Comparative analysis of N-glycans from wheat seeds

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Introduction

Grains currently bred as agricultural crops have been variously crossbred and improved by humans, resulting in higher yields and starch quality at harvest time. On the other hand, they have become vulnerable to changes in the global environment, and there is a need for stronger varieties that can cope with food problems caused by drought and other factors.

Wheat, one of the world's three major cereal grains, is used to make bread, noodles, and other products, and is produced in large quantities and is relatively drought-resistant. In Japan, however, wheat production is lower than in other countries because it is often grown in the back of rice paddies, making it difficult to optimize soil management, and because the ripening period of wheat falls during the rainy season, which can seriously affect yield and quality.
Therefore, varieties grown in Japan are produced with improved varieties that ripen earlier and are relatively resistant to high humidity and rainfall during the harvest season.

Glycan structures are closely related to all life phenomena such as cellular patterns and cellular states, and *N*-glycans are involved in all life stages. However, the *N*-glycan structure in wheat seeds is largely unknown.

Since glycans are easily affected by external factors such as the environment, we believe that analysis of glycan structures will clarify the effects of glycans on living organisms.

One of the wheat varieties, Norin 61 (N61), is a mainstay variety that has been bred to adapt to Japan's unique climate. On the other hand, *Triticum aestivum acc*. KU-11319B (#654), a variety native to Afghanistan, is relatively resistant to drought and can ensure a certain yield even under poor conditions.

Therefore, the purpose of this study is to clarify the *N*-glycan structure of wheat under storage conditions, to compare and analyze two varieties, N61 and #654, that differ in growth environment and varietal characteristics, to analyze how the glycan structure has been changed by breeding, and to clarify the process of adaptation and adaptation to the environment.

Objective

- Quantitatively clarify the structure of *N*-linked glycans in N61 and #654.
- Comparative analysis of N61 and #654 to clarify the characteristics of glycans between varieties and the effects of breeding.

Materials & Method

- **Samples**

N61 and #654 samples were collected at Yokohama City University in 2020.

- **Method**

Hydrazine decomposition
・*N*-glycans are chemically cut from glycoproteins

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N-acetylation
・Replace hydrogen atoms in the compound with acetyl groups

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Pyridyl amination
・Insertion of 2-aminopyridine

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Gel filtration chromatography
・Removal of excess pyridylamino glycans and separation/purification

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Size fractionation HPLC analysis
・Separate *N*-glycans according to molecular size

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Reversed-phase HPLC analysis
・Further separation and purification of *N*-glycans

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Mass spectrometry using MALDI-TOF/MS

Results

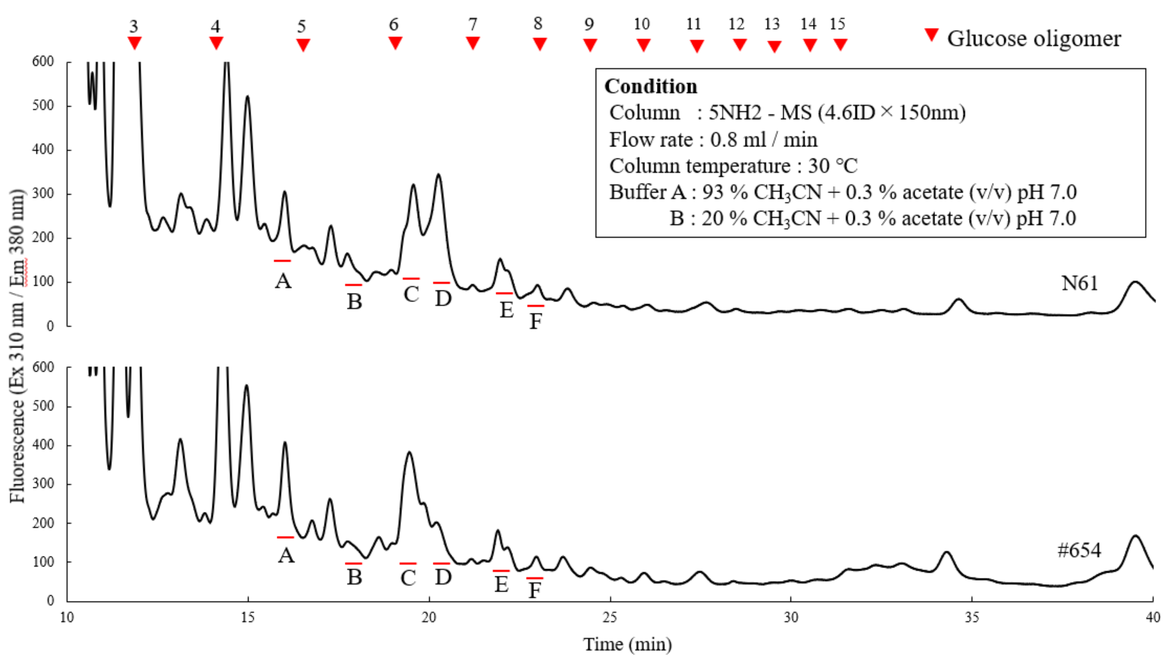

Figure 1. Size fractionation HPLC analysis of N61 and #654.

Table 1. Quantitative value of each *N*-glycans per mg of N61 and #654 (pmol / mg).

Gal Man GlcNAc Fuc Xyl —: α-linkage —: β-linkage

No.	Estimated structure		N61 Quantitative value (pmol / mg)	#654 Quantitative value (pmol / mg)
A	1× PA	[M2FX]	1.64	2.02
B	3× PA	[M4]	1.18	0.71
C	2× PA	[M3FX]	3.60	4.12
D	4× PA	[M5]	0.78	1.53
E	3× PA	[M6]	1.10	1.02
F	PA	[GN2M3FX]	2.10	2.31

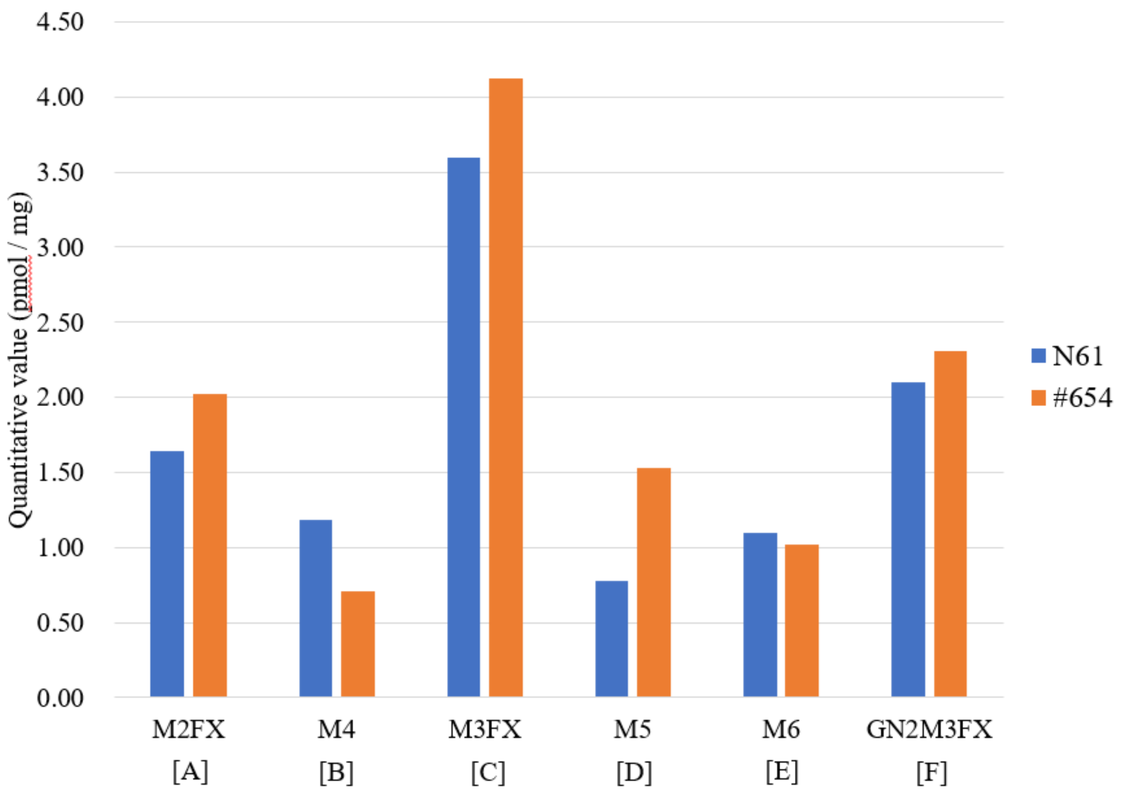

Figure 2. Comparison of quantitative values of *N*-glycans per mg of N61 and #654 (pmol / mg).

Conclusion

- M2FX, M4, M3FX, M5, M6, and GN2M3FX identified in this study have been reported in previous glycan studies on other plants. Therefore, it is assumed that they are also present in wheat seeds.
- The most major structure in both N61 and #654 was M3FX. The glycan structures that differed quantitatively between N61 and #654 were M4 and M5.
- The differences in *N*-glycans obtained in this study are presumably related to interspecific differences in environmental adaptation.
- The other *N*-glycans structures in N61 and #654 are currently being analyzed in detail.