

Growth and Characteristics of Fructan-Accumulating Transgenic Rice: Potential for Utilization in Forage

Midori Yoshida^{1*} • Akira Kawakami² • Yutaka Sato¹

¹ National Agricultural Research Center for Hokkaido Region, Hitsujigaoka 1, Sapporo, 062-8555, Japan

² National Agricultural Research Center, Kannondai, Tsukuba, 305-8666, Japan

Corresponding author: * midori@affrc.go.jp

ABSTRACT

The feasibility of using rice as whole crop silage and biomass material has recently been investigated in Japan. Some plants in Graminae such as wheat and temperate grasses accumulate fructan, whereas rice is not able to synthesize fructan. In order to increase the quality of rice for forage and biomass material, we introduced wheat fructosyltransferase genes into rice and succeeded in obtaining transgenic rice that accumulate fructan in tissues such as leaves, sheaths and seeds. A rice transformant with the 1-SST (sucrose:sucrose 1-fructosyltransferase) gene accumulates $\beta(2,1)$ linkage oligomer (DP3-5), and a rice transformant with the 6-SFT (sucrose:fructan 6-fructosyltransferase) gene accumulates mainly $\beta(2,6)$ linkage fructan. A rice transformant carrying both 1-SST and 6-SFT genes showed additional accumulation of fructan with $\beta(2,6)$ -linked fructosyl units attached to bifurcose. When the plants were grown in a greenhouse under summer light conditions, lines expressing 1-SST and both genes accumulated a considerable amount of fructan in tissues, and the content of total soluble sugar also increased in these lines. This fructan-accumulating rice has the potential for utilization for forage in northern regions.

Keywords: biomass, forage, fructan, transgenic rice, wheat fructosyltransferase

Abbreviations: DP, degree of polymerization; HPAEC-PAD, high-performance anion exchanged chromatography with a pulsed amperometric detector

INTRODUCTION

Rice is the most important crop in Japan and Southeast Asia, and its cultivation has recently become important in other countries, such as Australia and U.S.A. Rice has been for long a staple food for a huge population in the world and will also be an important source of carbohydrates as well as wheat in the future. Rice originated from the tropics and they do not synthesize fructan in their tissues through their life cycle.

There are several purposes for producing fructan-accumulating rice. The first is the addition of a functional quality to rice for the human diet. The second is to enhance resistance of rice to environmental stresses, particularly cold stress. We previously reported the enhancement of cold tolerance of rice seedlings by manipulation of fructan accumulation in seedlings (Kawakami *et al.* 2008). The third important target for producing fructan-accumulating rice is improvement of the forage-rice quality. High fructan content in forage is generally suitable for fermentation of silage (Smith *et al.* 1997), and for digestibility (Humphreys 1989) and palatability (Mayland *et al.* 2000) for livestock. The final purpose for producing rice forage in Japan is related to the problems of a low level of self-sufficiency in domestic forage and over-production of rice. The amount of rice consumed in Japan has been decreasing in recent years. The government is currently promoting reduction of rice cultivation acreage. Other crops are now being cultivated in those areas, but 400,000 ha still remain as fallow paddy fields. Utilization of those fields for cultivation of forage rice is now being promoted (Kato 2008). Rice grains and whole crop silage of rice are now being used for livestock feed (Ennahar *et al.* 2003; Kato 2008) and biomass material (Hideno *et al.* 2009) in Japan. In this report, we introduce basic data on the growth of fructan-accumulating transgenic rice and describe the characteristics of fructan-accumulating rice to show the feasibility of using rice as whole crop

silage and biomass material.

MATERIALS AND METHODS

Japonica rice, cv. 'Yukihikari' (*Oryza sativa* L.) with moderately cold tolerance was used as the non-transgenic plant. The method for producing a transformant that expresses the wheat 1-SST gene (*wft2*, AB029888) or 6-SFT (*wft1*, AB029887) (Kawakami and Yoshida 2002) was previously described by Kawakami *et al.* (2008). In addition, a transformant that expresses two wheat fructosyltransferases, 1-SST and 6-SFT, was produced in this study. For expression of both genes, the DNA sequence of maize ubiquitin-promoter-driven 1-SST gene (*wft2*) with the terminator of neomycin phosphotransferase gene was inserted upstream of the enhancer of 35S promoter-driven 6-SFT gene (*wft1*) in the same vector (Fig. 1). Seventeen lines of self-pollinated homozygous plants expressing a single gene (*wft2*, five I lines; *wft1*, five S lines) or both genes (*wft1* and *wft2*, seven S/F lines) were successfully obtained and used for examinations. The T₃ homo lines and the original cultivar plants were seeded and grown in a phytotron with sunlight. Temperatures were artificially controlled at 25°C/19°C (day/night) (15-h photoperiod). Plant growth of three independent plants of each line was measured at about 1 week-interval and panicles (seeds) were harvested after they were fully ripened. Sampling for analysis of carbohydrate was carried out at 1 p.m. in summer. The light intensities in the phytotron during the examination period were about 1250-1550 $\mu\text{E}/\text{m}^2/\text{S}$ at 1 p.m. under a sunny condition. Extraction and measurement of soluble sugar were carried out as previously described by Yoshida *et al.* (1998). Analysis of fructan composition was carried out by using a HPAEC-PAD (Dionex, DX500) with a CarboPack PA1 column (Dionex). Peaks of glucose, fructose, sucrose, 1-kestose, 1-nystose and 1-fructosyl-nystose were identified by authentic standards (Wako, Osaka, Japan) and by purified 6-kestose (Iizuka *et al.* 1993) as shown by Kawakami *et al.* (2008). Other unidentified peaks were compared with wheat fructans (Kawakami and Yoshida 2005).

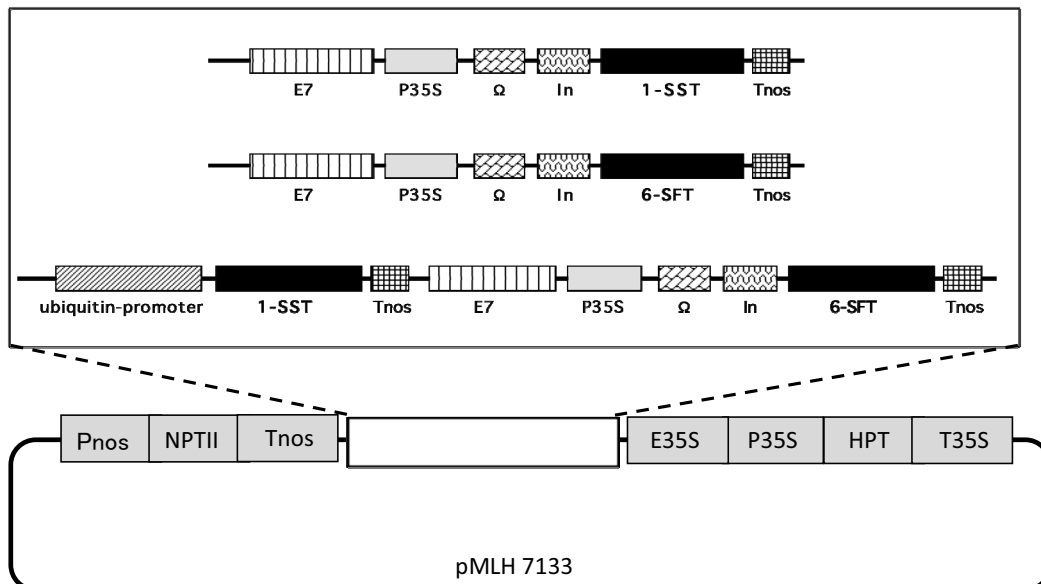


Fig. 1 Vector construction for rice transformation. P35S, CaMV 35S promoter core (-90 to -1); E7, seven-times repeats of enhancer in the 5' upstream region of CaMV 35S promoter; In, phaseolin first intron; Ω, TMV 5' upstream region; T35S, CaMV 35S terminator. Tnos, terminator of neomycin phosphotransferase gene in Ti plasmid; NPTII, neomycin phosphotransferase gene; 1-SST, coding region of sucrose:sucrose 1-fructosyltransferase; 6-SFT, coding region of sucrose:fructan 6-fructosyltransferase. The pMLH7133 vector was provided by Dr. Mitsuahara, National Institute of Agrobiological Science (Mitsuahara *et al.* 1996).

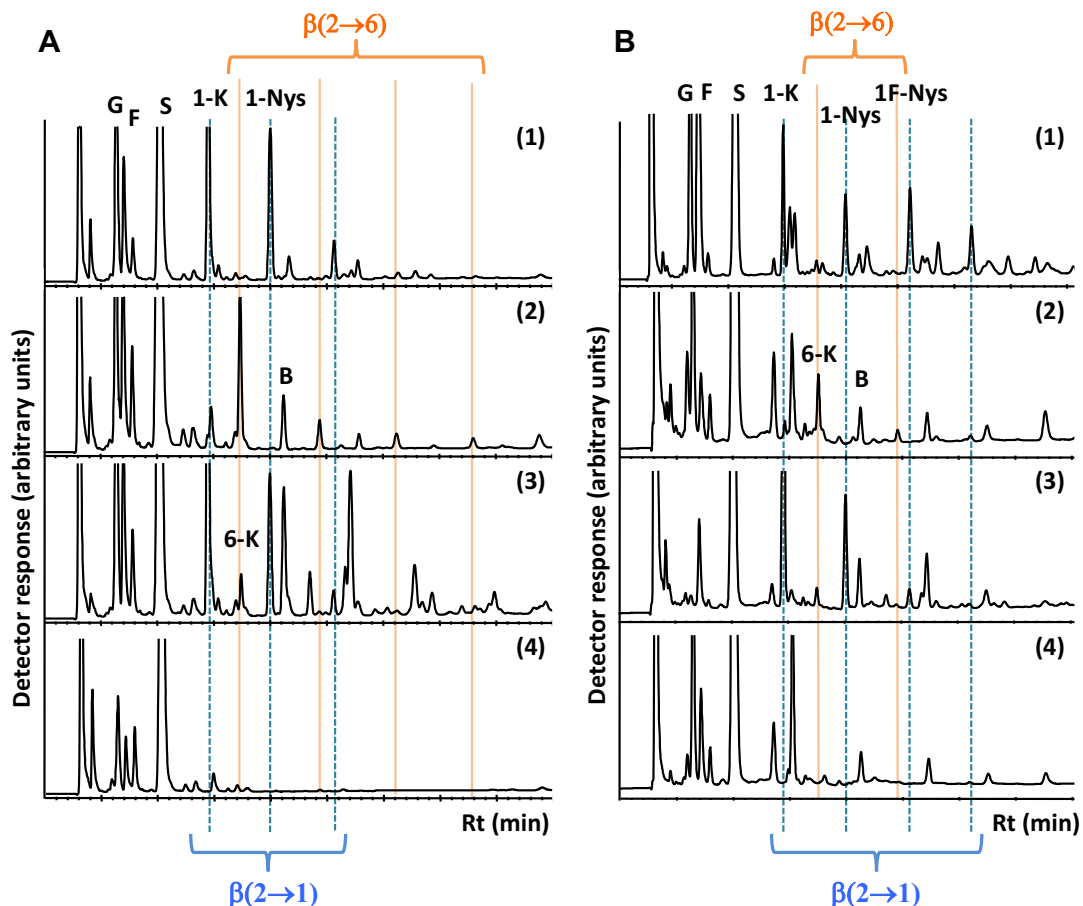


Fig. 2 HPAEC-PAD chromatogram of sugar composition of mature leaf blades (A) and seeds (B) of non-transgenic 'Yukihikari' (4) and transgenic rice plants expressing 1-SST (1), 6-SFT (2) and both 1-SST and 6-SFT (3). G, glucose; F, fructose; S, sucrose; 1-K, 1-kestose; 6-K, 6-kestose; 1-Nys, 1-nystose; 1F-Nys, 1-fructosylnystose; B, bifurcose. Peaks with blue lines and orange lines are $\beta(2,1)$ linkage and $\beta(2,6)$ linkage of fructan, respectively. Rt, retention time.

RESULTS

Fructan composition of transformant rice plants

Fig. 2 shows chromatographs of typical compositions of fructans in mature leaf blades and seeds in three different transformant lines. Fructan was accumulated in sheaths of

transformants (data not shown). In the leaf blade of 1-SST-expressing lines (1 lines in subsequent figures and tables), large amounts of 1-kestose and 1-nystose were accumulated and higher DP $\beta(2,1)$ -linked fructans, at least DP 7, were detected. On the other hand, in 6-SFT-expressing lines (S lines in subsequent figures and tables), a large amount of 6-kestose was detected and bifurcose was also produced.

Though detectable peaks were small, $\beta(2,6)$ -linked fructans for which polymerizations were over DP 15 were also produced (data not shown) (Kawakami *et al.* 2008). In lines expressing both genes-, 1-SST and 6-SFT (S/F lines in subsequent figures and tables), considerable amounts of 1-kestose, 1-nystose and bifurcose were detected, whereas the level of 6-kestose was very low compared with those three peaks in the chromatograph. The small peak with a right-end blue line in **Fig. 2A** is 1-fructosyl-nystose. The next two groups of three peaks may be kestopentaoses and kestohexaoses with both $\beta(2,1)$ and $\beta(2,6)$ linkages as estimated from the chromatograph of wheat fructan for which compositions were previously reported (Bancal *et al.* 1993; Kawakami and Yoshida 2005). These data indicate that rice transformants expressing both 1-SST and 6-SFT accumulated graminan type of fructan (**Fig. 2A**).

Fig. 2B shows chromatographs of fructan composition in seeds. Although some unidentified peaks of oligo- and polysaccharides that are not found in a chromatograph of leaves were detected even in non-transgenic plant seeds, identified fructan peaks (e.g. 1-kestose, 1-nystose and 1-fructosyl-nystose) were detected in transformant seeds. Compositions of accumulated fructans were similar to those of leaves.

Growth and morphology of fructan-accumulating rice lines

(1) Plant length, leaf blade length, tiller number and flowering day

Finally screened fructan-accumulating rice showed no morphological abnormalities, and most of the plants grew and ripened normally except a weakness of some seedlings of the transformant lines expressing both genes (e.g. S/F10-2-2 and S/F30-3-1) was observed. **Fig. 3** shows photographs of transformants and the original cv. 'Yukihikari' at the booting and the ripening stage. Heading and flowering day were delayed in many fructan-accumulating transgenic lines (**Table 1**). **Figs. 4** and **5** show growth of transformant lines expressing a single enzyme (1-SST or 6-SFT) and both enzymes (1-SST and 6-SFT), respectively. No significant differences were observed in plant lengths, but the lengths of flag leaf blades or penultimate leaf blades of some lines expressing a single gene were larger than those of the non-transgenic original plants and two of those lines had one more leaf before the final flag leaf (**Fig. 4B**). The number of tillers of an individual plant varied among the lines, but those of many transformant lines were less than those of the non-transgenic plants (**Figs. 4C, 5B**).

(2) Number of branches, ripening rate and seed weight

The morphologies of panicles and seeds of transgenic lines also appeared normal (Kawakami *et al.* 2008), but the numbers of branches of a panicle, ripening rates and seed weights of transgenic lines decreased slightly (**Table 2**). Gene mutation by transformation generally results in a decrease in the ripening rate of rice. It is not clear whether these differences are due to the influence of fructan accumulation or transformation itself. Decrease in total harvest seed weight might be due to the decrease in the numbers of tillers of transformants. Germination of seeds of almost all transformant lines was also normal. However, weakness of seedling growth in the early stage was observed in some lines expressing both genes together as described before.

Sugar and total mass quality

(1) Soluble sugar content of tissue

Fig. 6 shows water-soluble sugar contents of mature leaf blades (penultimate leaves) of transgenic lines. When the plants were grown in a phytotron under summer light con-

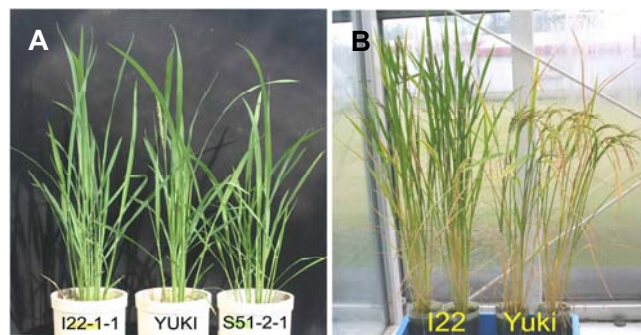


Fig. 3 Photographs of non-transgenic ('Yukihikari') and transgenic rice plants (I22, 1-SST expressing line; S51, 6-SFT expressing line) at the booting stage (A) and ripening stage (B).

Table 1 Difference in the periods from seeding to heading or flowering of non-transgenic 'Yukihikari' and fructan-accumulating rice. Plants of single gene, 1-SST (I) or 6-SFT (S), expressing lines (1) and double genes, 1-SST and 6-SFT (S/F), expressing lines (2) were sown in a phytotron on August 29 and June 27, 2006, respectively. Data were obtained from primary stems of three plants (mean \pm SD).

	Days from seeding	
	Heading	Flowering
(1)		
Yukihikari	58.3 \pm 2.3	59.3 \pm 2.3
I 16-2-1	61.0 \pm 1.7	62.7 \pm 0.6
I 22-1-1	63.7 \pm 1.2	64.7 \pm 1.2
I 24-6-1	60.3 \pm 1.2	62.0 \pm 0
I 29-5-1	65.3 \pm 3.5	66.7 \pm 3.1
I 31-10-1	58.7 \pm 0.6	61.0 \pm 1.7
S 33-5-1	59.3 \pm 1.5	61.0 \pm 1.7
S 50-5-1	61.0 \pm 0	62.0 \pm 0
S 51-2-1	61.0 \pm 0	62.0 \pm 0
S 24-2-5	60.0 \pm 1.7	62.3 \pm 0.6
S 38-5-1	61.0 \pm 0	62.0 \pm 0
(2)		
Yukihikari	65.3 \pm 2.3	66.3 \pm 2.3
S/F 10-2-4	68.0 \pm 0	69.0 \pm 0
S/F 11-3-4	66.3 \pm 1.5	67.0 \pm 1.7
S/F 22-1-1	64.6 \pm 1.2	65.3 \pm 0.6
S/F 26-1-1	68.3 \pm 0.6	69.3 \pm 0.6
S/F 30-1-1	67.0 \pm 2.6	68.0 \pm 2.6
S/F 30-3-1	68.3 \pm 3.5	69.3 \pm 3.5

ditions, fructan contents in leaves of the transgenic lines with wheat 1-SST and with both genes ranged 17-23 mg and 26-37 mg/g fresh weight, respectively. The content of total soluble sugars also increased in these lines. On the other hand, levels of fructan accumulation in the transgenic lines with wheat 6-SFT gene were low (about 2-7 mg/g fresh weight). Similar differences between introduced genes were obtained for other tissues (data not shown). In rice, sucrose content is higher in source tissues and the content is decreasing in sink tissues. Fructan contents in transformant lines were also higher in source tissues and lower in sink tissues. For example, fructan contents of the sheaths of flag leaves and primitive panicles of I22-1-1 lines at the booting stage were about 7-10 mg/g fresh weight.

(2) Total plant weight

Fig. 7 shows fresh weight and dry weight of a whole plant just after flowering. The values varied among transformant lines, but a decrease in weight mass was mainly observed in lines expressing both genes.

DISCUSSION AND CONCLUSION

Success in genetic engineering for synthesis of fructan in rice may have great benefits for human or animal health because of the status of rice in foods in the world. We showed that the amount of fructan accumulation was large in vegetative tissue of rice when a suitable promoter was

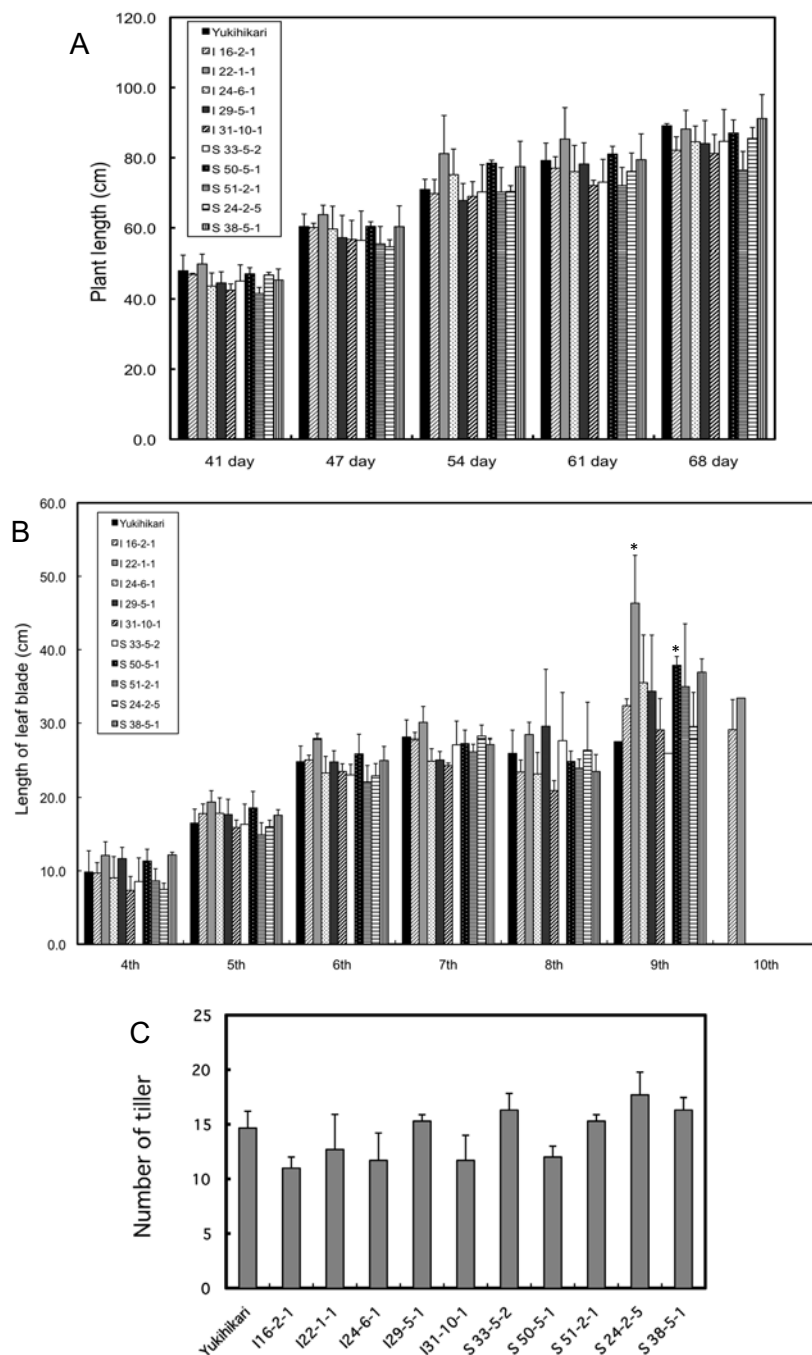


Fig. 4 Changes in lengths of individual plants (A), final length of each leaf blade (B) and final number of tillers (C) of rice lines expressing a single gene, 1-SST (I line) or 6-SFT (S line), and non-transgenic plants ('Yukihihikari'). The plants were sown on August 29, 2006 in a phytotron. X-axes of A and B indicate growth days and leaf number, respectively. Vertical bars represent the standard deviations of three plants. Significant differences between the data for transgenic lines and data for non-transgenic rice ('Yukihihikari') were analyzed by Student's *t*-test. Asterisk indicates the value at $P < 0.01$.

used. Fructan accumulation was greater in the line expressing both genes (wheat 1-SST gene and 6-SFT gene), but the manipulation of the 1-SST gene also resulted in a large amount of fructan accumulation and synthesis of $\beta(2,1)$ -linked oligo-fructan. The 6-SFT uses fructan rather than sucrose as a fructosyl acceptor. That might be the reason why lower levels of fructan in transformants expressing 6-SFT were observed. In our experiment, the levels of fructan were higher in source tissues. This is due to high sucrose content in rice source tissues. Fructan accumulation levels of 20 to 30 mg in tissues of plants that do not accumulate fructan originally are very high compared with the levels of other transgenic plants having fructosyltransferase, although an exception was reported in root tissues of transgenic beet (Sévenier *et al.* 1998, Weyens *et al.* 2004). Fructan composition and accumulation of a single gene (1-SST or 6-SFT)-expressing transgenic rice were discussed in detail in our previous paper (Kawakami *et al.* 2008). Trans-

genic rice lines expressing both genes also accumulated fructan oligomers *in vivo*. It has been proved that purified chicory root 1-SST possesses 1-FFT activity *in vitro* (Van den Ende and Van Laere 1996), and Fig. 2A shows that transformants expressing both 1-SST and 6-SFT produced putative kestopentaoses and kestohexaoses with both $\beta(2,1)$ and $\beta(2,6)$ linkages similar to wheat fructans (Yoshida *et al.* 2007). These results are important for understanding the characteristics of wheat fructosyltransferase *in vivo* (Yoshida *et al.* 2007).

Morphological abnormalities were not observed in finally screened transformant lines. It has been reported that rice have some invertase possessing fructan exohydrolase activity (Ji *et al.* 2007). Thus, fructan that were accumulated in rice vacuoles might play a role of a temporary carbohydrate storage as energy source like in wheat and temperate grasses. In this study, we reconfirmed that high levels of fructan accumulation in rice tissues have sig-

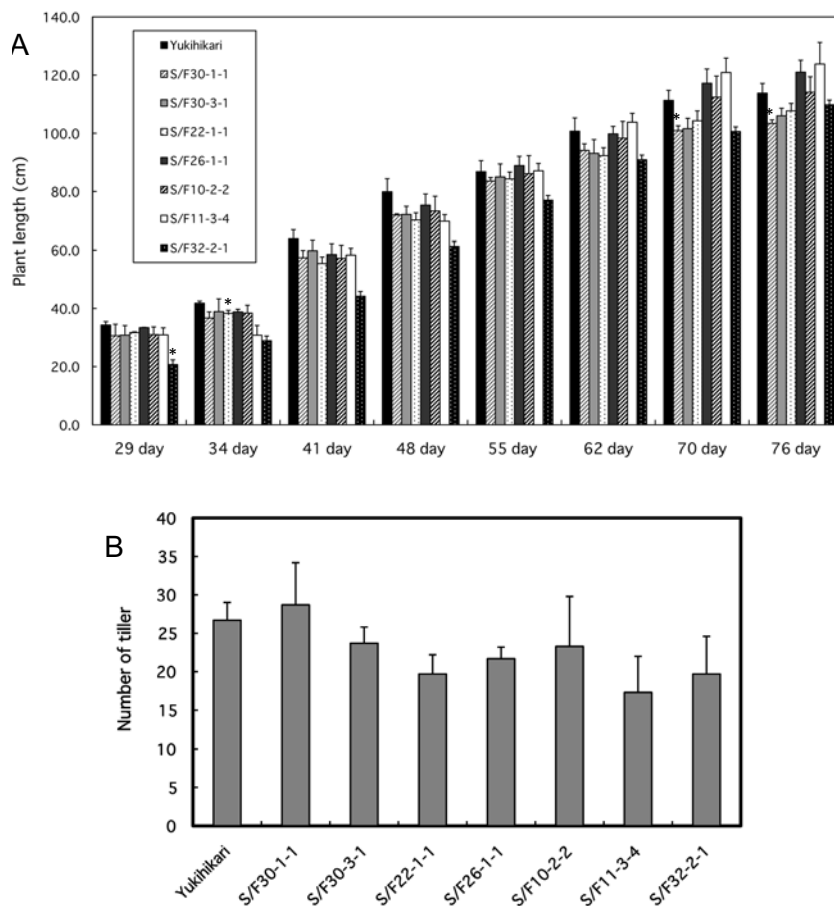


Fig. 5 Changes in lengths of individual plants (A) and number of tillers (B) of rice lines expressing both genes, 1-SST and 6-SFT (S/F line), and non-transgenic plants ('Yukihikari'). The plants were sown on June 27, 2006 in a phytotron. The x-axis of (A) indicates growth days. Vertical bars represent the standard deviations of three plants. Significant differences between the data for transgenic lines and data for non-transgenic rice ('Yukihikari') were analyzed by Student's *t*-test. Asterisk indicates the value at $P < 0.01$.

Table 2 Number of branches of a panicle, ripening rate, seed weight and total seed harvest of non-transgenic 'Yukihikari' and transgenic rice plants of single gene, 1-SST (I) or 6-SFT (S), expressing lines (1) and double gene, 1-SST and 6-SFT (S/F), expressing lines (2) were sown in a phytotron on August 8, 2008 and June 27, 2006, respectively. Data presents mean \pm SD (3 plants). *Data was obtained from one plant.

	Number of branches	Ripening rate (%)	Seed weight (g/100 seeds)	Total seed harvest (g/plant)
(1)				
Yukihikari	20.5 \pm 7.6	84.7 \pm 19.1	2.07 \pm 0.02	23.7 \pm 0.38
I 16-2-1	21.4 \pm 7.5	73.4 \pm 17.5	2.01 \pm 0.03	17.4 \pm 4.23
I 22-1-1	25.9 \pm 10.0	84.7 \pm 9.7	1.85 \pm 0.03	19.7 \pm 0.52
I 24-6-1	15.7 \pm 6.9	63.6 \pm 13.4	1.63 \pm 0.02	9.9 \pm 2.30
I 31-10-1	21.3 \pm 6.9	56.5 \pm 23.4	1.71 \pm 0.02	11.8 \pm 4.27
S 50-5-1	20.7 \pm 8.0	83.5 \pm 9.8	2.00 \pm 0.03	20.0 \pm 0.92
S 51-2-1	19.9 \pm 8.9	63.1 \pm 13.0	1.62 \pm 0.02	14.5 \pm 0.98
S 24-2-5	21.5 \pm 6.9	81.3 \pm 7.7	2.08 \pm 0.01	19.6 \pm 1.19
S 38-5-1	17.6 \pm 8.5	76.3 \pm 12.1	2.00 \pm 0.01	14.1 \pm 2.29
(2)				
Yukihikari	25.2 \pm 8.7	85.4 \pm 14.7	1.96 \pm 0.07	37.8 \pm 2.7
S/F26-1-1	22.4 \pm 7.8	80.1 \pm 13.0	1.83 \pm 0.05	31.8 \pm 3.0
S/F10-2-2	27.6 \pm 8.0	66.8 \pm 11.7	1.98 \pm 0.05	32.1*
S/F30-1-1	18.7 \pm 10.3	80.4 \pm 13.4	1.70 \pm 0.02	34.2*
S/F30-3-1	19.7 \pm 9.6	81.8 \pm 12.1	1.79 \pm 0.03	29.7 \pm 0.2
S/F22-1-1	21.3 \pm 9.0	37.7 \pm 21.5	1.45 \pm 0.04	12.5 \pm 2.0

nificant unfavorable effects neither on rice growth and maturity nor on seed germination under a controlled environment.

Our results suggest that fructan-accumulating rice might become high-quality forage. However, further studies to evaluate basic forage qualities, starch content and cell wall

composition of fructan-accumulating rice are needed. We have also reported enhancement of the cold tolerance of rice seedlings by fructan (Kawakami *et al.* 2008). Direct seeding of rice has been necessary for reducing costs and saving labor for rice cultivation. However, in northern regions, there are still some problems for direct seeding. One problem is exposure to low temperatures (below 10°C) at the early growth stage in spring. It might be possible to unravel this problem by using fructan-accumulating rice. We observed a decrease in harvested seeds of our transformants. This might be influenced by the transformation itself or gene mutation during cell culture. As we know, technical improvements or backcrossing with non-transgenic plants is still needed. On the other hand, rapid advances for the increase of total harvest have also recently been made in conventional breeding for forage rice. Although our research is still at the primary step, fructan-accumulating rice might be a good candidate not only for forage but also for biomass material.

ACKNOWLEDGEMENTS

We are grateful to Dr. Masaru Iizuka, Kobe Shoin Women's University, Japan for providing the 6-kestotriose used as a standard for identification, and we also thank Dr. Mitsuhashi for providing pMLH7133 vector. This research was partially supported by a Grant-in-Aid for Scientific Research (2006) from the Ministry of Education, Science, Sports and Culture (No.17580241).

REFERENCES

- Bancal P, Gibeaut DM, Carpita NC (1993) Analytical methods for the determination of fructan structure and biosynthesis. In: Suzuki M, Chatterton NJ (Eds) *Science and Technology of Fructan*, CRC Press, Boca Raton, USA, pp 83-118

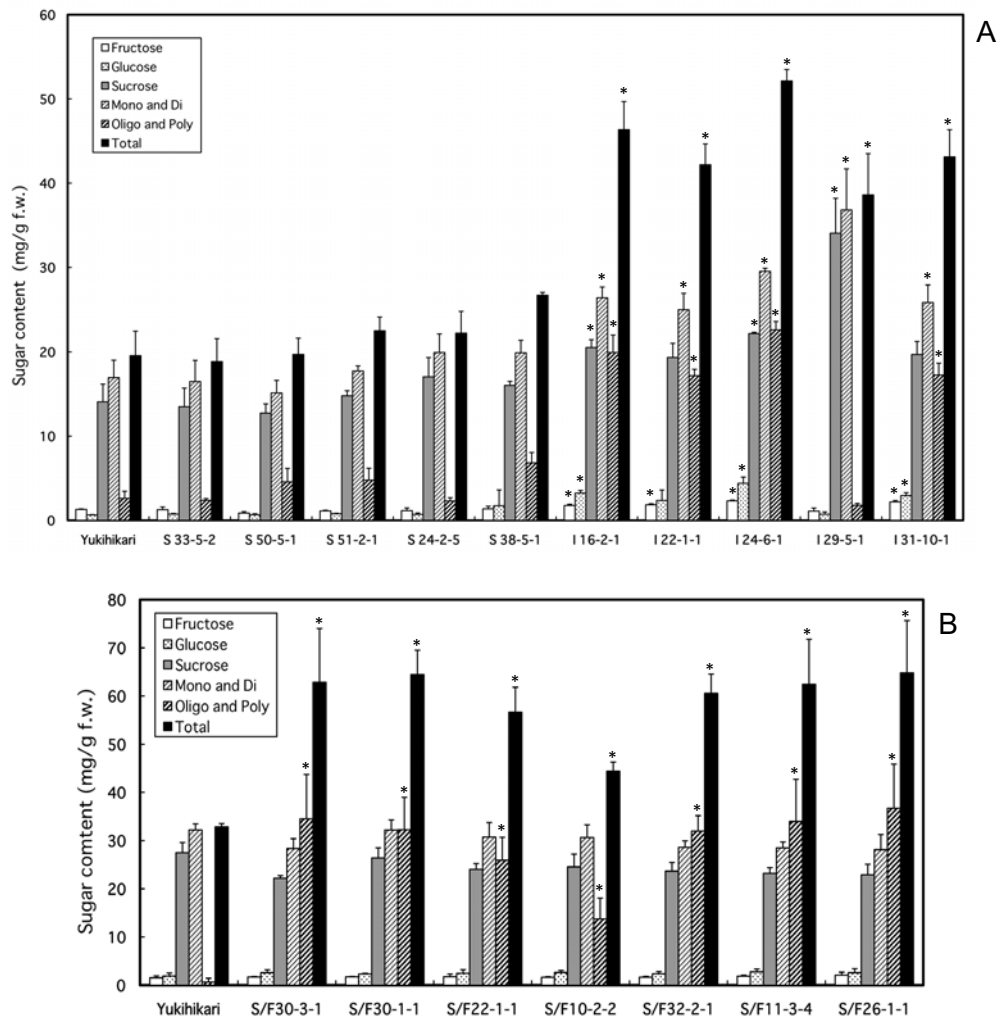


Fig. 6 Water-soluble sugar contents of penultimate leaves of fructosyltransferase-expressing rice lines and non-transgenic plants ('Yukihikari') at the flag leaf stage. Plants of single gene, 1-SST (I line) or 6-SFT (S line), -expressing lines (A) and double genes, 1-SST and 6-SFT (S/F line), -expressing lines (B) were sown in a phytotron on August 29 and June 27, 2006, respectively. Mono and Di, total sugar content of mono- and disaccharides; Oligo and Poly, total sugar content \geq DP3; Total, total water-soluble sugar content. Vertical bars represent the standard deviations of three plants. Significant differences between the data for transgenic lines and data for non-transgenic rice ('Yukihikari') were analyzed by Student's *t*-test. Asterisk indicates the value at $P < 0.01$.

Ennahar S, Cai Y, Fujita Y (2003) Phylogenetic diversity of lactic acid bacteria associated with paddy rice silage as determined by 16S ribosomal DNA analysis. *Applied and Environmental Microbiology* **69**, 444-451

Hideno A, Inoue H, Tsukahara K, Fujimoto S, Minowa T, Inoue S, Endo T, Sawayama S (2009) Wet disk milling pretreatment without sulfuric acid for enzymatic hydrolysis of rice straw. *Bioresource Technology* **100**, 2706-2711

Humphreys MO (1989) Water-soluble carbohydrate in perennial ryegrass breeding: III. Relationships with herbage production, digestibility and crude protein-content. *Grass and Forage Science* **44**, 423-430

Iizuka M, Yamaguchi H, Ono S, Minamiura N (1993) Production and isolation of levan by use of levansucrase immobilized on the ceramic support SM-10. *Bioscience, Biotechnology and Biochemistry* **57**, 322-324

Ji X, Van den Ende W, Schroeven L, Clerens S, Geuten K, Cheng S, Bennett J (2007) The rice genome encodes two vacuolar invertases with fructan exohydrolase activity but lacks the related fructan biosynthesis genes of the Pooideae. *New Phytologist* **173**, 50-62

Kato H (2008) Development of rice varieties for whole crop silage (WCS) in Japan. *Japan Agricultural Research Quarterly* **42**, 231-236

Kawakami A, Sato Y, Yoshida M (2008) Genetic engineering of rice capable of synthesizing fructans and enhancing chilling tolerance. *Journal of Experimental Botany* **59**, 793-802

Kawakami A, Yoshida M (2002) Molecular characterization of sucrose:sucrose 1-fructosyltransferase and sucrose:fructan 6-fructosyltransferase associated with fructan accumulation in winter wheat during cold hardening. *Bioscience, Biotechnology and Biochemistry* **66**, 2297-2305

Mayland HF, Shewmaker GE, Harrison PA, Chatterton NJ (2000) Non-structural carbohydrates in tall fescue cultivars: relationship to animal preference. *Agronomy Journal* **92**, 1203-1206

Kawakami A, Yoshida M (2005) Fructan:fructan 1-fructosyltransferase, a key

enzyme for biosynthesis of graminan oligomers in hardened wheat. *Planta*, **21**, 1-15

Mitsuhara I, Ugaki M, Hirochika H, Ohshima M, Murakami T, Goto Y, Katayose Y, Nakamura S, Honkura R, Nishimiya S, Ueno K, Mochizuki A, Tanimoto H, Tsugawa H, Otsuki Y, Ohashi Y (1996) Efficient promoter cassettes for enhanced expression of foreign genes in dicotyledonous and monocotyledonous plants. *Plant Cell Physiology* **37**, 49-59

Sévenier R, Hall RD, van der Meer IM, Hakkert HJC, van Tunen AJ, Koops AJ (1998) High level fructan accumulation in a transgenic sugar beet. *Nature Biotechnology* **16**, 843-846

Smith KF, Reed KFM, Foot JZ (1997) An assessment of the relative importance of specific traits for the genetic improvement of nutritive value in dairy pasture. *Grass and Forage Science* **52**, 167-175

Van den Ende W, Van Laere A (1996) Variation in the *in vitro* generated fructan pattern from sucrose as a function of the purified chicory root 1-SST and 1-FFT concentrations. *Journal of Experimental Botany* **47**, 1797-1803

Weyens G, Ritsema T, Van Dun K, Meyer D, Lommel M, Lathouwers J, Rosquin I, Denys P, Tossens A, Nijs M, Turk S, Gerrits N, Bink S, Walraven B, Lefebvre M, Smeekens S (2004) Production of tailor-made fructans in sugar beet by expression of onion fructosyltransferase genes. *Plant Biotechnology Journal* **2**, 321-327

Yoshida M, Kawakami A, Van den Ende W (2007) Graminan metabolism in cereals: Wheat as a model system. In: Shiomi N, Benkeblia N, Onodera S (Eds) *Recent Advances in Fructooligosaccharides Research*, Research Signpost, Kerala, India, pp 201-212

Yoshida M, Abe J, Moriyama M, Kuwabara T (1998) Carbohydrate levels among winter wheat cultivars varying in freezing tolerance and snow mold resistance during autumn and winter. *Physiologia Plantarum* **103**, 8-16

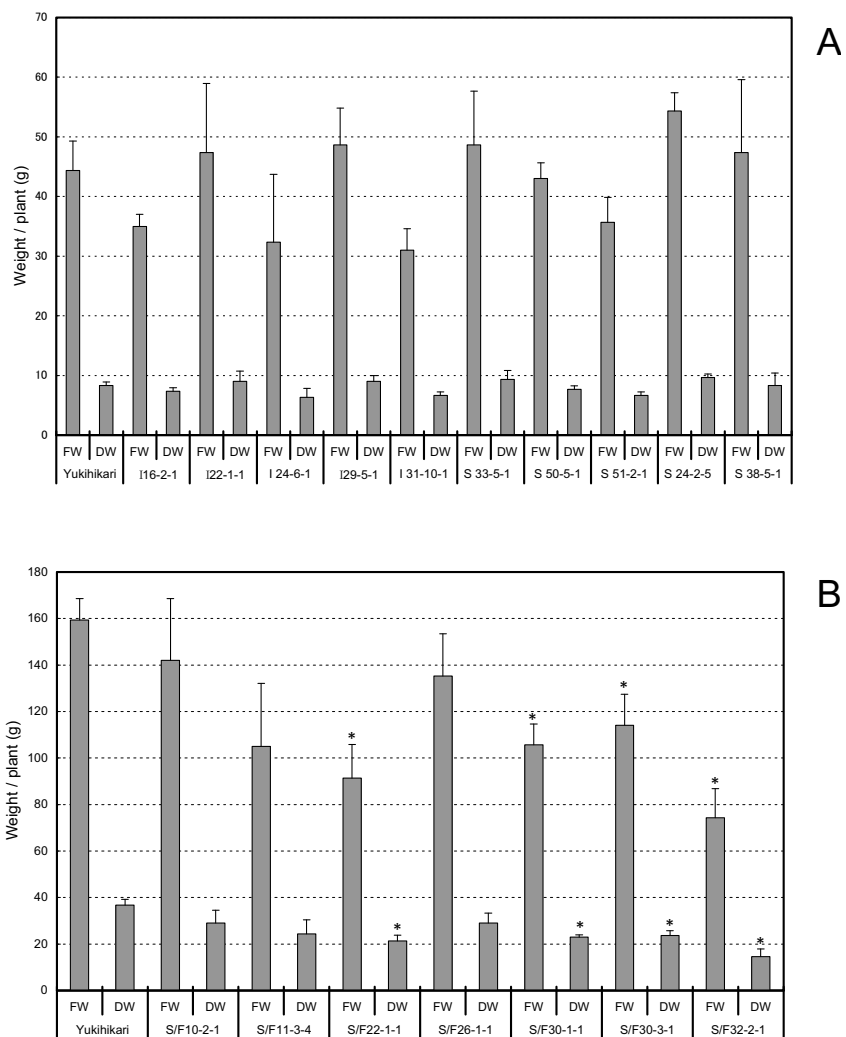


Fig. 7 Fresh weights and dry weights of whole plants of non-transgenic ('Yukihikari') and fructan-accumulating rice just after flowering. Plants of single gene, 1-SST (I line) or 6-SFT (S line), -expressing lines (A) and double genes, 1-SST and 6-SFT (S/F line), -expressing lines (B) were sown in a phytotron on August 29 and June 27, 2006, respectively. Vertical bars represent the standard deviations of three plants. Significant differences of the data of transgenic lines for the data of non-transgenic rice ('Yukihikari') were analyzed by Student's *t*-test. Asterisk indicates the value at $P < 0.01$.