Molecular improvement of processing quality in wheat 1B/1R translocation lines

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Outline

1、Origin and spread of 1BL/1RS translocations

2、Quality Defects of 1B/1R Translocation lines

3、Reasons for poor processing quality and Ways to improve

4、Some of our research work on 1B/1R translocations

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Origin and spread of 1BL/1RS translocations

The 1R(1B) substitution line 'Zorba',developed in Germany

↓

a number of 1BL.1RS translocation lines in Germany

1R(1B) substitution line, 'Salzmunder Bartwiezen' developed in Germany 1RS from rye cultivar 'Petkus'

a number of 1BL.1RS translocation lines in Eastern Europe

1930s

a 1BL.1RS translocation line 'Kavkaz' in Russia

1970s

New 1BL.1RS translocation lines in the world

1BL.1RS translocation line 'Salmon' derived from crosses between two octaploid triticales in Japan

ADVANTAGES OF THE 1BL/1RS:

1. Disease resistance: powdery mildew, stripe rust, leaf rust and stem rust.
2. high grain yield potential across a wide range of environments

R. A. Graybosch, *Journal of Cereal Science,* 2001, **33:** 3–16

Frequencies of 1B/1R translocation lines in wheat cultivars from 20 countries

Origin	Cultivar number	Rate(%)	Origin	Cultivar number	Rate(%)
Argentina	33	9.1	Iran	30	3.3
Australia	73	0.0	Japan	10	10.0
Austria	5	0.0	Mexico	7	28.6
Bulgaria	15	20.0	Norway	22	0.0
Canada	24	4.2	Romania	24	12.5
Chile	41	26.8	Russia	18	22.2
China	49	28.6	Serbia	11	0.0
France	94	17.0	Turkey	85	10.6
Germany	75	16.0	Ukraine	13	7.7
Hungary	10	40.0	USA	79	13.9

Jin et al., *Crop & Pasture Science*, 2011, **62**, 746–754

Cultivation of Non-Petkus-derived 1B/1R Translocation Lines

- Some disease resistance of Petkus-derived 1B/1R translocations was lost (Shi et al，2001)

- Because of the high yiejd potential in some eviroments, they are still been used

- Besides some non-Petkus-derived disease resistant 1B/1R translocation lines have been cultivated constantly (Lei et al，2012 ；Yang et al，2014；Li et al，2016)

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Quality Defects of 1B/1R Translocation lines

- **Low bread volume**
- **‘sticky’ dough**
- **Low overmixing tolerance**
- **Low SDS sedimentation**

(*Graybosch, Journal of Cereal Science, 2001, 33:3–16*)

- less extensible
- lower resistance to extension

(Barbeau etal, Journal of the Science of Food and Agriculture, 2003, 83:29–38)

- Not **suitable for processing high quality bread**
- Not **suitable for processing high quality noodles**

（Liu et al., ACTA AGRONOMICA SINICA，2004）

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- **Reasons for poor processing quality of 1B/1R translocation wheat**

  ☆ 1BS: loss of the *Glu-B3* locus

  ☆ 1RS: Introduction of ω-secalins: monomer molecules, strong water absorption

- **Ways to improve processing quality of 1B/1R translocation wheat**

  ☆ Importing high-quality glutenin subunit genes or increasing glutenin expression in 1B/1R translocation lines (5+10, 7^{OE})

  ☆ Removal of ω-secalin genes in 1B/1R translocation lines (deletion line

  ☆ Silencing the expression of ω-secalin genes by RNA interference

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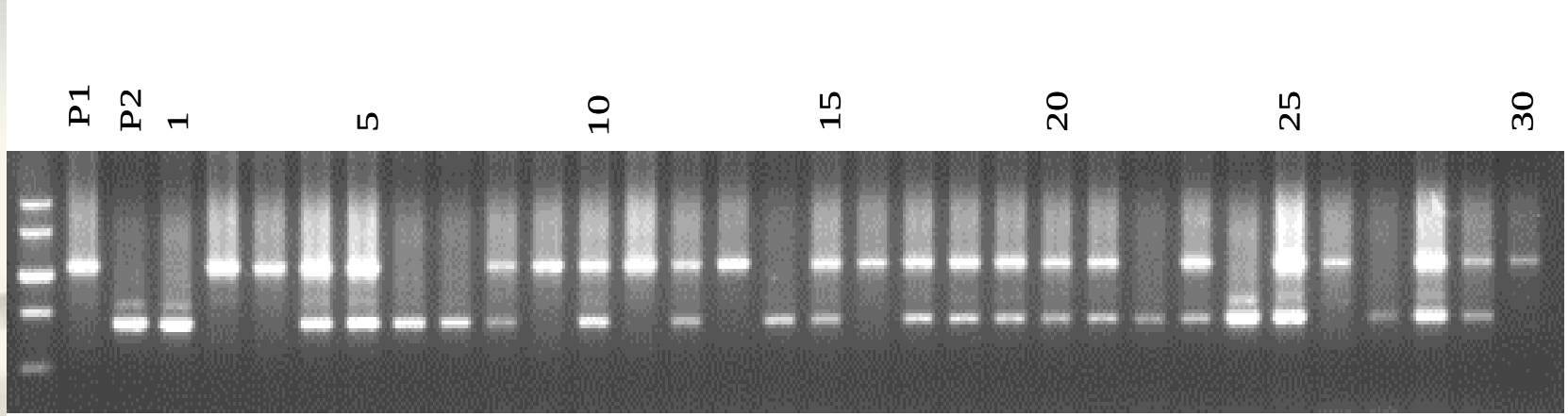
1. A co-dominant molecular marker for detection of homozygosity and heterozygosity of 1B/1R translocation



Detection of a F2 segregating population using developed molecular markers

(P1: Lumai 14,1B/1R; P2: Glenlea,non 1B/1R; 1-30: F2 plants)

Chai等，Plant Breeding， 2006，125(3):302-304

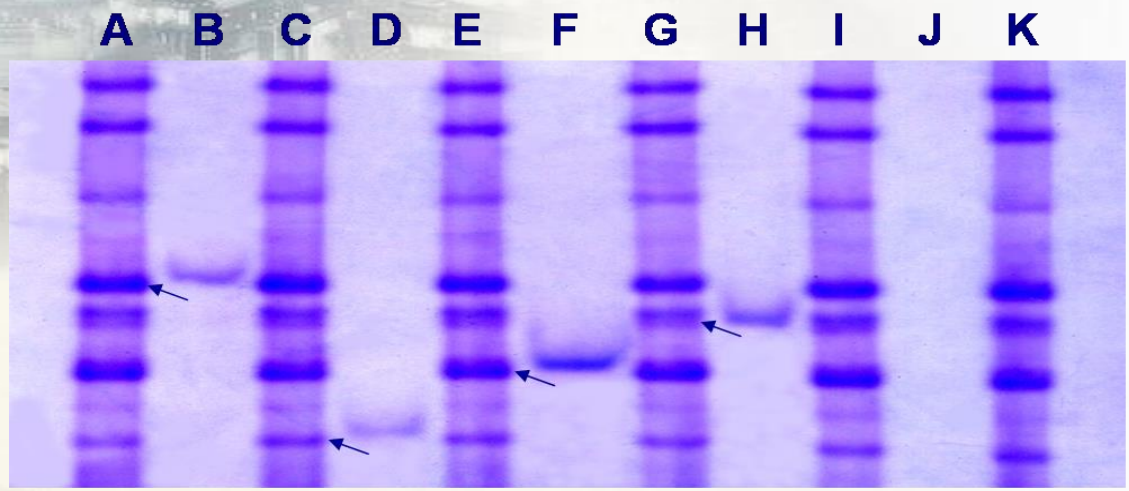
2. Gene length of ω-secalin gene family and transcriptional activity

Gene Length of ω-secalin gene family	Transcriptional activity
1150bp	No
1076bp	Yes
1075bp	No
1052bp	Yes
1004bp	Yes

Chai et al., Cell Research, 2005，15(8):658-664.

Via homoeologous cloning of genomic and cDNA
based on a 1076bp ω-secalin gene Hull et al.，Plant Mol Biol, 1991

3. Characterization of expression activity of ω-secalin genes with transcription activity



Comparison of Prokaryotic expressed recombinant proteins and gliadin expressed in seeds of the 1B/1R translocation line cultivar Lankao 906 on A-PAGE

（A, C, E, G, I, K: Lankao 906; B: Sec-1-1; D: Sec-1-2; F: Sec-1-3; H: Sec-1-4; J: -ck）

Identification of excised protein bands by LC-MS/MS

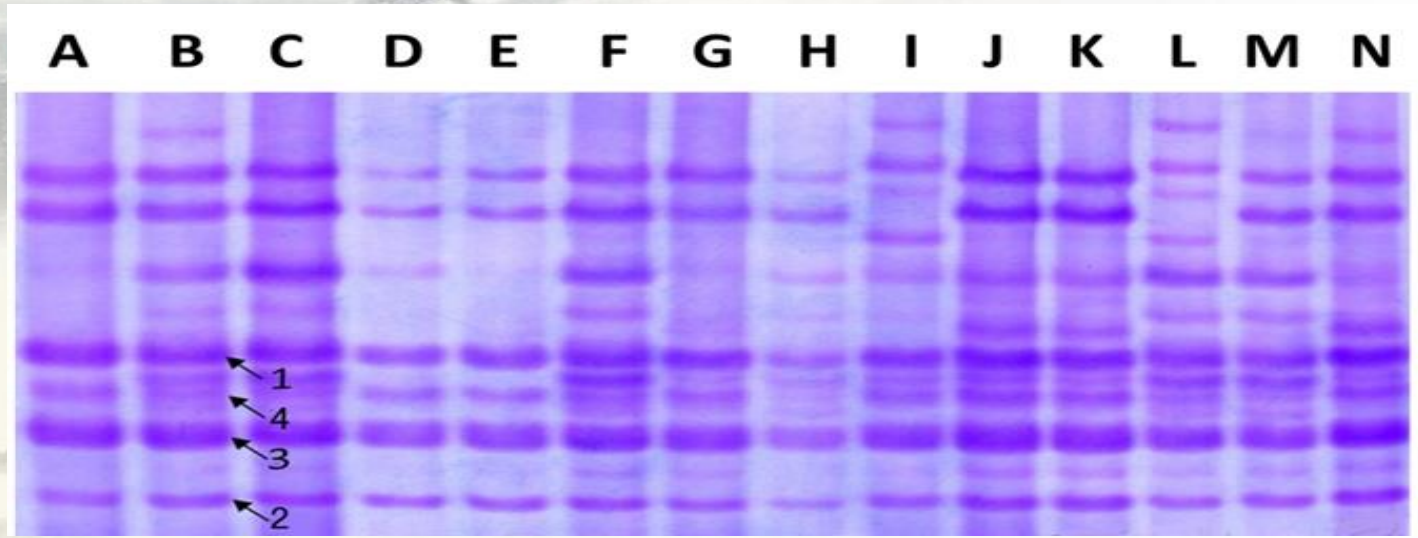
Table 1 Identification of excised protein bands from acid polyacrylamide gel electrophoresis (A-PAGE) by liquid chromatography tandem mass spectrometry (LC-MS/MS)

Protein bands on A-PAGE[1)]	PepCount sequence	Matched proteins
Band 1	QLNPSEQELQSPQQPVPK	ω-Secalin (*Secale cereale*), ACQ625.1 ω-Secalin (*Triticum aestivum*), ACQ83637.1
Band 2	PFGQQPEQIISQR	
	QLNPSEQELQSPQQPVPK	ω-Secalin (*S. cereale*), ACQ625.1 ω-Secalin (*T. aestivum*), ACQ83637.1
Band 3	PFGQQPEQIISQR	
	PQQPFPLQPK	
	QLNPSEQELQSPQQPVPK	ω-Secalin (*S. cereale*), ACQ625.1 ω-Secalin (*T. aestivum*), ACQ83637.1
Band 4	PFGQQPEQIISQR	
	QLNPSEQELQSPQQAVPK	ω-Secalin (*T. aestivum*), ACQ83638.1 ω-Gliadin (*T. aestivum*), ACN62214.1

Table 2 Association of indicated protein bands on A-PAGE with the four secalin proteins

Protein bands on A-PAGE	PepCount sequence	Secalin protein	Start	End
Band 1	QLNPSEQELQSPQQPVPK	sec-1-1	2	19
	PFGQQPEQIISQR		237	249
Band 2	QLNPSEQELQSPQQPVPK	sec-1-2	2	19
	PFGQQPEQIISQR		237	249
	PQQPFPLQPK		252	261
Band 3	QLNPSEQELQSPQQPVPK	sec-1-3	2	19
	PFGQQPEQIISQR		231	243
Band 4	QLNPSEQELQSPQQAVPK	sec-1-4	2	19

Theω-secalin bands could be detected in different 1BL/1RS translocations

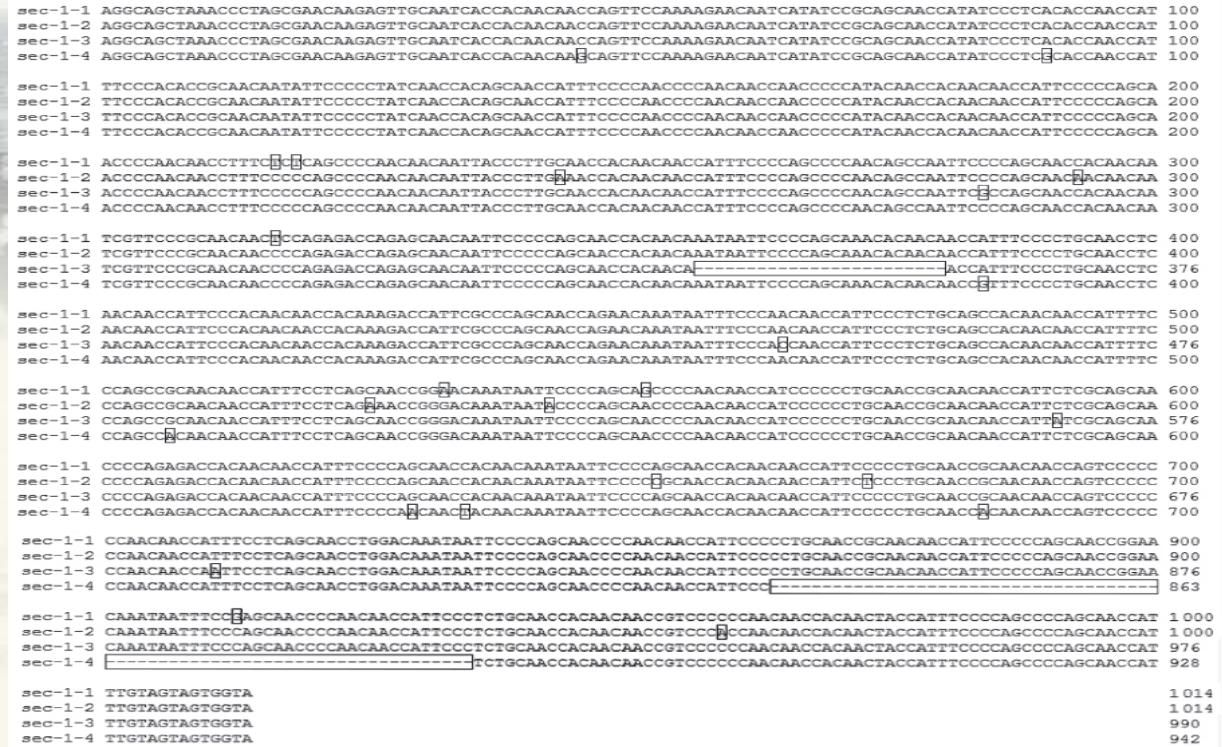


Detection of ω-secalin bands in different 1BL/1RS translocations on A-PAGE gel.

（A: Heng 7228; B: Lankao 906; C: Zhoumai 13; D: Yumai 70; E: Lumai 14; F: Laizhou 953; G: Zhongmai 9; H: Jinghua 1; I: Jingdong 8; J: Chuanmai 17; K: Chuanmai 12; L: Huaimai 18; M: Jinmai 45; N: Predgornaia. Arrows indicate ω-secalin bands.）

Chai et al., Journal of Integrative Agriculture，2016，15(12): 2712–2718

sequence comparison of mature protein-coding regions of four ω-secalin genes with espression activity



The sequence similarity of four ω-secalin genes was above 98%

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4. RNA interference against rye base genes

RNAi vector:

Ubi promoter from corn

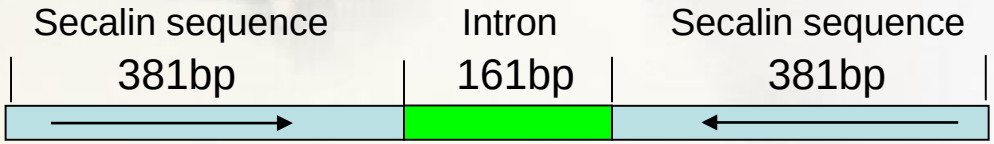
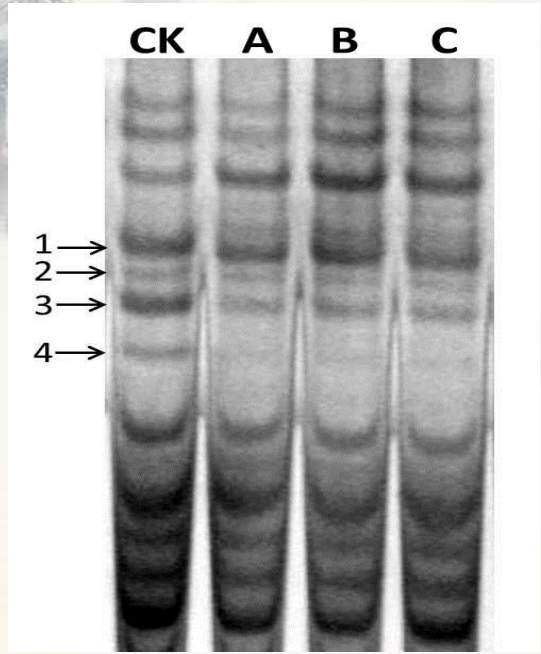


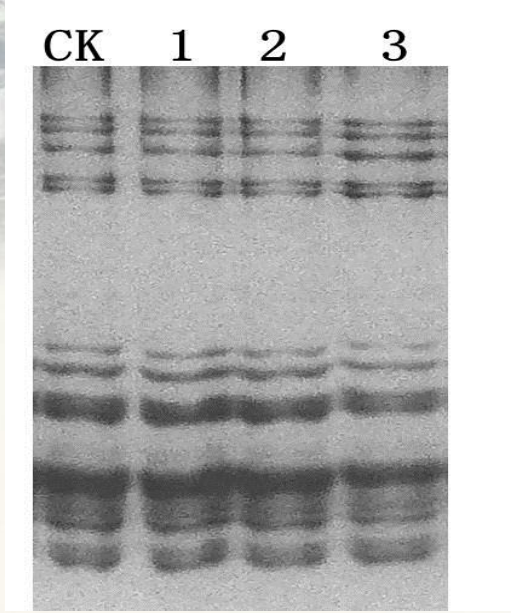
Illustration of interference fragment of ω-secalin gene

(The intron sequence is derived from the first intron of the wheat Waxy gene)



Detection of secalin in transgenic wheat seeds by A- PAGE

(CK: recipient cultivar; A–C: three pure transgenic lines; 1–4: four ω-secalin bands.)



Detection of glutenin subunits in transgenic wheat seeds by SDS-PAGE

(CK: recipient cultivar; 1–3: three homozygous transgenic lines.)

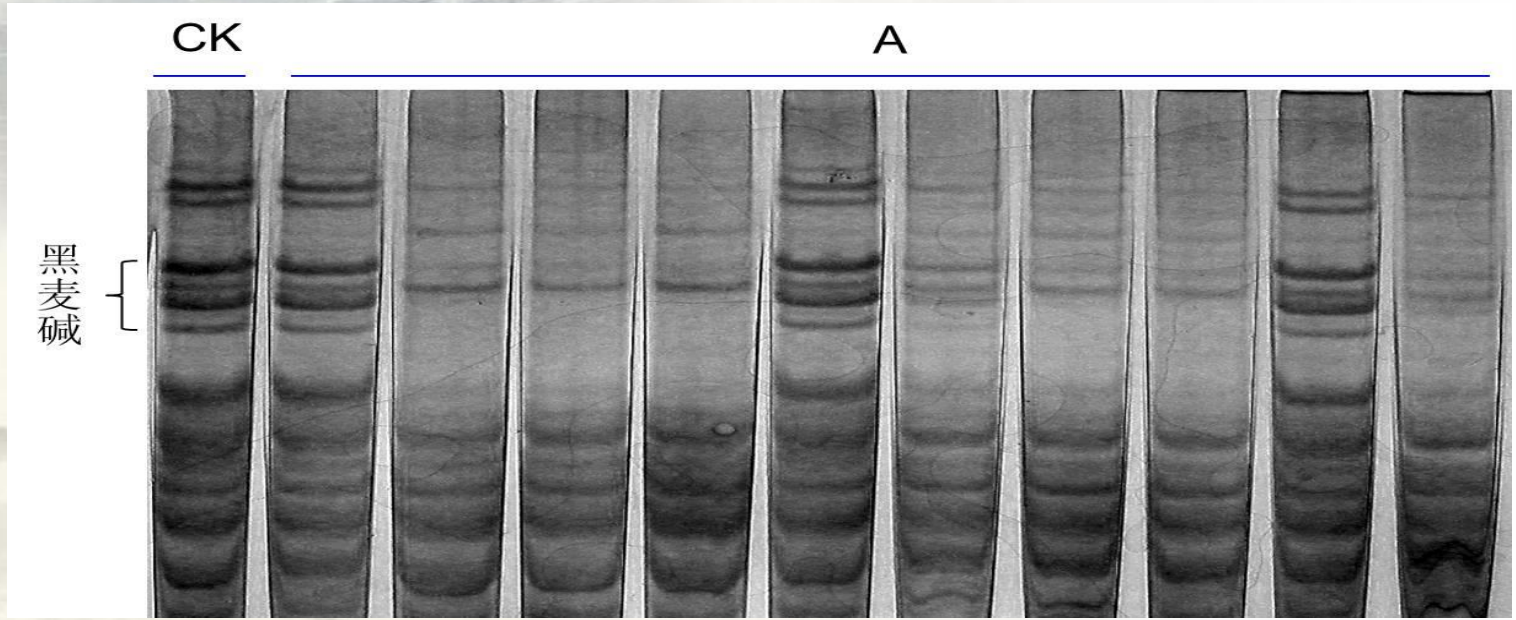
Grain processing quality of transgenic lines and their recipient cultivar

Line	Protein content %	Water absorption rate %	Wet gluten content %	Gluten index	Zeleny Sedimentation Value ml	Stabilization time min
CK	14.7±0.4a	61.7±0.2a	30.9±1.5a	36.7±2.3a	17.5±0.8a	1.2±0.3a
K1-5	14.4±0.3a	62.6±0.6a	27.6±1.3a	43.0±3.6ab	21.6±0.6b	2.6±0.3b
K1-15	14.7±0.0a	62.2±1.9a	29.4±0.9a	48.0±5.2b	23.7±1.2bc	2.5±0.3b
K1-16	14.7±0.1a	61.7±0.6a	31.5±1.3a	45.7±1.2b	25.0±1.1c	2.4±0.2b

Note: CK: recipient cultivar Jinhe9123; K1-5,K1-15,K1-16:three transgenic lines

Chai et al.，ACTA AGRONOMICA SINICA，2016，42(5): 627-632

Seed specific promoter Bx7



Detection of secalin in transgenic wheat seeds by acid PAGE

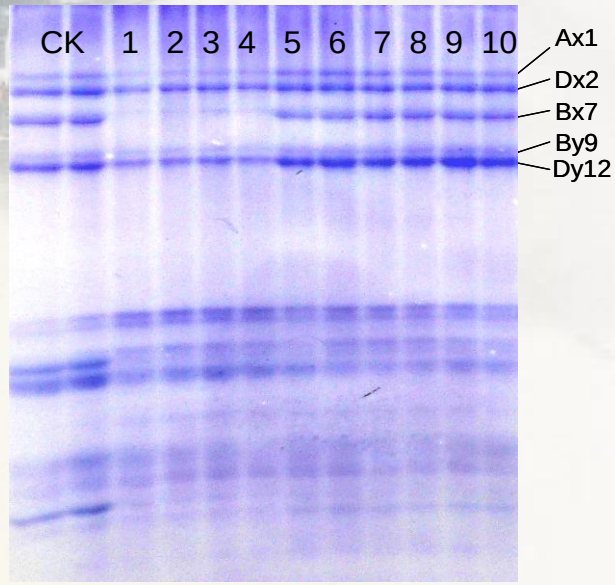
（CK: Receptor variety Kenong 199；A: Different transgenic lines）

Grain processing quality of transgenic lines and their receptor

Line	Wet gluten content （%）	Gluten index	Wet gluten content x Gluten index	Zeleny Sedimentation Value （ml）	Stabilization time (min)	Protein content （%）
Kenong 199 (the receptor)	32.2	46	1418. 2	29. 0	3. 4	13. 55
#8-2	20.9	23	480. 7	18. 9	2. 4	14. 24
#8-6	21.9	21	459. 9	18. 8	2. 0	14. 12
D34	/	/		20. 3	2. 0	/
#13-3	21.7	99	2148. 3	38. 4	9. 2	14. 19
#13-7	19.1	100	1910. 0	37. 8	8. 0	14. 27
#13-8	21.8	99	2158. 2	38. 9	9. 2	14. 46

Comparison of food production quality between transgenic line #13-7 and the recipient Kenong 199

material	Bread volume（mL）	Bread total score	Noodle total score
Kenong199	759.06	76. 3	81
#13-7	849.6	84.8	87
Increased proportion	+11.9%	+11.1%	+7.4%



SDS - PAGE analysis of seed glutenin

（CK: Receptor variety Kenong 199 ； 1-10:Different transgenic lines，
1-2:#8-2；3-4:#8-6；5-6:#13-3；7-8:#13-7；9-10:#13-8)

Next work

The superior transformation event will be combined with HMW 5+10 high-quality genes to cultivate strong or extra-strong gluten wheat

There exists this possibility:

1. There is a significant negative correlation between the **ω-**gliadin content and the processing quality in the genetic background with 5+10 genes(Zhao et al.,2014).

2. The **ω-secalin** interference gene preferentially silences the expression of the 7 subunit but not the expression of the 5 subunit (Blechl et al.,2016).

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