Fructan Content in *Aegilops cylindrica* and its Relationship to Snow Mold Resistance and Freezing Tolerance

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Snow mold is caused by several different psychrophilic fungi that grow under a persistent snow cover and can extensively damage overwintering crop species (Bruehl, 1982). Resistance to snow mold is an essential trait of winter wheat varieties grown in Hokkaido, Japan. Useful genetic resistance has been identified in a limited number of winter wheat cultivars (Amano and Ozeki, 1981; Bruehl, 1982; Kleijer, 1988; Litschko et al., 1988; Gaudet and Kozub, 1991; Iriki and Kuwabara, 1992; Kuwabara et al., 1996), and these sources are currently employed in breeding programs. Additionally, snow mold resistance has been identified in wheat relatives including *Aegilops/Triticum*. Several *Ae. cylindrica* accessions that exhibit snow mold resistance similar to that of highly resistant wheat cultivar PI 173438, have been identified (Iriki et al. 2001).

The only known physiological association with snow mold resistance to date is the ability of snow mold resistant winter wheat cultivars to accumulate a large amount of carbohydrates, particularly fructans, before snow cover, and to maintain them under the snow throughout winter and early spring (Kiyomoto and Bruehl, 1977; Kiyomoto, 1987; Yoshida et al., 1998). Carbohydrates, particularly simple sugars, have also been implicated in the development of freezing tolerance in winter wheat (Yoshida et al., 1998). The physiological basis of the snow mold resistance in *Ae. cylindrica* has not been characterized yet. If this species possesses a different physiological basis for snow mold resistance compared to wheat, it may be possible to introduce novel snow mold resistance genes and freezing tolerance genes into a wheat background. This could create new genetic combinations and ultimately result in improved snow mold resistance and low temperature tolerance. Here, we report on the contents of simple and complex carbohydrates in *Ae. cylindrica* accessions during the winter, before and under snow, and relate these results to snow mold resistance and freezing tolerance at Sapporo where *Typhula ishikariensis* is the predominant snow mold pathogen.

Materials and Methods

1. Plant materials

To evaluate the relationship between the resistance to snow mold caused by *T. ishikariensis* and fructan content of the plant, we grew nine snow mold resistant *Ae. cylindrica* accessions (KU 2441, KU 2486, 400476, 400477, 400384, 400454, KU 2438, 400452, KU 7-5) (Iriki et al., 2001), six susceptible accessions (00089763, KU2401,KU2418, KU2661, 00089764, 00089770) whose snow mold resistance was considered to be similar to or lower than that of wheat cultivar Chihokukomugi (Iriki et al., 2001), and winter wheat cultivars PI 173438 (snow mold resistant) and Chihokukomugi (susceptible) in 2001, 2002/2003 and 2003/2004 at Sapporo, Japan. Another eight *Ae. cylindrica* accessions (00089775, KU 2421, KU2402, KU2901, PI 2669814, PI 276974, PI 276977, PI 330484.), varying in freezing tolerance (Limin and Fowler, 1985; Iriki, unpublished data) and winter wheat cultivars Valujevskaya (cold hardy), Chihokukomugi (moderately cold hardy) and PI 173438 (cold tender) (Yoshida et al., 1998) were also examined in 2001 to evaluate the association of freezing tolerance and carbohydrate contents. To measure carbohydrate contents, six plants of each accession and cultivar were seeded into a 17cm diameter plastic pot containing commercial potting medium (Hokkai Sankyo, Sapporo, Japan; N 374mg, P₂O₅ 1485mg, K₂O 242mg/kg) and grown outdoors. Seeding dates were September 10, 2001, September 19, 2002, and September 17, 2003, respectively. To evaluate freezing tolerance, we seeded fifty seeds of
Table 1. Fructan contents of *Aegilops cylindrica* accessions and winter wheat cultivars sampled in early December and early March.

| Treatment       | Sampling Month | December | March |
|-----------------|----------------|----------|-------|
| 2001            |                |          |       |
| Resistant group | 65.0 ± 14.9¹   |          | –     |
| Susceptible group | 62.5 ± 10.7    |          | –     |
| PI 173438       | 155.0 ± 4.2    |          | –     |
| Chihokukomugi   | 94.7 ± 7.4     |          | –     |
| 2002/2003       |                |          |       |
| Resistant group | 66.2 ± 10.4    | 43.6 ± 4.4| –     |
| Susceptible group | 62.6 ± 5.9     | 24.9 ± 7.3| –     |
| PI 173438       | 153.8 ± 14.4   | 102.6 ± 8.9| –     |
| Chihokukomugi   | 87.6 ± 3.5     | 33.5 ± 4.5| –     |
| 2003/2004       |                |          |       |
| Resistant group | 69.3 ± 8.2     | 26.6 ± 7.7| –     |
| Susceptible group | 39.9 ± 7.9     | 12.3 ± 4.1| –     |
| PI 173438       | 134.2 ± 8.7    | 52.2 ± 10.1| –     |
| Chihokukomugi   | 67.5 ± 9.2     | 16.6 ± 1.4| –     |

¹ Mean (mg/g · fw) ± standard deviation.

Each accession or cultivar into flats (30 × 45 × 7 cm) containing the commercial potting medium on September 25, 2001, and grew them outdoors.

2. Carbohydrate analysis

At the beginning of December, before the persistent snow cover, in 2001, 2002, and 2003, plants were sampled from pots. The plants under snow cover were sampled again in March, 90 days after the establishment of snow cover in 2002/2003 and 2003/2004. The plants were washed, and, after removing their roots, they were reserved at −80 ºC until use for carbohydrate extraction. Water-soluble carbohydrates (fructan, mono-and disaccharides) were quantified according to Yoshida et al. (1998). Crowns plus stem tissues 1.5 cm in total length were cut into pieces and boiled for 1 h in distilled water containing 1mg/mL³ propylene glycol as internal standard. The extract solution was passed through a 0.45 μm pore filter. Ten µL of the filtrate was then injected into Shodex columns of KS 802 and KS 803 combined (Shodex Co., Ltd., Tokyo) warmed at 50ºC for analysis by HPLC. Deionized water was used as solvent at a flow rate of 0.8 ml min⁻¹, and carbohydrates were detected using a refractive index detector.

3. Evaluation of freezing tolerance

The LT₅₀ (the temperature at which 50% of the plants killed (Pomeroy and Fowler, 1973) was evaluated according to the procedure of Yoshida et al. (1997). Plants were excavated on December 11, 2001, prior to the onset of continuous snow cover, washed and trimmed. The crowns plus 3 cm of stem tissues of each cultivar or accession were randomly divided into 5 temperature treatments, each treatment consisting of ten plants. Plants were put onto moistened absorbent cotton, which were folded and wrapped in aluminum foil, and exposed to −3.0ºC for 8h. Temperatures were then decreased at a rate of 1ºC h⁻¹. When the temperature reached −16ºC, −18ºC, −20ºC, −22ºC or −24ºC, samples were removed and placed overnight at 2 ºC. Plants were then transplanted into flats containing vermiculite and allowed to recover in a greenhouse. Percent survival was recorded after 2 weeks. LT₅₀ values were calculated by probit analysis.

Results and Discussion

In early December 2001 and 2002/2003, the mean fructan content of the crowns of snow mold resistant *Ae. cylindrica* accessions was not significantly different from that of susceptible accessions (Table 1). The mean fructan content in the susceptible accessions was significantly lower in 2003/2004 than in 2001 or 2002/2003 (P<0.001). The fructan content of snow mold resistant check PI 173438 was consistently higher than that of the susceptible check Chihokukomugi in the three years. The fructan contents of check wheats were considerably lower in 2003/2004 than in 2001 or 2002/2003. Since the cultivation method and growing conditions affect the accumulation of fructan (Yukawa et al., 1994; Yukawa et al., 1995), the environmental conditions in autumn and early winter in 2003/2004 were assumed to be unfavorable for check wheats and susceptible accessions of *Ae. cylindrica* for accumulation of fructan before snow cover. In the two years, fructan contents were consistently higher in the resistant group of *Ae. cylindrica* accessions than in the susceptible group in March (P<0.01). Similarly, the fructan content was consistently higher in snow mold resistant check wheat PI 173438 than in the susceptible check Chihokukomugi.

Differences in freezing tolerance expressed as LT₅₀ were observed among *Ae. cylindrica* accessions and check wheat. The LT₅₀ value of Valujevskaya (cold hardy), Chihokukomugi (moderately cold hardy) and PI 173438 (cold tender) was −23.0ºC and −19.2ºC, and −15.5ºC, respectively. The most freezing tolerant *Ae. cylindrica* accession was PI 276974 (LT₅₀= −22.0ºC), followed by PI 330484 (−21.6ºC), PI 276977 (−21.5 ºC), 400454(−21.5ºC), and PI 266814 (−20.5ºC). The most tender accession was KU 2421 (−15.0ºC). Limin and Fowler (1985) reported that the four PI accessions showed freezing tolerance similar to that of the cold hardy check wheat cultivar Norstar. However, in our experiment using cold hardy check wheat cultivar Valujevskaya whose freezing tolerance is similar to that of Norstar (Yoshida et al., 1998), the freezing
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Fructan content in winter was slightly lower in the PI accessions than in Valjevskaya. Among *Ae. cylindrica* accessions, freezing tolerance was not correlated with fructan, mono- and di-saccharides or total sugar content (Fig. 1).

Amano and Osanai (1983) reported that snow mold resistant cultivars such as PI 173438 accumulate large amounts of sugar before snow cover and the rate of sugar metabolism under snow was similar to that in susceptible cultivars. Kiyomoto (1987) observed a positive correlation between the sugar content before snow cover and the snow mold resistance. Kiyomoto and Bruehl (1977) reported that ranking of total sugar content in the field in early autumn did not correspond to the ranking of cultivars for snow mold resistance, and noted that the total carbohydrate content in early autumn is considerably different from that in late autumn. Overall, sugar content before snow cover seems to be an important factor for wheat to survive under snow. Sugars accumulated in wheat plant consist of mono- and disaccharides, and fructan (Yoshida et al., 1998). Among them, fructan appears to play an important role in surviving under snow (Yoshida et al., 1998); snow mold resistant cultivars accumulate large amounts of fructan before snow cover and appear to maintain high fructan contents in winter compared to susceptible cultivars.

Although we confirmed the high fructan content of snow mold resistant cultivar PI 173438 before snow cover, there was no significant difference in the mean fructan content between snow mold resistant and susceptible *Ae. cylindrica* accessions in two of the three years we studied. In 2002/2003, the fructan content of three snow mold resistant accessions (KU 2441, KU 2486, 400476) was similar to that of susceptible

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![Fig. 1. Relationships between LT50 and sugar contents (A, fructan; B, total mono- and disaccharides; C, total sugar).](image1)

![Fig. 2. Comparison of fructan content between snow mold resistant and susceptible accessions in *Aegilops cylindrica* before snow cover and after 90 days incubation under snow. A: 2002/2003, B: 2003/2004.](image2)
accessions before snow cover, but in 2003/2004, it was higher than that of susceptible accessions. In the two years studied, the resistant accessions retained a high fructan content after snow cover compared to the susceptible accessions (Fig. 2). These indicate that the fructan content before snow cover is not related to the content after snow cover in *Ae. cylindrica*. Thus, the fructan content before snow cover is not a critical factor for *Ae. cylindrica* accessions to survive under snow: the fructan content after prolonged snow cover is crucial. Since the levels of accumulation and depletion of fructan in the three resistant accessions differed between the two years, the three resistant accessions seems depletes fructan slowly under certain environmental conditions as observed in 2002/2003. Therefore, it is possible that different physiological mechanisms for snow mold resistance exist among *Ae. cylindrica* accessions and that accumulation and utilization of fructan during the winter may reflect the presence of genetic components different from those in wheat.

This study demonstrated that snow mold resistant accessions of *Ae. cylindrica* retain a high fructan content under snow. Furthermore, it is probable that some accessions deplete fructan slowly under snow to retain fructan until the snow melts under certain environmental conditions. This is in contrast with the snow mold resistant wheat PI 173438 that appears to have a high fructan content before snow cover.

Although Yoshida et al. (1998) reported a positive relationship between the freezing tolerance and simple sugar content of winter wheat, there appeared to be no association between freezing tolerance and total sugar, simple sugar, or fructan content in *Ae. cylindrica*. The mechanism involved in the freezing tolerance appears to be different between the two species. Because the mechanisms of snow mold resistance and freezing tolerance in *Ae. cylindrica* appear differ from those in wheat, *Ae. cylindrica* could be a novel genetic resource to improve snow mold resistance and freezing tolerance of wheat.

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