cm long, and both plants were under water. In tall cultivars such as MR2, leaves that are above the water can absorb oxygen from the air, transport it to the submerged plant parts, and reduce oxygen deficiency stress (Kende et al. 1998). We previously reported the large genetic diversity of DW avoidance among 165 temperate *japonica* cultivars, having detected *qPL3*, the quantitative trait locus that is associated with the plant length in a DW environment by conducting a genome-wide association study (Iwasa et al. 2023). MR2 carries the *qPL3* allele, which indicates a greater plant length (data not shown) and may lead to its high DW avoidance. In addition, the tiller number is also related to DW avoidance in the early growth stage for weed control (Iwasa et al. 2023). Although we did not analyze the tiller number in DW in this study, DW avoidance of MR2 could increase further if the tillering ability were improved.

4. Properties of NIL-SD1

MR2 carries the *SD1* haplotype of Koshihikari as well as NIL-SD1 (Fig. 1d). Next, we discuss whether the advantages of MR2 are caused by the *SD1* haplotype from the data for NIL-SD1. Compared with Takanari, NIL-SD1 showed greater plant length and slightly higher above-ground biomass with GM, although there were no significant differences (Tables 1, 2). NIL-SD1 also showed high survival rates and above-ground biomass under DW conditions (Table 3; Figs. A4, A5). These properties were comparable to those of MR2. Therefore, the greater plant height due to the *SD1* in MR2 is attributable to the improved growth in GM plots and enhanced DW avoidance. In contrast, NIL-SD1 did not exhibit high weed competitiveness, contrary to our expectations (Figs. 3, A2, A3) and the results of MR2. Several studies have shown that greater plant length suppresses weed growth (Bastiaans et al. 1997, Koarai & Morita 2003, Tachibana et al. 2015b), but our results suggest it does not. Furthermore, MR2 plant height was greater than NIL-SD1; this could not be explained only by the effect of *SD1* (Fig. 3). Many other genes related to increased plant length, including *OsGA20ox1* and *Ghd7* (Yano et al. 2012, Abe et al. 2012, Xue et al. 2008), have been reported. The effects of these genes in MR2 for the long culm and the adaptability of the three aspects in this study should be confirmed in future studies.

Conclusion

This study showed that MR2 had higher growth in GM plots and higher DW avoidance at depths of 20 cm for three weeks immediately after transplanting, which are essential for organic farming. MR2's high growth was responsible for the higher NAR, probably due to increased canopy photosynthesis rather than leaf area expansion. The greater plant length was responsible for the higher DW avoidance, which avoids hypoxia. These advantages are partially explained by the presence of the *SD1* gene, with a larger plant size relative to semidwarf cultivars. Our findings suggest that rice lines with greater plant lengths will be useful for GM application and DW management in organic farming. With further improvement, MR2 can become superior breeding material for organic farming.

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Authors' contributions

MI and TO designed this study. MI, SA, KK, TN, and TO conducted the field survey, and MI and SA contributed to the haplotype analysis. MI, SA, and TO wrote the manuscript. All authors have read and approved the final manuscript.

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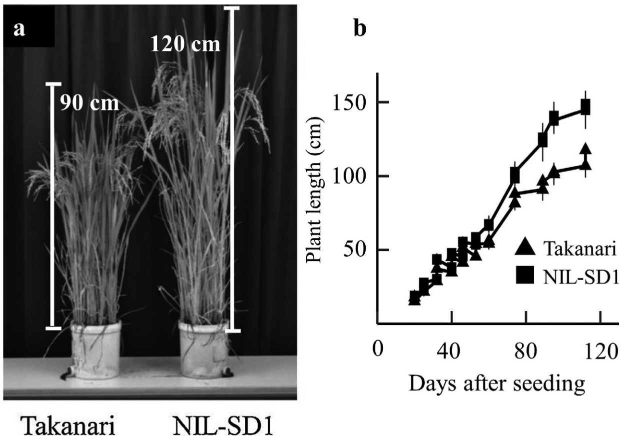

Fig. A1. Characteristics of NIL-SD1

(**a**) Gross morphological comparisons of Takanari and NIL-SD1 (Picture is taken from San et al. 2020).

(**b**) Changes in plant length of Takanari and NIL-SD1 from transplantation to heading stage with chemical fertilizer (CF) in 2021.

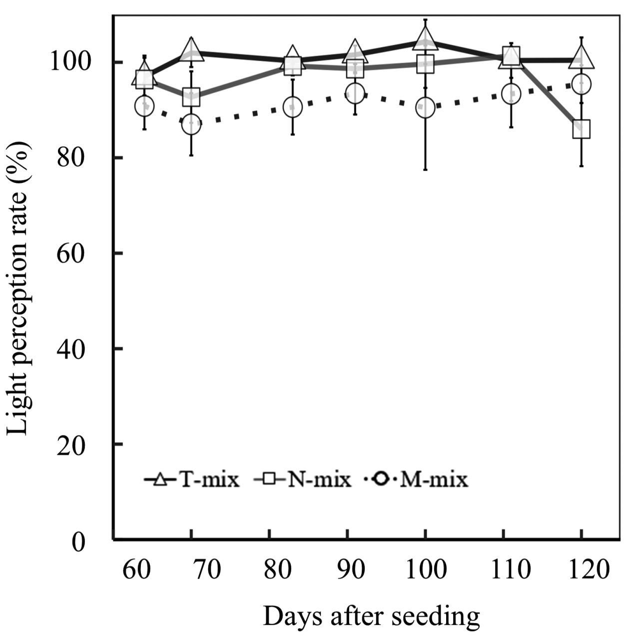

Fig. A2. Light perception rate of late watergrass in mixed stands

Error bars indicate standard deviation (n = 3). M-mix (○, dotted black line), N-mix (□, solid gray line), and T-mix (△, solid black line) indicate the values of late watergrass mixed with Monster Rice 2 (MR2), NIL-SD1, and Takanari, respectively.

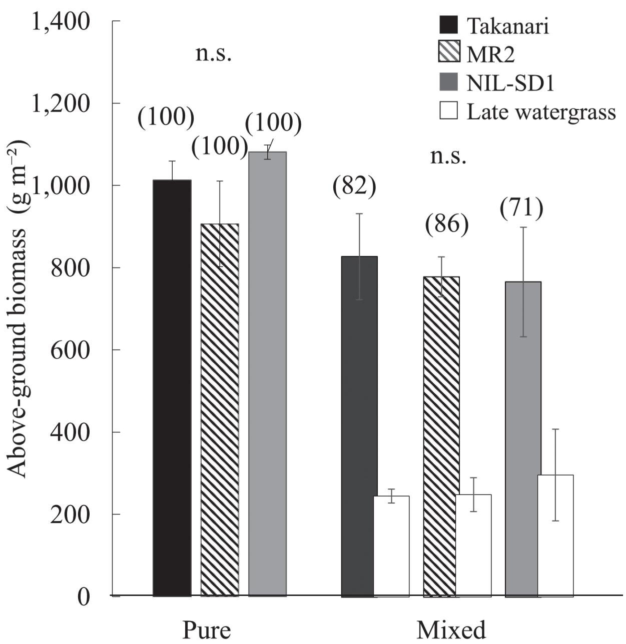

Fig. A3. Differences in above-ground biomass of Takanari, Monster Rice 2 (MR2), NIL-SD1, and late watergrass in August 2019

Rice plants were grown in pure stands without late watergrass (pure) and mixed with late watergrass (mixed). Error bars indicate standard deviation (n= 3). Values inside the parentheses indicate the ratio of mixed to pure.

Calculation: [Biomass of rice line in mixed plot (g m^{-2})] / Biomass of rice line in pure plot (g m^{-2})] × 100 (%)

"n.s." indicates the results of the analysis of the Tukey-HSD test among rice lines or late watergrass that were mixed with each rice line for each plot (p < 0.05, n = 3).

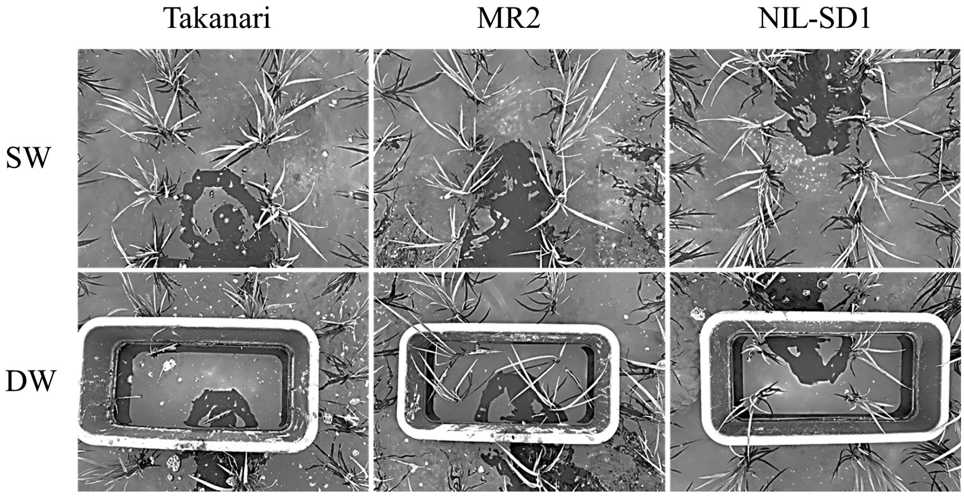

Fig. A4. Effect of water treatment on growth of Takanari, Monster Rice 2 (MR2), and NIL-SD1 in 2019

SW and DW indicate shallow-water and deepwater conditions, respectively.

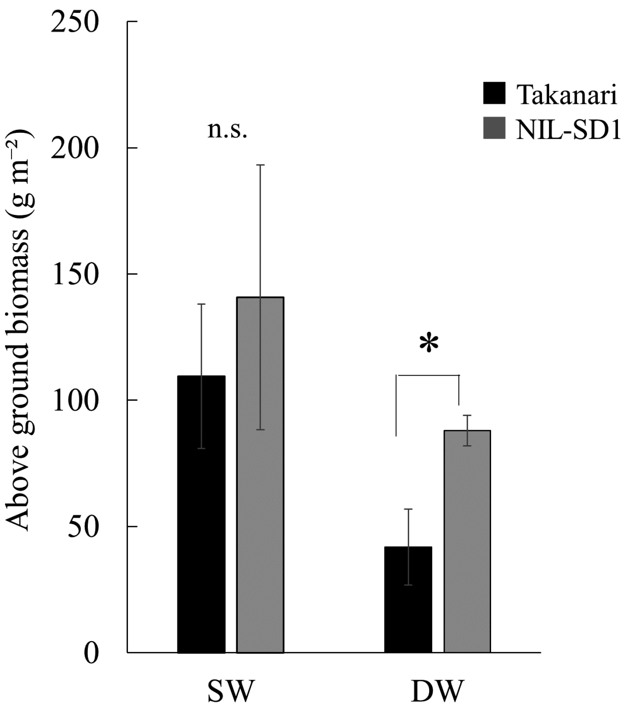

Fig. A5. Effect of water treatment on above-ground biomass of Takanari and NIL-SD1 in 2021

Rice plants were grown in shallow-water (SW) and deepwater (DW). Error bars indicate standard deviation. The statistical difference between Takanari (black) and NIL-SD1 (gray) was analyzed by the Student's t-test ($p < 0.05$, n = 3).

Table A1. Comparison of total nitrogen (N) and carbon (C) input for green manure (GM) and chemical fertilizer (CF) plots

		Total N input (kg N 10a^{-1})	Total C input (kg C 10a^{-1})
GM	Hairy vetch	7.05	98.65
	Oat	4.66	157.18
	Total	11.71	255.83
CF		6.0	-