Structural analysis of glycans derived from wheat strain, “Yumeshihou” seeds

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◆ Research background

Wheat = Nutritious ＆ suitable for storage ➡ They are optimized (evolved) for human convenience !

It is an easy to breed and have improved yields and nutritional balance.

However, those branded wheat became vulnerable to sudden changes in the environment due to repeated breeding.

In addition,

Extreme climate change ＆ deteriorating international situation
= soaring prices of imported grains

We need to do it soon !!!

There is an urgent need to improve the grain self-sufficiency rate in Japan.

Objective

Development of wheat that is more resistant to abnormal environmental changes than conventional domestic wheat, and that can be used to make high-quality bread with a strong texture and flavor.

By comprehensively understanding the structures and expression levels of all N-glycans, we will try to determine how plants evolved and clarify their functions.

◆ What is YUMESHIHOU ?

“YUMESHIHOU” is an early-maturing, high-yielding bread wheat that can be cultivated in warm regions such as the Kanto region.

○ Features

Excellent bread-making properties.
High gluten content.
Hard to lodging down and slightly higher yields than common wheat.

○ Main growth area

Ibaraki and Mie.

※YUMESHIHOU sample (harvested in 2020) was supplied from Professor Tomohiro Ban of Yokohama City University.

◆ Method

○ Pyridylamino (PA) method

Fluorescence labeling method of reducing end of glycans using 2-aminopyridine.

1. Hydrazinolysis (cutting extraction of the glycan from protein)
↓
2. N-acetylation
↓
3. Pyridyl amination (introduction of the fluorescent labeling)
↓
4. Gel filtration chromatography (the excessive PA removal)
↓
5. GL-Pak Carbograph (concentration)
↓
6. Size-fractionation HPLC analysis
↓
7. Reversed-phase HPLC analysis
↓
8. MALDI-TOF/MS
↓
9. Enzyme processing

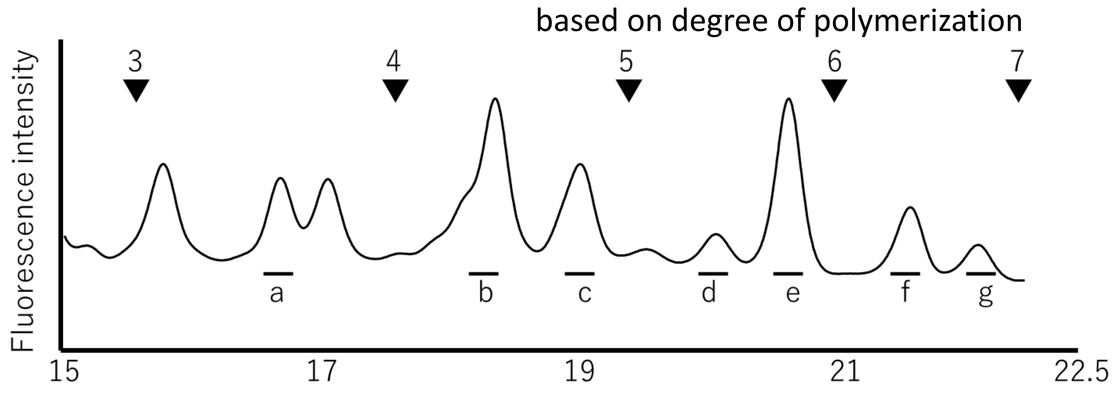
◆ Discussion

- Since the content of M3FX glycan in YUMESHIHOU was particularly high as in other wheat species, it was suggested that M3FX is a major glycan in the early growth process of wheat.
- As a feature of the YUMESHIHOU, a large amount of M6 glycan was specifically detected, however, there was no significant difference in the content of other N-glycans.
- From this result, it was suggested that the amount of M6 is related to lodging resistance and high yield.

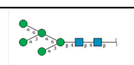
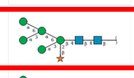
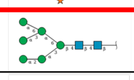
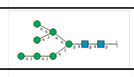
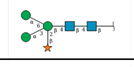
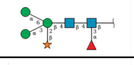
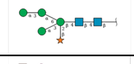
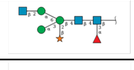
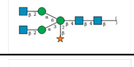
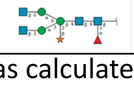
◆ Results

◎ Elution pattern of N-glycans of YUMESHIHOU seeds by size-fractionation HPLC

Numbered arrowhead ▼: Elution position of PA-isomaltooligosaccharides based on degree of polymerization



◎ Glycan structure determined by structural analysis

Peak No.	Structure	Abbreviation	Ratio(%)	type
c1		M5A	18.28	High mannose
d2		M5X	2.20	
e1		M6	19.27	
g1		M7	2.93	
a1		M3X	18.72	Pauci mannose
b1		M3FX	21.96	
b2		M4X	6.24	
d1		GNM3FX	3.25	Complex
d3		GN2M3X	1.49	
* f1		GN2M3FX	5.66	

The relative ratio was calculated with the sugar chain whose structure could be determined as 100%.

*the structure was confirmed by additional experiments.

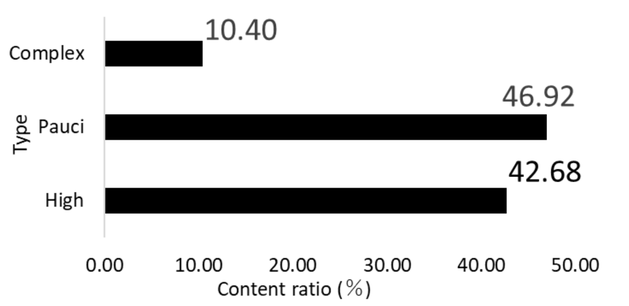
In this study, the structure of **M5X** was confirmed from YUMISHIHOU, but there were few reports of such a structure.

The amount of **M6**-type sugar chain was **higher** than that of other wheat species.

◎ Comparison of sugar chain structures by sugar composition (xylose and fucose)

- High Mannose
- Paucimannose
- Complex

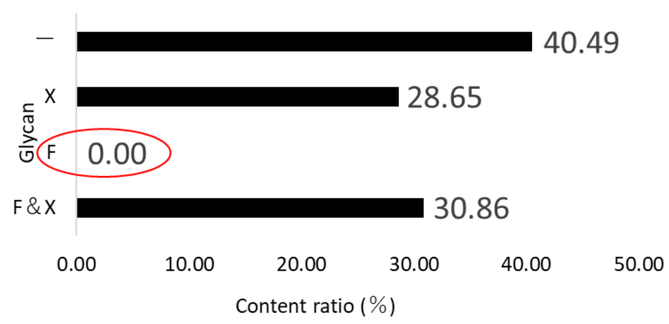
Type Content



Pauci mannose type was the most common

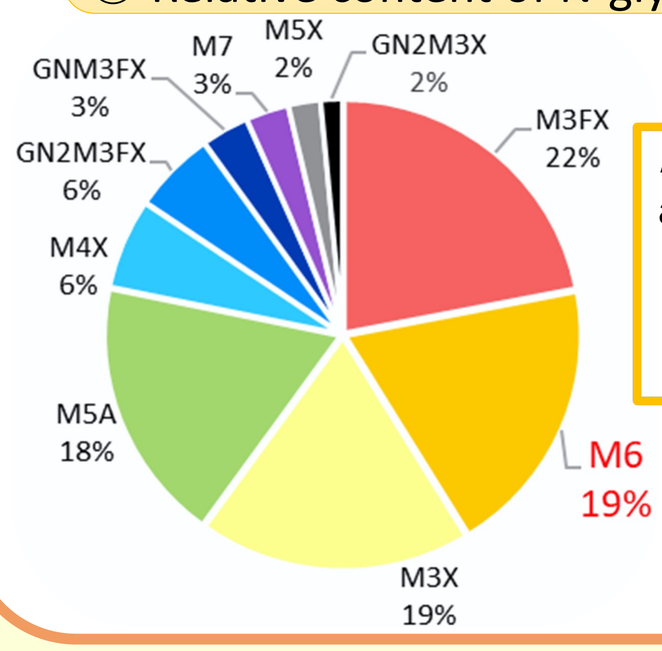
- Neither Fucose nor Xylose (—)
- Only Fucose available (F)
- Only Xylose available (X)
- Both Fucose and Xylose are available (F &X)

Fuc, Xyl Content



A glycan with only fucose bound could not be confirmed

◎ Relative content of N-glycans by structure



Abundant N-glycan structures are shown as below (%)

1. M3FX	21.96
2. M6	19.27
3. M3X	18.72