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REVIEW

Research on Fructan in Wheat and Temperate Forage Grasses in Japan

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Abstract

In autumn, winter wheat and temperate forage grasses, such as orchardgrass, timothy, and perennial ryegrass, accumulate fructan, which is a kind of fructose-based oligo- and polysaccharide. The fructan content in their crown tissue reaches more than 30% of their dry weight before snow cover, and this increase in fructan content is associated with both the freezing tolerance and the snow mold resistance of winter crops in the northern region of Japan. These crops mainly accumulate the β(2,6)-linked levan type of fructan, and their structure and composition vary among wheat and grasses. We cloned several kinds of genes encoding fructosyltransferase and fructan exohydrolase from these plants, and analysis of the expression of fructosyltransferase revealed varietal differences in wheat under field conditions and low-temperature responses in grasses. Further investigation of the control of fructan metabolism through molecular biology and genetics should lead to the development of methods for improving the over-wintering ability of these crops.

Discipline: Plant breeding
Additional key words: freezing tolerance, snow mold resistance

Introduction

In northern and sub-arctic agricultural regions, winter cereals and forage grasses are exposed to severe winter conditions, such as minus temperatures and long periods of persistent snow cover. Wheat and temperate forage grasses are very important crops in Hokkaido, the northernmost island of Japan located at about 41° to 45° latitude. There is snow cover for 4-5 months in agricultural fields in Hokkaido[29]. Snow molds, which are psychrophilic fungi, exclusively develop in dark and humid conditions at around 0°C under snow cover[12] and cause fatal damage to crops, resulting in extensive loss of yield. Over-wintering crops in Hokkaido suffer from sub-lethal temperatures before snow cover and from snow mold infection. Therefore, enhancement of both the freezing tolerance and snow mold resistance of these crops for winter survival is one of the main objectives for breeding to increase yields[7].

Plants that can acclimate to the cold increase their freezing tolerance in low temperature conditions. This physiological process is called 'hardening'. Over-wintering plants accumulate carbohydrates in their tissues for energy storage during hardening. Wheat and temperate forage grasses are plants that produce fructan, a fructose-based polysaccharide, as temporary storage for photo assimilates in vacuoles instead of starch in chloroplasts, and they accumulate a considerable amount of fructan in their tissues from autumn to early winter before snow cover. The amount of accumulated fructan and the composition of the fructan have been considered important factors in the wintering ability of these crops involving both freezing tolerance and snow mold resistance[4,13,20,26,29]. In the past decade, many genes of enzymes involved in fructan metabolism have been isolated from wheat (*Triticum aestivum* L.) and temperate forage grasses (e.g., *Lolium perenne* L.) and have been functionally characterized. In this review, we outline the recent advances in research on fructan in wheat and temperate forage grasses from the viewpoint of wintering ability.

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Wheat (*Triticum aestivum* L.)

1. Relationships of fructan accumulation with freezing tolerance and snow mold resistance among wheat cultivars

Both the freezing tolerance and snow mold resistance of field winter wheat increase during hardening and decrease under snow cover[14,29]. The content of non-structural water-soluble carbohydrate in wheat crown tissue has been shown to be associated with winter survival[1]. Yukawa and Watanabe[31] reported the relationships between fructan content and the wintering abilities of winter wheat cultivars in the northern temperate zone with deep snow in winter. Yoshida et al.[29] revealed that the freezing tolerance of wheat cultivars excluding snow mold-resistant cultivars were correlated both with the total content of monosaccharides and disaccharides and with fructan content in crown tissue before snow cover. And it was also shown that snow mold-resistant cultivars continued to accumulate more fructan until snow cover, while freezing-tolerant cultivars ceased accumulating fructan to increase mono- and disaccharides during second hardening[28] with exposure to sub-zero temperatures (Table 1)[29]. Consumption of fructan by highly snow mold-resistant cultivars under snow was shown to be less than that by other cultivars (Table 1)[29]. This variation in the wintering ability of wheat might be caused by the difference in the balance of fructan synthesis and degradation in response to low temperatures[32].

2. Molecular characteristics of genes coding enzymes for fructan metabolism in wheat

Wheat accumulates a mixed type of fructan with β(2,1)- and β(2,6)-linked fructosyl units called graminan[11,30]. Graminans are produced from sucrose by sucrose:sucrose 1-fructosyltransferase (1-SST, EC 2.4.1.99), sucrose:fructan 6-fructosyltransferase (6-SFT), and fructan:fructan 1-fructosyltransferase (1-FFT, EC 2.4.1.100) (Fig. 1), and they are degraded by fructan exohydrolases (1-FEH, EC 3.2.1.153; 6-FEH, EC 3.2.1.154; 6&1-FEH)[11,30]. The degree of polymerization (DP) of wheat fructan is around 3 to 20. Kawakami and Yoshida[8,9] cloned 4 cDNAs that cover all kinds of fructan synthetic enzymes in wheat. Several clones of wheat fructan exohydrolases, 1-FEH[22,25], 6-FEH (6-KEH)[23,24], and 6&1-FEH[10], have been isolated and functionally analyzed. Plant fructosyltransferases and fructan exohydrolases show high homology to vacuolar and cell-wall invertases, respectively (Fig. 2). Kawakami and Yoshida[8] demonstrated that expression levels of *wft1* (6-SFT) and *wft2* (1-SST) in leaf and crown tissue were correlated with fructan accumulation levels both in seasonal changes and varietal differences during hardening. On the other hand, it is known that there are multiple genes for fructan exohydrolases in a plant. The FEH genes encoding enzymes that can degrade the main composition of graminan in wheat have not been reported yet.

Forage grasses

1. Relationships between fructan accumulation and winter hardiness in temperate forage grasses

As in wheat, the amounts and DPs of fructan in temperate forage grasses increase during autumn[15]. The concentration of fructan in timothy (*Phleum pratense* L.) reaches more than 30% of dry weight in early December in Hokkaido (data not shown). Yamamoto et al.[26] reported decreases in phlein [β(2,6)-linked fructan] in timothy and orchardgrass (*Dactylis glomerata* L.) under snow during the frigid winter in Hokkaido. In early spring, fructan in forage grasses is degraded further and is used as an energy reserve for regrowth[19,26]. Degradation of fructan also occurs following defoliation such as cutting[27]. Studies on the ecotypes of orchardgrass showed a significant correlation between fruc-

Table 1. Relationships of fructan contents with wintering abilities among wheat cultivars

Wheat variety	Before snow cover			The end of snow cover	
	Freezing tolerance (LT50, minus °C)	Snow mold resistance	Fructan content in crown (mg/g.fw.)	Consumption of fructan under snow	Fructan content in crown (mg/g.fw.)
Hardy winter cultivars	24 - 25	moderately resistant	70 - 100	very high & moderate	15 - 20
Snow mold-resistant cultivars	16 - 20	highly resistant	90 - 130	very low to high	55 - 80
Japanese snow mold-resistant cultivars	20 - 23	resistant	90 - 110	moderate	35 - 60
Hokkaido leading winter cultivar (1990's)	about 20	moderately resistant	about 65	moderate	about 10
Japanese northern winter cultivars	17 - 19	susceptible	50 - 70	low & moderate	5 - 20
Spring wheat cultivars	< 13	susceptible	30 - 40	very low	0 - 3

The values shown are approximate and refer to Yoshida et al.[29].

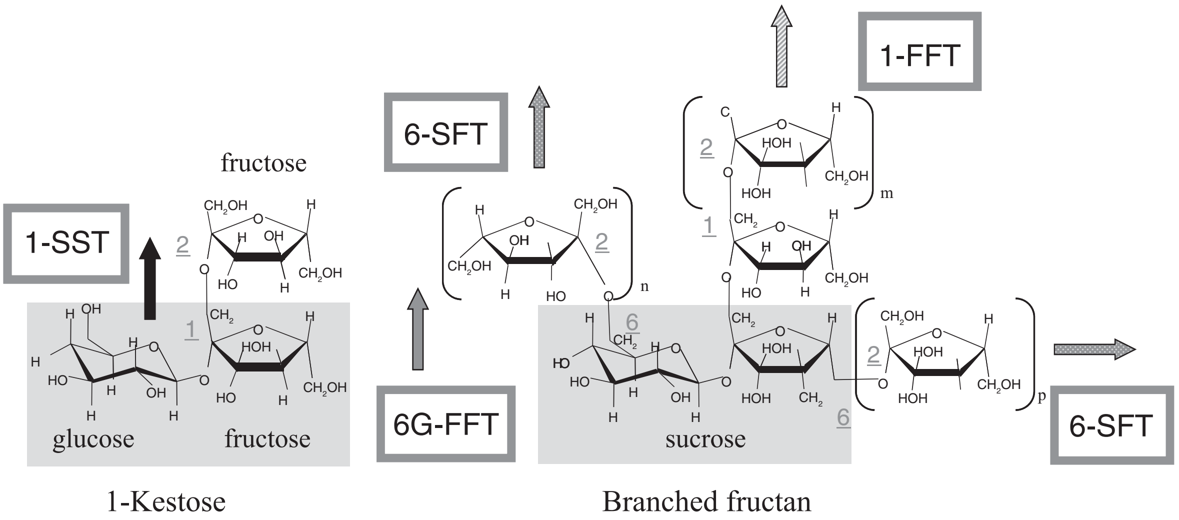

Fig. 1. Structures of fructans in wheat and temperate forage grasses and enzymes of fructan synthesis.
1-SST, sucrose:sucrose 1-fructosyltransferase; 6-SFT, sucrose:fructan 6-fructosyltransferase; 1-FFT, fructan:fructan 1-fructosyltransferase; 6G-FFT, fructan:fructan 6^{G}-fructosyltransferase. Arrows indicate the direction of transferring reaction of each enzyme.

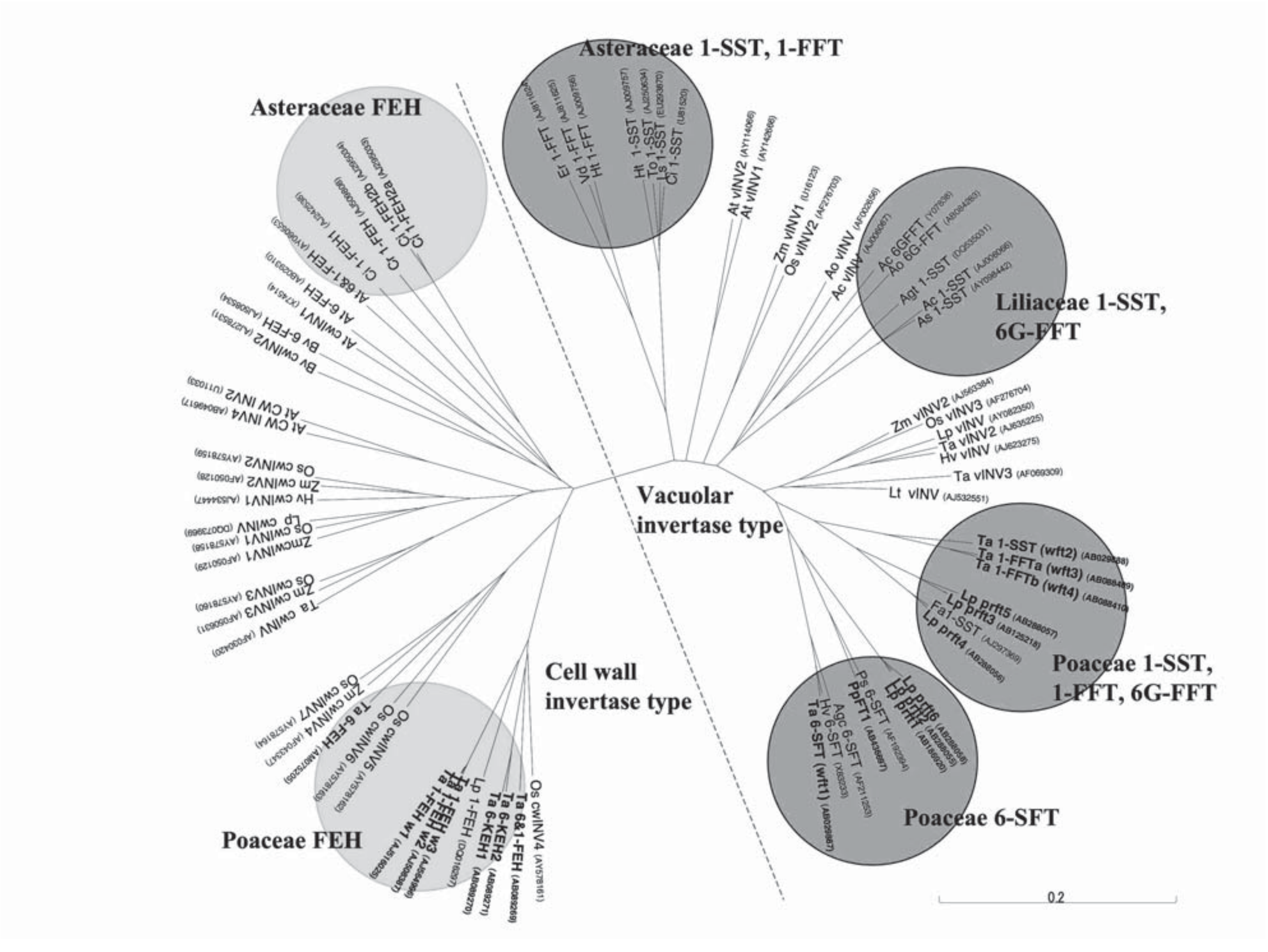

Fig. 2. Phylogenetic tree of amino acid sequences of plant fructosyltransferases and fructan exohydrolases.
Abbreviations for enzyme names not described in the text are: cwINV, cell wall invertase; vINV, vacuolar invertase. Abbreviations for binomial scientific names are: Ac, *Allium cepa*; Agc, *Agropyron cristatum*; Agt, *Agave tequilana*; Ao, *Asparagus officinalis*; As, *Allium sativum*; At, *Arabidopsis thaliana*; Bv, *Beta vulgaris*; Ci, *Cichorium intybus*; Cr, *Campanula rapunculoides*; Er, *Echinops ritro*; Fa, *Festuca arundinacea*; Ht, *Helianthus tuberosus*; Hv, *Hordeum vulgare*; Lp, *Lolium perenne*; Ls, *Lactuca sativa*; Lt, *Lolium temulentum*; Os, *Oryza sativa*; Pp, *Phleum pratense*; Ps, *Poa secunda*; Ta, *Triticum aestivum*; To, *Taraxacum officinale*; Vd, *Viguiera discolor*; Zm, *Zea mays*.

tan concentration in autumn and winter hardiness[18]. Transgenic perennial ryegrass plants expressing wheat 1-SST and 6-SFT exhibit an increased level of fructan accumulation as well as an increased level of freezing tolerance[5]. These findings provide evidence of an association between fructan accumulation and winter hardiness, including freezing tolerance.

2. Structure and degree of polymerization of fructans in temperate forage grasses

There is variation in the structure of fructan among species of temperate forage grass. Timothy and orchardgrass are known to accumulate a series of simple levans[2,3]. On the other hand, the fructan profile of perennial ryegrass includes levan neoseries and inulin neoseries, which contain some internal glucose molecules in the β(2,6)- or β(2,1)-fructose linkages, in addition to inulins[17]. Precursors of fructan neoseries are produced by fructan:fructan 6^G-fructosyltransferase (6G-FFT, EC 2.4.1.243) catalyzing the transfer of a fructose unit to C6 of a glucose unit of another fructan or sucrose (Fig. 1). DPs of fructans accumulated in temperate forage grasses also vary greatly among species[16,20]. The HPLC profile of carbohydrates in crown tissue in late autumn indicates that timothy accumulates fructans with DPs higher than those of other species, while fructans in perennial ryegrass and meadow fescue (*Festuca pratensis* Huds.) contains low DP fructans, which are shown as single peaks in the chromatographs of Fig. 3. Fructan in timothy has been reported to have a DP of up to 90 in leaf tissue[2].

3. Molecular characteristics of genes coding enzymes for fructan metabolism in perennial ryegrass and timothy

Based on the fructan profile, it has been proposed that at least four enzymes are involved in fructan biosynthesis in perennial ryegrass: 1-SST, 1-FFT, 6G-FFT, and 6-SFT (or 6-FFT)[17]. Hisano et al.[6] identified cDNAs of 1-SST (*prft4*), 6G-FFT (*prft3* and *prft5*), and 6-SFT homologues (*prft1*, *prft2* and *prft6*) from perennial ryegrass (Fig. 2). Two distinct patterns of mRNA expression of *prft* genes were observed during cold treatment: levels of mRNA expression of *prft1* and *prft2*, 6-SFT candidates, in leaf and crown tissue increased gradually in parallel with the accumulation of fructans over a period of 30 days, while *prft3* (6G-FFT) and *prft4* (1-SST) transcripts increased abruptly within 24 hours of cold treatment, followed by a decrease after several days of cold treatment, and then increased again during further cold treatment over at least 30 days. These expression patterns are consistent with the roles of (putatively) encoding enzymes in fructan synthesis. Tamura et al.[21] identified a *PpFT1* cDNA encoding an enzyme with 6-SFT activity from timothy. Phylogenetic trees of plant 6-SFTs discriminated *PpFT1* and putative 6-SFT in *Poa secunda*, which accumulates high DP levans, from the *Lolium* and Triticeae 6-SFT groups (Fig. 2). PpFT1 has a high affinity for 6-kestose to produce levans and a low affinity for 1-kestose, different from wheat 6-SFT, Wft1. Moreover, PpFT1 has a higher affinity for fructans with high DPs as acceptors than

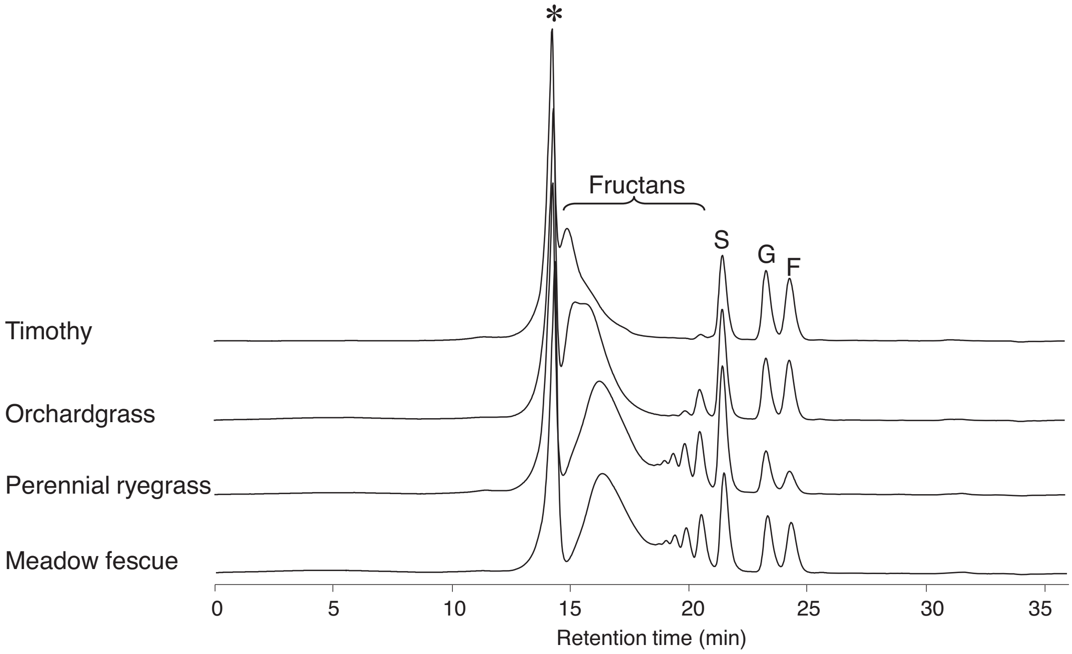

Fig. 3. HPLC profiles of carbohydrates in forage grasses.

Sugar extracts were prepared from crown tissues sampled in November in the field of NARCH, Sapporo, Japan. HPLC analysis was performed using Shodex KS-802 and KS-803 columns. Abbreviations for sugar peaks are: G, glucose; F, fructose; S, sucrose. *Asterisk* indicates unknown peaks deviated from other than carbohydrates.

Wft1 and consequently produces levans with higher DPs. Increase in *PpFT1* transcripts in cold conditions suggests the involvement of *PpFT1* in the accumulation of fructans during cold hardening in timothy.

Conclusion

There is no doubt about the importance of fructan accumulation and consumption for the over-wintering ability of wheat and temperate forage grasses. However, at present, identification of genes for key enzymes and regulators in fructan metabolism has been limited to several species. To improve the freezing tolerance and snow mold resistance of these wintering crops, it is necessary to investigate the gene-expression system in fructan metabolism. Reverse genetic studies, such as transgenic analysis, will reconfirm the physiological roles of fructan in freezing tolerance and snow mold resistance. Moreover, these genes are expected to be candidate genes for quantitative trait loci related to fructan metabolism, which might contribute to the development of beneficial tools for breeding programs.

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