第 32 卷 第 4 期
2006 年 4 月 625～629 页

作 物 学 报
ACTA AGRONOMICA SINICA

Vol.32, No.4
pp. 625－629 Apr., 2006

研究简报

小麦资源胚乳蛋白 *Glu-1*、*Glu-3*、*Gli-1* 基因位点变异特点

王 瑞[1] 张改生[1] F J Zeller[2] S L K Hsam[2]

([1] 西北农林科技大学农学院小麦研究所,陕西杨凌 712100; [2]德国慕尼黑技术大学植物育种研究所,德国慕尼黑 D-85350 Freising-Weihenstephan)

摘 要: 141 个普通小麦品种及农家种中,由 *Glu-1* 位点控制的高分子量谷蛋白亚基共 27 种图谱,最常见的图谱是(N,7+8,2+12)占 22%和(N,7+9,2+12)占 19.9%, *Glu-A1*、*Glu-B1*、*Glu-D1* 位点控制的均为正效应亚基,其图谱(1,7+8,5+10),(1,14+15,5+10),(1,13+16,5+10),(1,17+18,5+10),(2*,7+8,5+10),(2*,13+16,5+10)占 13.4%; 由 *Glu-3* 位点控制的低分子量谷蛋白亚基共 48 种以上的图谱,最常见的图谱是(a,j,c), *Glu-A3* 位点存在 6 个以上等位基因,新发现的占 5.7%, *Glu-B3* 位点存在 10 个以上等位基因,新发现的占 2.8%, *Glu-D3* 位点存在 3 个等位基因;由 *Gli-1* 位点控制的醇溶蛋白共 81 种以上图谱, *Gli-1A1* 位点存在 7 个以上等位基因,新发现的占 7.1%, *Gli-B1* 位点存在 12 个以上等位基因,新发现的等位基因占 3.5%, *Gli-D1* 位点存在 10 个等位基因, *Gli-B1* 位点的 1 为 1B/1R 易位系,占总数的 33.6%; 由 *Gli-1* 位点控制的醇溶蛋白和由 *Glu-3* 位点控制的低分子量谷蛋白亚基基因变异远比由 *Glu-1* 位点控制的高分子量谷蛋白亚基复杂和丰富。

关键词: 普通小麦;高分子量谷蛋白亚基;基因变异

中图分类号: S512

Characterization of *Glu-1*, *Glu-3* and *Gli-1* Alleleic Variation of Storage Protein in 141 Hexaploid Wheat Cultivars in China

WANG Rui[1], ZHANG Gai-Sheng[1], F J Zeller[2] and S L K Hsam[2]

([1] Wheat Research Center, Agronomy College, Northwest Sci-Tech University of Agriculture and Forestry, Yangling 712100, Shaanxi, China; [2] Plant Breeding Institute, Technical University of Munchen, D-85350 Freising-Weihenstephan, Germany)

Abstract: The banding patterns of HMWgs, LMWgs and HMW gliadins of 141 common wheat cultivars (lines) were analyzed using two-step SDS-PAGE and a modified A-PAGE, respectively checked out according to the 20 standard cultivars' patterns. 27 HWMgs band patterns were encoded by *Glu-1* locus, all positive effect subunits such as (1,7+8,5+10),(1,14+15,5+10),(1,13+16,5+10),(1,17+18,5+10),(2*,7+8,5+10),(2*,13+16,5+10) were about 13.4% in the 141 cultivars (lines) which were encoded by *Glu-A1*, *Glu-B1* and *Glu-D1*. Over 48 LMWgs band patterns were encoded by *Glu-3* locus, the pattern with the highest frequency was (a,j,c), more than 6 alleles were at *Glu-A* locus and 5.7% of them were newly discovered, above 10 alleles were at *Glu-B1* locus and 2.8% of them were newly discovered, 3 alleles were at *Glu-D1* locus; 81 HMW gliadin band patterns were encoded by *Gli-1* locus, more than 7 alleles were at *Gli-A1* locus, and 7.1% of them were newly discovered, more than 12 alleles were at *Gli-B1* locus, and 3.5% of them were newly discovered, 10 alleles were at *Gli-D1* locus; 33.6% alleles encoded by *Gli-B1* were 1B/1R translocation lines. So HMW gliadin alleleic variation encoded by *Gli-1* and LMWgs alleleic variation encoded by *Glu-3* are much more complex and abundant than HMWgs alleleic variation, these results also showed that LMWgs, gliadins and these similar to HMWgs are seldom located on chromosome 1A. Moreover, the A-PAGE patterns or SDS-PAGE patterns are possibly different even if their HMWgs, LMWgs and HMW gliadin compositions are the same, in other words, their LMW gliadins compositions may be different.

Key words: Common wheat; HMW; Gene variation

基金项目: 德国慕尼黑技术大学植物育种研究所资助,在德国慕尼黑技术大学植物育种研究所完成。

作者简介: 王瑞(1963-),女,西北农林科技大学副研究员,在职博士,主要从事小麦育种研究,1994、1998 年先后赴 CIMMYT、德国慕尼黑技术大学从事合作研究。Tel:029-87081471, E-mail: R631123@public.xa.an.cn

Received(收稿日期):2005-02-04; Accepted(接受日期):2005-09-18.

小麦胚乳贮藏蛋白组成与小麦加工产品的类型和产品的质量有重要关系，面包型、面条型小麦质量与其富含有益的谷蛋白成分赋予的弹性和富含有益的醇溶蛋白成分赋予优良的易拉、耐煮品质是密不可分的，面包、面条皆需要谷蛋白和醇溶蛋白共同给予的高强度面筋[1-4]。

位于小麦同源群1染色体长臂上的高分子量谷蛋白亚基基因（*Glu-1* 位点控制）可用SDS-PAGE标定，目前认为 *Glu-A1* 位点编码的1、2*，*Glu-B1* 位点编码的7+8、13+16、14+15和17+18，*Glu-D1* 位点编码的5+10对品质具有正效应，其他亚基则相反[1,4-7]。

低分子量谷蛋白亚基（*Glu-3* 位点控制）和高分子量醇溶蛋白（*Gli-1* 位点控制）的编码基因位于小麦同源群 *1* 染色体的短臂上，由于同一位点编码此二者的基因紧密连锁[8-9]，且分子量接近，在SDS-PAGE图谱中高度重叠，尤其是低分子量谷蛋白亚基谱带多，难以辨读[9-10]。Gupta等[11]采用分步法首先剔除掉麦粉中的醇溶蛋白，从而提取到单纯的谷蛋白，再用SDS-PAGE分析其高、低分子量谷蛋白亚基组成，可有效地辨读低分子量谷蛋白亚基，再配合改良的A-PAGE方法[12-13]，为深入地研究小麦低分子量谷蛋白和醇溶蛋白组成特点提供了基础。

本文研究了我国一些有影响的小麦品种、农家种及优质、抗性资源高、低分子量谷蛋白亚基和醇溶蛋白组成特点，以期为小麦优质育种提供基础和参考信息。

1 材料与方法

1.1 材料

选用小麦品种（品系）和农家种共141个，其中包括许多重要的资源材料如小偃6号、陕优225、PH82-2-2、绵阳19、陕253、中优16、郑农33、宛798、陕451、陕优412、80356、阎麦8911和冀5099等。

1.2 方法

1.2.1 高分子量醇溶蛋白遗传组成 采用A-PAGE分析，参考Jackson等提出的位点图谱[9]，依据20个标准品种的A-PAGE图谱进行辨读（图1，表1）。

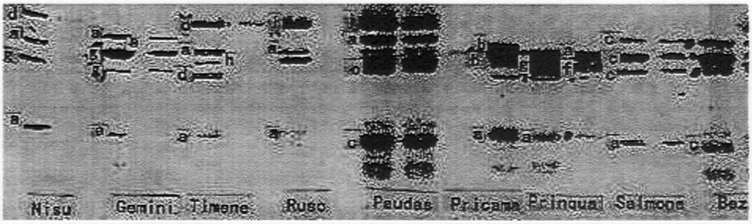

图1 一些标准品种SDS-PAGE中Glu-3（LMWgs）组成

Fig.1 Two-step SDS-PAGE patterns (mainly mark Glu-3 allele variation) of some standard cultivars

表1 20个标准品种 *Glu-3*、*Gli-1* 组成图谱

Table 1 *Glu-3*, *Gli-1* gene patterns of 20 standard cultivars

Cultivar	*Glu-A3*	*Glu-B3*	*Glu-D3*	*Gli-A1*	*Gli-B1*	*Gli-D1*
Apostle	f	g	a	b	f	g
Brimstone	a	g	c	f	f	f
Longbow	d	g	c	o	f	l
Aralon	a	b	c	f	b	b
Beaver	f	j	c	b	l	b
Riband	d	f	c	o	g	b
Alpel	a	b	b	b	b	b
Nisu	g	d	a	f	h	d
Flereal	a	d	c	a	h	l
Liecorne	e	b	a	m	b	k
Lives	e	b	c	m	b	i
Gemini	a	g	a	a	e	d
Timene	d	h	a	o	d	b
Ruso	e	I	a	m	k	a
Paudas	a	I	c	a	m	f
Pricama	e	b	a	m	b	a
Prinqual	f	g	a	f	c	a
Salmene	a	c	c	l	s	b
Chinese Spring	a	a	a	a	a	a
Hereward	f	g	c	b	f	b

1.2.2 高、低分子量谷蛋白亚基组成

1.2.2.1 谷蛋白Gupta分步法 谷蛋白采用Gupta分步法SDS-PAGE进行分离，按照20个标准品种的图谱进行辨读（图1，表3）。将半粒种子研碎置于离心管中，加250 μL 55%异丙醇过夜，次日在65℃水浴中振摇10 min，再在65℃水浴中提取30 min，离心（13 000×*g*）5 min。将上清液弃去，再加55%异丙醇250 μL，重复上述过程3次，直到醇溶蛋白被剔除完，残留物为纯化谷蛋白。

上述残留物中加入55%（*V*/*V*）异丙醇+0.08 mol/L Tris-HCl（pH 8）溶液+10% DTT（*W*/*V*）（100 μL中加入1 mg DTT），充分摇匀，在65℃振荡器中振荡10 min，再在65℃水浴中提取30 min，离心。再加含55%（*V*/*V*）异丙醇+0.08 mol Tris-HCl+10%（*W*/*V*）碘化乙酰胺，在65℃水浴中提取30 min，离心5 min，吸上清液100 μL于另一离心管中，加100 μL样品缓冲液（2% SDS，0.02%溴酚蓝+0.08 mol/L Tris-HCl+40%丙三醇）摇匀，在65℃水浴中保温15 min，离心，备用。

1.2.2.2 SDS-PAGE凝胶制备 分离胶含55%（*W*/*V*）丙烯酰胺，0.376%（*W*/*V*）甲叉双丙烯酰胺，pH 8.8，4.5% Tris，4 mol/L HCl，0.1% SDS，100 μL 10% APS，10 μL TEMED，制好后过夜。浓缩胶含55%（*W*/*V*）丙烯酰胺，1.5%（*W*/*V*）甲叉双丙烯酰胺，pH 6.8 1.71% Tris，4 mol/L HCl，0.1% SDS，80 μL

表4 *Glu-3* 基因图谱及其频率(48种谱带)

Table 4 *Glu-3* gene patterns and their frequencies (48 patterns)

Glu-A3	*Glu-B3*	*Glu-D3*	频率(%)	*Glu-A3*	*Glu-B3*	*Glu-D3*	频率(%)	*Glu-A3*	*Glu-B3*	*Glu-D3*	频率(%)
a	b	a	1.4	d	g	c	2.8	f	i	c	1.4
a	c	c	0.7	d	h	c	0.7	f	j	a	6.4
a	d	a	2.1	d	j	c	0.7	f	j	c	1.4
a	d	c	3.5	d	j	a	3.5	f	b	a	0.7
a	b	c	0.7	d	new	a	0.7	f	d	a	1.4
a	f	c	1.4	d	new	c	0.7	o	h	a	0.7
a	g	c	5.0	e	c	a	0.7	new	f	c	0.7
a	g	a	6.4	e	b	c	0.7	new	g	c	2.8
a	i	c	0.7	e	g	c	0.7	new	h	a	0.7
a	j	a	4.3	e	i	c	2.1	new	new	a	0.7
a	j	c	14.2	e	j	a	2.1	f	f	c	1.4
a	h	a	2.8	e	j	c	1.4	new	i	c	0.7
a	new	a	0.7	a	i	a	0.7	e	j	d	0.7
d	f	a	0.7	f	a	c	0.7	f	d	c	0.7
d	f	c	0.7	d	g	a	1.4	f	g	c	7.8
f	g	a	2.1								

表5 *Glu-3* 位点基因变异及其频率

Table 5 The allele variation at *Glu-3* locus and frequencies

Glu-A3(1AS)		*Glu-B3*(1BS)		*Glu-D3*(1DS)	
变异位点 Locus	频率 Frequency(%)	变异位点 Locus	频率 Frequency(%)	变异位点 Locus	频率 Frequency(%)
a	45.4	a	1.5	a	42.6
d	15.6	b	3.5	c	56.7
e	7.8	c	1.4	d	0.7
f	24.8	d	9.2		
o	0.7	f	5.2		
new	5.7	g	29.1		
		h	7.1		
		i	6.4		
		j	34.0		
		new	2.8		

注：j* 为 1B/1R 易位而来。

表6 *Gli-1* 基因图谱及其频率(81种谱带)

Table 6 *Gli-1* gene patterns and their frequencies (81 patterns)

Gli-A1	*Gli-B1*	*Gli-D1*	频率 Frequency (%)	*Gli-A1*	*Gli-B1*	*Gli-D1*	频率 Frequency (%)	*Gli-A1*	*Gli-B1*	*Gli-D1*	频率 Frequency (%)
a	l	f	1.4	new	d	a	0.7	a	f	l	2.1
a	l	i	2.1	m	s	g	0.7	b	f	f	0.7
a	l	b	0.7	f	s	i	0.7	b	f	b	1.4
b	l	i	0.7	b	h	a	1.4	new	f	i	0.7
b	l	d	0.7	a	h	g	1.4	o	new	i	2.1
b	l	a	7.8	o	h	i	2.8	new	new	g	0.7
b	l	g	2.1	f	h	i	2.1	o	g	l	0.7
f	l	g	0.7	f	h	b	0.7	a	g	f	0.7
f	l	l	1.4	a	h	a	1.4	b	g	l	1.4
f	l	a	3.5	b	k	l	0.7	a	g	l	0.7
f	l	i	5.0	new	k	l	0.7	o	g	a	0.7
m	l	d	0.7	f	e	i	1.4	new	g	i	0.7
m	l	i	1.4	f	e	f	1.4	new	c	f	0.7
m	l	a	1.4	m	k	li	2.1	new	c	i	0.7
o	l	a	0.7	a	k	a	0.7	f	c	i	0.7
o	l	I	0.7	o	k	l	0.7	a	c	b	1.4
o	l	d	1.4	new	k	i	0.7	b	c	b	0.7
o	l	j	1.4	b	k	i	0.7	o	c	i	1.4
b	e	i	1.4	f	b	a	0.7	a	c	i	0.7
m	e	i	0.7	l	b	i	1.4	a	c	a	1.4
l	l	c	0.7	a	b	k	0.7	b	c	f	0.7
new	l	a	0.7	f	f	a	0.7	o	c	l	0.7
l	d	a	0.7	f	f	d	0.7	a	c	g	1.4
o	d	I	1.4	b	f	l	2.1	f	c	a	0.7
f	d	a	0.7	b	f	i	1.4	a	new	k	0.7
a	d	b	0.7	b	f	a	0.7	o	a	a	1.4
a	d	a	1.4	a	f	a	0.7	a	a	a	0.7

表 7　*Gli-1* 位点变异及其频率

Table 7　*Gli-1* allele variation and frequencies

Gli-A1	频率 Frequency(%)	*Gli-B1*	频率(%) Frequency	*Gli-D1*	频率 Frequency(%)
a	21.3	a	2.1	a	29.8
b	24.8	b	2.8	b	5.7
f	19.9	c	12.1	c	0.7
m	7.1	d	5.7	d	3.5
l	2.8	e	4.3	f	4.3
o	16.3	f	11.3	g	7.1
new	7.1	g	5.0	i	34.8
		h	9.9	j	1.4
		k	6.4	k	1.4
		l	35.5	l	11.3
		s	1.4		
		new	3.5		

References

[1] Payne P I, Lawrence G J. Catalogue of alleles for the complex gene loci, *Glu-A1*, *Glu-B1*, *Glu-D1* which code for high-molecular-weight subunits of glutenin in hexaploid wheat. *Cereal Res Commun*, 1983, 11: 29-35

[2] Singh N K, Shepherd K W. The cumulative allelic variation in LMW and HMW glutenin subunits to physical dough properties in progeny of two bread wheat. *Theor Appl Genet*, 1989a, 77: 57-64

[3] Labuschagne M T, Van Deventer C S. The effect of Glu-B1 high molecular weight glutenin subunits on biscuit-making quality of wheat. *Euphytica*, 1995, 83: 193-197

[4] Wang R(王瑞), Ning K(宁锟), Pena R J. The correlation between high-molecular-weight subunit compositions of some high-quality bread wheat and their hybrid progenies and bread-making quality. *Acta Agriculturae Boreali-occidentalis Sinica*(西北农业学报), 1995, 4(4): 25-30

[5] Pan X-L(潘幸来), Smith D B, Jackson E A. Diversity of Glu-1, Glu-3 and Gli-1 of 16 wheat cultivars bred in Huanghuai wheat growing region in China. *Acta Genet Sin*(遗传学报), 25(3): 252-258

[6] Ma Chuanxi, Xu Feng. A comparative study of bread-making quality among Chinese wheat cultivars and those from those some other countries. *Cereal Res Commun*, 1997, 25(2): 149-153

[7] Redelli R, Ng P K, Ward R W. Eletrophoretic Characterization of storage proteins of 37 Chinese landraces of wheat. *J Genet & Breed*, 1997, 51: 239-246

[8] Payne P I, Jackson E A, Holt L M, Law C N. Genetic linkage between endosperm protein genes on each of the short arms of chromosomes 1A and 1B in wheat. *Theor Appl Genet*, 1984a, 67: 235-243

[9] Jackson E A, Holt L M, Payne P I. Characterization of high-molecular-weight gliadin and low-molecular-weight glutenin subunits of wheat endosperm by two-dimensional eletrophoresis and the chromosomal location of their controlling genes. *Theor Appl Genet*, 1983, 66: 29-37

[10] Shepherd K W. Low-molecular-weight glutenin subunits in wheat: their variation, inheritance and relation with bread-making quality. In: Proc 7th Int Genet Symp, Cambridge, England, 1988. pp 943-949

[11] Gupta R B, Shepherd K W. Two step one-dimensional SDS-PAGE analysis of LAW subunits glutelin. *Theor Appl Genet*, 1990, 80: 65-74

[12] Arangoa M A, Campanerct M A. Evaluation and characterization of Gliadin Nanob particle and isolates by Reversed-phase HPLC. *J Cereal Sci*, 2000, 31: 223-228

[13] Hoefer Scientific Instruments. Protein Electrophoresis, Applications Guide, 65 Minnesota Street, San Francis Co. CA. 1994