

# Breeding and introduction of waxy wheat Nuomai No.12

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## ABSTRACT

Waxy wheat contains <1% amylose in the endosperm starch. This research has utilized full waxy wheat from USDA and the main cultivated varieties in South China as parents. Over a period of 5 years and 10 seasons, we have developed some special waxy wheats, such as: Nuomai No.12. The research included the use of molecular marker-assisted selection, combine limited backcross (cultivated varieties in South China as recurrent parents), summer multiplication as well as the assistance of biochemistry molecular marker selection. The starch in the endosperm of Nuomai No.12 contains 100% amylopectin (Amylose-free). This special wheat line has integrated the good characters of waxy starch, high yield and widely adaptability. The parameters of Peak Viscosity, Trough Viscosity and Final Viscosity in Nuomai No.12 are 36.4, 8.3, 12.7 RVU respectively. Nuomai12 has abundance of mineral elements such as Fe, Na, Mg, K. The seed of Nuomai No.12 is rich in pentosan, a healthful ingredient for human body. In addition, Nuomai No.12 has possessed good agronomy character and strong spring habit and satisfying early maturity (special early maturity, 10 days earlier than the common cultivars). The yield of Nuomai No.12 equals to the current cultivars named Chuanyu 12 which with high yield in South China. The Tang-yuan (sweet dumplings, one kind of Chinese traditional food made from waxy rice ) made of Nuomai No.12 is nutritious and smooth to the taste while the soup is not muddy. All the good characters above could ensure Nuomai No.12 a wide market in the future.

**Keywords:** Waxy wheat; Special wheat; Molecular marker-assisted selection; Special early maturity

## INTRODUCTION

High-amylopectin (Amylose<1%, Amylose-free) wheat is referred to as waxy wheat. It is known that wheat amylose synthesis is regulated by a granule-bound starch synthase (GBSSI) which is encoded by *Wx* gene. Common wheat is hexaploid (*Triticum aestivum* L., AABBDD), thus it has 3 types of *Wx* protein subunit: *Wx-A1*, *Wx-B1* and *Wx-D1*. Subunit *Wx-A1* is encoded by allele *Wx-A1* on 7AS loci; Subunit *Wx-B1* by allele *Wx-B1* on 4AL loci while subunit *Wx-D1* by allele *Wx-D1* on 7DS loci. Fully waxy wheat has null, or non-functioning alleles at each of these three loci and thus its amylose content decreases to 0. This unique trait of waxy wheat confers to a great prospect in industrial areas such as food and starch processing. Therefore, the

development and application of waxy wheat becomes one of the research tasks in many countries.

## MATERIALS AND METHODS

### Materials

98Y1441(*Wx-A1b*, *Wx-B1b*, *Wx-D1b*, Kindly provided by Dr. Robert Graybosch) is the donor of full waxy wheat germplasm. It is the offspring of the cross between Kanto107(*Wx-A1b*, *Wx-B1b*) and Baihuomai (*Wx-D1b*), Chuanyu12 (*Wx-A1a*, *Wx-B1a*, *Wx-D1a*) developed by our institute works as receptor of recessive waxy alleles as well as the high yield donor.

### Methods

1. Normal crosses combined with genetic assay to identify the seed of interest: perform the normal crosses, screen seeds for all recessive *Wx* alleles by Iodine-Dye and PCR assay on the brush portion, then plant the germ part of the selected seeds .
2. Backcross the individuals in segregate with the high-yield parent and select the waxy ones in the next generation to combine the waxy property with the high yield potential.
3. Plant in another high land place in summer to speed up the development and to increase the adaptability of waxy offspring lines.
4. Measure the amylopectin content by iodine-colorimetry assay.
5. Study starch gelatinization property by RVA apparatus.
6. Study the HMW-GS composition by SDS-PAGE.
7. Identify the genotype of *Wx* loci with the aid of allelic-specific PCR (Nakamura et al., 2002 ; Shariflou et al., 2001), PCR reaction conditions see (Shu et al.2006).

The primer sequence of *Wx-A1* loci :  
TCGTGTTTCGTCGGCCGAGATGG  
CCGCGCTTGTAGCAGTGAAGTACC

The primer sequence of *Wx-B1* loci :  
CTGGCCTGCTACCTCAAGAGCAACT  
CTGACGTCCATGCCGTTGACGA

The primer sequence of *Wx-D1* loci :  
ACAGGATCTCTCTGGAAG  
GCAAGGAAAATAGTGAAG

8. Study the mineral element by ICP technique.
9. Measure the pentosan content in the whole wheat flour by spectrophotometer.

## RESULTS AND DISCUSSION

Parent Combination of Nuomai12:

Chuanyu12//98Y1441/Chuanyu12//Chuanyu12 (BC2F6) . Limited backcross (Parent Chuanyu12 has served as male parent twice and female parent once in the three artificial hybrid crosses).

Starch property of Nuomai No.12:

Amylopectin percentage: Raw starch content of Nuomai12 is 64.65% and the amylopectin percentage is 100.00%.

RVA viscosity property: RVA curve and data (table 1) shows that the viscosity curve changes smoothly after the trough. Little rebound is observed and the rebound value is rather small (the difference between the final viscosity and the trough viscosity). This fact indicates that Nuomai No.12 has strong resistance for coalescence and precipitation. Consequently, the speed and degree of coalescence and precipitation is small and weak. Thus, the application of Nuomai12 on the bread and quick frozen product could decrease the speed of coalescence (precipitation) and in turn prolong shelf time.

Genotype of *Wx* loci of Nuomai No.12 :

The Result shows that the genotype of Nuomai No.12 is *Wx-A1b*, *Wx-B1b*, *Wx-D1b* at *Wx* loci, indicating that Nuomai No.12 is fully waxy wheat.

Protein parameters of Nuomai No.12:

The composition of High Molecular Weight Glutenin Subunit (HMW-GS) is *Glu-A1*(1), *Glu-B1*(7+8), *Glu-D1*(5+10). The quality score for *Glu-1* is up to 10 (full score), indicating that Nuomai No.12 has the superior genetic background for processing quality. Meanwhile its SDS value is as high as 55ml, indicating a good gluten quality.

Mineral elements content of Nuomai No.12:

Nuomai No.12 has a higher mineral elements content than its parent Chuanyu12. The Fe and Na content is 158.9% and 78.0% more than that of Chuanyu12. Besides Chuanyu12, Nuomai No.12 has higher Na, Mg and K content than the other cultivars (Table 2).

Pentosan content of Nuomai No.12:

The pentosan content of Nuomai12, Chuanyu12 and control cultivar Chuanyu107 is 6.04%, 3.96% and 4.81% respectively. Thus, the pentosan content of Nuomai No.12 is 1.53 times of Chuanyu12 and 1.26 times of control cultivar Chuanmai107, showing that Nuomai No.12 is richer in pentosan, a gradient beneficial for human health.

The main agronomic traits of Nuomai No.12:

The agronomic traits of Nuomai No.12 is better than its parents, especially the 1000-grain weight and spike weight. The 1000-grain weight has increased by 11.4% and the spike weight has increase by 24.1% compared with the parent cultivar Chuanyu12 (Table 3). Nuomai No.12 has a strong spring habit in that the sowing date-heading date is 126 days, about 10 days mature ahead of Chuanmai107, the one widely planted in Sichuan province now . Therefore, the Nuomai No.12 could advance the harvest day.

## CONCLUSION

The amylopectin percentage of starch of Nuomai No.12 reaches 100% which expands its utility and enhanced its commercial value.

The better agronomic traits of Nuomai No.12 include a similar biological phenotype to its high-yield recurrent parent cultivar Chuanyu12. In addition, with the aid of summer multiplication in high altitude and selection for the adaptability, Nuomai No.12 has a broader range of planting area. Thus, Nuomai No.12 has gained the characteristics of superiority, high yield potential and wide adaptability and combining the waxy property with high yield capacity.

The mature period of Nuomai No.12 is earlier than the other common wheat obviously. Its harvest time is 10 days ahead of the other widespread wheat cultivars, thus shortens the growing length. Therefore, mature earlier and high yield of Nuomai No.12, which could increase the utility efficiency of cultivated land, especially the land of high efficiency.

Nuomai No.12 is rich in nutrition and has superior gluten quality and good processing quality. Previously, it was hard to improve starch and protein quality simultaneously. Here, Nuomai No.12 is good in both starch and gluten composition. Nuomai12 contains 100% of amylopectin and its SDS, the parameter reflecting the gluten quality and quantity, is as high as 55ml. The Tang-yuan (one kind of Chinese traditional food made of waxy rice) made of Nuomai12 is tasty, smooth and delicate, while the soup remain clear all through.

The analysis of mineral elements and pentosans of Nuomai No.12 shows that Nuomai No.12 is rich in Fe, Na, Mg, K et al and pentosan, which are all beneficial for health. Therefore, Nuomai12 may server as the raw material for functional food to improve the health condition.

**Table 1. RVA starch viscosity property of Nuomai No.12 (water content 14%)**

Parameter	Peak viscosity (Cp)*	Trough Viscosity (Cp)	Break down(Cp)	Final Viscosity (Cp)	Setback (CP)	Peak time(min)	Pasting temperature□
Nuomai No. 12	436.8	99.5	337.2	152.7	53.1	3.13	68.8

Cp: viscosity unit

**Table 2. Mineral element content of Nuomai No.12 (mg/100g whole wheat flour)**

Elements	Ca(mg)	Fe(mg)	K(mg)	Mg(mg)	Mn(mg)	Na(mg)	Zn(mg)	Se(ug)
Nuomai No.12	46.81	8.57	477.25	146.75	2.32	6.39	4.7	272.53
Chuanyu12	37.35	3.31	412.39	118.99	1.75	3.59	3.71	229.98
Increase %	+25.3	+158.9	+15.7	+23.3	+32.6	+78.0	+26.7	+18.5

**Table 3. The comparison of Nuomai12 and its parent cultivar in terms of agronomic traits.**

Parameter	Plant height(cm)	spike per plant	No. of spikelets per spike	No. of grains per spike	1000-grain weight(g)	Spike weight(g)
Nuomai No.12	92	4	22	84	42.9	3.6
98Y1441	115	3	21	41	38.0	1.6
Chuanyu12	89	4	21	75	38.5	2.9

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