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Studies on Fructan Accumulation in Wheat (*Triticum aestivum* L.)

IV. Fructan accumulation under cold treatments and its varietal difference in relation to the activities of sucrose-sucrose fructosyl transferase and fructan exohydrolase*

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Abstract : The mechanisms of fructan accumulation by changes in sucrose-sucrose fructosyl transferase (SST; EC 2.4.1.99) and fructan exohydrolase (FEH; EC 3.2.1.80) under cold treatments (2°C, 6°C) were investigated using varieties of wheat (*Triticum aestivum* L. cv. Norin 61 and Yukichabo) differing in fructan accumulation. Fructan concentration increased with the increase in SST activity and decrease in FEH activity under cold treatments. The acumulation of fructans was accelerated with high activity of SST at 2°C treatment, suggesting the participation of SST in fructan accumulation. The variety of Norin 61, which has lower fructan accumulation, contained higher activity of FEH, and the fructan concentration of Norin 61 decreased with increasing FEH activity from the 10th day under 6°C treatment, suggesting that the varietal difference in fructan accumulation is affected largely by the level of FEH activity. The relationship between fructan accumulation and growth habit in the varieties is discussed.

Key words : Cold acclimation, Cold hardiness, Fructan, Fructan exohydrolase, Nonstructural carbohydrate, Sucrose-sucrose fructosyl transferase, Wheat

コムギのフルクタン蓄積に関する研究 第4報 低温処理によるフルクタンの蓄積とフルクタン合成および分解酵素との関係およびその品種間差：湯川智行・小林 真**・渡辺好昭***・山本紳朗****（北陸農業試験場，**国際農林水産業研究センター，***東北農業試験場，****帯広畜産大学）

要 旨：秋季におけるフルクタン蓄積量が異なるコムギ2品種（農林61号，ユキチャボ）を用いて，低温処理下（2，6°C）におけるフルクタン合成酵素（スクローススクロースフルクトシルトランスフェラーゼ；EC 2.4.1.99）と分解酵素（フルクタンエキソハイドロラーゼ；EC 3.2.1.80）の変化を調査し，フルクタンの蓄積機構と品種間差の発現機構について検討した．フルクタン含有率は，低温処理による合成酵素活性の増加と分解酵素活性の低下のもとで増加した．特に処理温度の低い2°C条件下でフルクタン含有率が高く，このときに合成酵素活性も高いことから，高濃度のフルクタンの蓄積には高い合成酵素活性が必要と考えられた．また，6°C条件下において，フルクタン含有率の低い農林61号はユキチャボに比較して分解酵素活性が高く，また処理10日目以降のフルクタン含有率の低下と分解酵素活性の増加とが関連することから，品種間差の発現には分解酵素が強く関与していると考えられた．さらに，フルクタンの蓄積と品種の生育特性との関連について論議した．

キーワード：コムギ，低温順化，非構造性炭水化物，フルクタン，フルクタン合成酵素，フルクタン分解酵素

Fructans, the principal storage carbohydrate in many winter cereals and grasses, are accumulated at low temperature in late autumn[1,3,10,12,20]. In the previous papers[22,24], we reported on the varietal differences in concentration and degree of polymerization of fructan of wheat in late autumn. The accumulation of fructans in late autumn has been connected with the increase of cold resistance[13,17] and snow tolerance[19,22]. Therefore, clarification about the mechanisms of fructan accumulation is important to improve the wintering ability of crops.

The synthesis of fructan of trisaccharide and beyond is known to be catalyzed by sucrose-sucrose fructosyl transferase (SST; EC 2.4.1.99) from two sucrose molecules and fructan-fructan fructosyl transferase (FFT; EC

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2.4.1.100), respectively [4,9,11]. The SST is considered to be the most important enzyme in fructan accumulation since it increases concomitantly with fructan accumulation[26] and it is the first enzyme for fructan synthesis[14]. On the other hand, fructan is degraded from the terminal fructose residue by fructan exohydrolase (FEH; EC 3.2.1.80)[9,11,15].

Jeong and Housley[6] reported on changes in SST and FEH activities of wheat at 10°C treatment in connection with fructan accumulation. However, little is known about the fluctuation of these enzymes at lower temperature. Furthermore, varietal difference in SST and FEH at low temperature is not known yet. This report deals with changes in the concentrations of fructans and activities of SST and FEH in two varieties of wheat given cold treatments to clarify the mechanisms of fructan accumulation.

Materials and Methods

1. Plant Materials

Two wheat (*Triticum aestivum* L.) varieties, Yukichabo and Norin 61, known to accumulate large and small amounts of fructan in late autumn, respectively, were used[22,24]. Plants were sown in plastic pots (0.75 L) containing artificial soil for horticulture (Kureha chemical; 0.4 N, 1.5 P_2O_5 and 0.4 K_2O g/kg) and grown in a chamber maintained at 20/15°C (light/dark) with a 16-h photoperiod. Light intensity of 40 W/m^2 at plant surface (1.5 m below lamps) was provided by incandescent lamps (Toshiba D400). Twenty-two days after sowing, the plants were transferred to conditions at a constant 2 or 6°C with the same photo conditions as before.

2. Analysis of fructan concentration and enzyme activity[23,25]

Shoots (blades and sheaths) were sampled for analysis of fructan concentration, as well as of SST and FEH activities. Plant length and weight of shoot were also measured at sampling.

Samples were homogenized with 10% insoluble polyvinylpyrrolidone in 0.2 M K-phosphate (pH 7.4) containing 20 mM 2-mercaptoethanol using a stainless steel grinder. The homogenates were centrifuged for 10 min at 4°C (30,000 g) and each supernatant was divided into two portions. After submerging in boiling water for 3 minutes, one portion was used for the determination of fructan concentration by high-performance liquid chromatography (HPLC), using water as eluant at a flow rate of 1 ml/min. A column with an exclusion limit of 4×10^5 (Shodex KS804, 8 mm$\times$300 m) and two columns with an exclusion limit of 1×10^4 (Shodex KS802, 8 mm$\times$300 mm) were combined and maintained at 65°C. The determination of fructans was conducted by a refractometer (Shodex SE-61) as fructose equivalent. Glucose, fructose and sucrose (mono- and disaccharides) concentration were also measured by the HPLC.

Solid $(NH_4)_2SO_4$ was added to another portion so as to give 80% saturation. The precipitate was collected by centrifugation and dialyzed against 10 mM K-phosphate to obtain crude enzyme. Fructans prepared from wheat (cv. Yukichabo)[8,21] and sucrose were used as substrate for determination of FEH and SST, respectively. After incubation in McIlvaine buffer (pH 5.5) for 4-h at 30°C, the production of trisaccharide and fructose was measured by HPLC for estimation of SST and FEH activities, respectively. HPLC was conducted using a combination of two columns (Shodex KS802, 8 mm$\times$300 mm).

Results

1. Plant growth

The elongation of plant length was suppressed by 2°C and 6°C cold treatments. In Yukichabo the suppression was larger at 2°C than at 6°C, while scarcely any difference was detected between the cold treatments in Norin 61. Plant length of Norin 61 was greater than that of Yukichabo at both cold treatments (Fig. 1-A).

The increase in fresh weight of the shoot was suppressed for 7 days after initiation of 2°C treatment, and thereafter increased. The suppression was not detected by 6°C treatment. Accordingly, the fresh weights of two varieties were larger at 6°C than at 2°C. The fresh weight of Yukichabo was larger than that of Norin 61 (Fig. 1-B).

The increase in dry weight was not suppressed by either of the cold treatments. There was no significant difference between 2°C and 6°C in the two varieties. The dry weight of Yukichabo was larger than that of Norin 61, similarly to the fresh weight (Fig. 1-C).

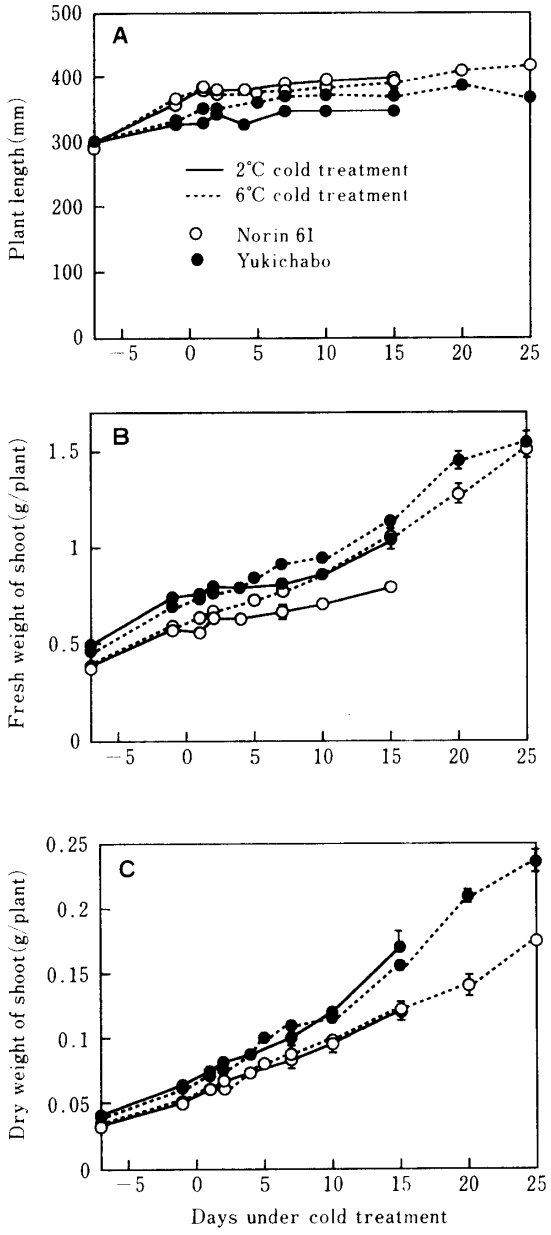

Fig. 1. Changes in plant length (A), fresh weight (B) and dry weight (C) of shoot in two wheat varieties by two cold treatments (2 and 6°C). The bars represent ±SE.

2. Changes in fructan concentration

Fructan concentrations increased rapidly at 2°C treatment and no varietal difference was detected (Fig. 2-A).

By treatment at 6°C, fructan concentration increased in both varieties until the 7th day, but thereafter increased slightly in Yukichabo and decreased in Norin 61 (Fig. 2-B). The maximum value of fructan concentration in Yukichabo and Norin 61 at 6°C was approximately one fourth and one eighth that at 2°C, respectively.

3. Changes in mono- and disaccharide concentration

The concentrations of mono- and disaccharides increased rapidly at 2°C treatment, and a varietal difference was not detected (Fig. 3-A).

In the 6°C treatment, the concentration of these saccharides increased gradually in both varieties until the 7th day, and thereafter increased slightly in Yukichabo and decreased slightly in Norin 61 (Fig. 3-B). The fluctuations of mono- and disaccharides in the two varieties at 6°C were similar to those of fructans. The concentrations of mono- and disaccharides at 2°C were higher than those at 6°C.

4. Changes in SST and FEH by cold treatment

In the 2°C treatments, SST activities in both varieties increased until the 10th day, while FEH activities decreased during that time (Fig. 4-A).

In the 6°C treatment, SST activities in both varieties increased slightly until the 7th day, while FEH activities in two varieties decreased drastically until the 7th day. FEH activity in Norin 61 increased from the 10th day after the on set of 6°C treatment (Fig. 4-B). Both SST and FEH were higher in Norin 61 than in Yukichabo under the two cold treatments.

Discussion

Jeong and Housley[6] indicated that concentration of fructans by 10°C treatment incraesed concomitantly with a decrease in FEH activity and an increase in SST activity. The present experiment also indicated that a similar increase of SST and a decrease of FEH resulted at 2°C and 6°C. Furthermore, the increase in fructan concentration was higher at 2°C than at 6°C under the large increase in SST activities, suggesting that high SST activity is necessary for high fructan accumulation. Santoiani et al.[16] reported that 4°C treatment had no significant effects on FEH activity of wheat root. The difference between Santoiani's and our result seems to be difference in the part of plant or variety used for the experiment.

Varietal difference in fructan concentration was not detected at 2°C treatment under higher activities of SST and FEH in Norin 61

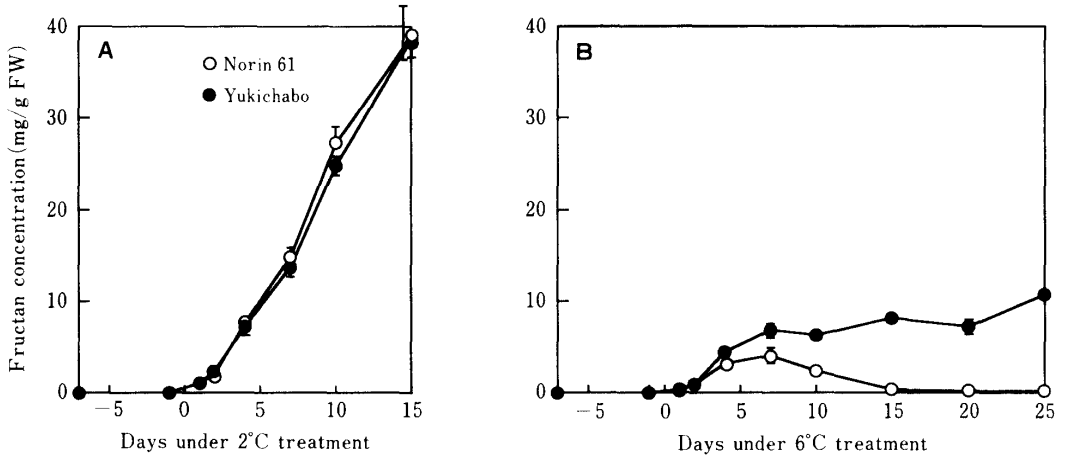

Fig. 2. Changes in fructan concentration in wheat by cold treatment. Values are the means of three replications. The bars represent ±SE.

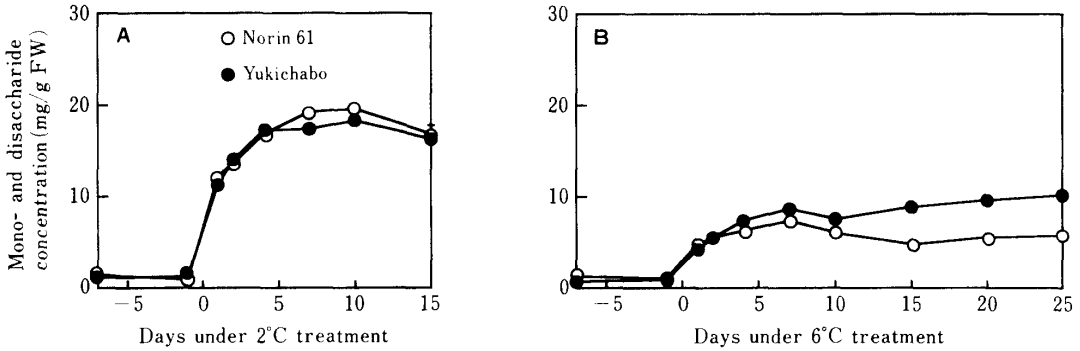

Fig. 3. Changes in concentration of mono- and disaccharides in wheat by cold treatment. The mono- and disaccharides include glucose, fructose and sucrose. Values are the means of three replications. The bars represent ±SE.

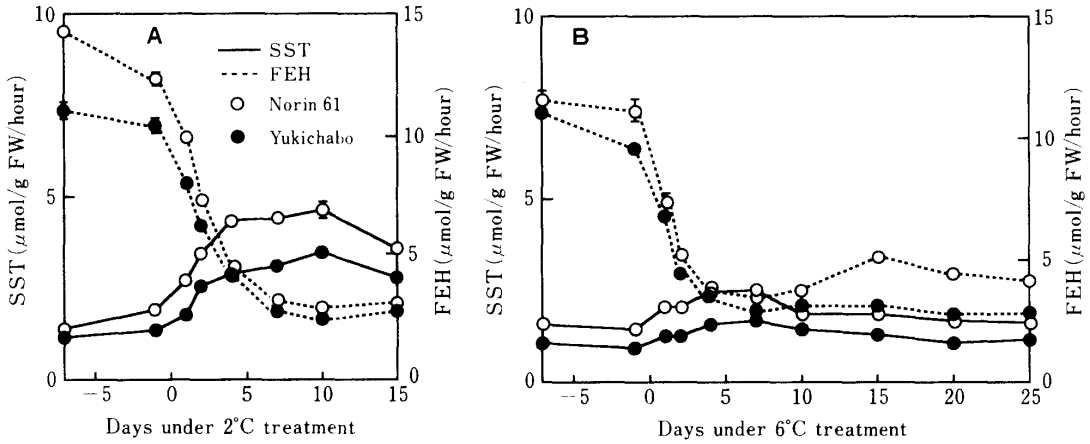

Fig. 4. Changes in SST and FEH activities in wheat by cold treatment. Values are the means of three replications. The bars represent ±SE.

than in Yukichabo, suggesting that much synthesis and hydrolysis occurred in Norin 61. At 6°C treatment, the fructan concentration of Norin 61 was lower than that of Yukichabo under fluctuations of enzyme activities similar to those observed at 2°C, and the concentration of Norin 61 decreased from the 10th day under the increase of FEH activity. The low

level and decrease in fructan concentration in Norin 61 at 6°C seems to correspond with the high level and increase in FEH activity, respectively. Those results suggest that the changes in FEH activity are responsible for the varietal difference in fructan accumulation.

In the present experiment, mono- and disaccharide concentration increased rapidly prior to the increase in fructan concentration, and the fluctuations of mono- and dissaccharides at 6°C were similar to those of fructans, suggesting a close relationship between the concentration of mono- and disaccharides and the accumulation of fructans in wheat. Those results in this experiment are consistent with Housley and Pollock's results[5] using ^{14}C tracer.

Nelson and Spollen[9] reported that fructan-storing but not starch-storing species continue active photosynthesis at low temperatures. In the present study, no difference in dry weight was detected between 2 and 6°C, while concentrations of fructans and mono- and disaccharides were higher at 2°C than at 6°C, suggesting that large amounts of photosynthates were converted to nonstructural carbohydrates at 2°C under little change in the photosynthetic capacity.

Dry matter production of Yukichabo was larger than that of Norin 61 at both temperatures. Takeda[18] reported a varietal difference in photosynthetic ability in winter cereals. The difference in dry matter production also seems to relate to the growth habit of the two varieties, since Norin 61 uses much photosynthate for elongation and Yukichabo accumulates fructans more efficiently contributing to dry matter production.

The present study deals with FEH and SST relating to fructan degradation and synthesis, respectively. For degradation of fructan, we reported in previous paper[25] that barley contains two kind of hydrolase. Wheat also seems to contain two kind of it because of the linkage structure of fructan[2]. In addition, we know participation of FFT for fructan synthesis[7,9]. Further investigation concerning FEH and FFT will be necessary to clarify fructan accumulation in more detail.

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