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西尔斯山羊草(*Aegilops searsii*)α-醇溶蛋白编码基因的克隆及原核表达

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摘 要:

α- β- γ- ω- α- 15% 30%
α- α-
α-
PCR α- KC421089
pEASY-E1 BL21 DE3 IPTG
Aegilops searsii 7 α-
849 954 bp 282 317 α-
α-
SNPs
7 α- KC421089

关键词: α- ; ; ;
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Cloning and Prokaryotic Expression of α-Gliadin Genes from *Aegilops searsii*

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Abstract: Gliadins are the major influence factors of wheat processing quality, which determine dough extensibility. On the basis of the electrophoretic mobility by acidic polyacrylamide gel electrophoresis, gliadin can be separated into α- β- γ- and ω-gliadin. Among them, α-gliadins are the most abundant and accounted for 15% 30% of the wheat storage proteins. On the other hand, α-gliadins can cause different diseases. The present study aimed at cloning and analyzing the α-gliadin genes from *Aegilops searsii*, expressing it in E.*coli* and obtaining high purified proteins. The α-gliadin genes were amplified by PCR and then inserted the gene KC421089 into pEASY-E1. The recombinant plasmids were expressed in the E.*coli* BL21(DE3) and then the purified proteins were obtained by cutting the gel slices. Seven novel α-gliadin genes were cloned from *Aegilops searsii.* Their length of the open reading frames ranged from 849-954bp, encoding the putative proteins with 282-317 amino acid residues, respectively. A BLAST search showed that these sequences have the typical structure of α-gliadin genes and SNPs and In/Dels. Moreover, the target proteins were expressed by *E. coli* and highly purified proteins were obtained by cutting the gel slices. Seven novel α-gliadin genes were cloned from *Aegilops searsii*, and the gene KC421089 were expressed by *E. coli* and highly purified proteins were obtained by cutting the gel slices. This study laid a good foundation for wheat quality improvement.

Keywords: α-gliadin; *Aegilops searsii*; Sequence analysis; prokaryotic expression

50% 60%[1]
α- β- γ- ω- [2,3]
α- 15 30%[4-6] 31 kD
Gli-2 *Gli-A2* *Gli-B2* *Gli-D2* [7]
(Celiac disease, CD)

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[8] α-
CD T
CD α-
- [9] CD
CD

Claudia [10] Ferrante [11] *E. coli*
γ-
α-

α-

1 材料与方法

1.1 材料来源

Y2131 *Aegilops searsii*, S^sS^s, 2n=2x=14

1.2 基因组 DNA 提取

CTAB DNA [12]

1.3 基因的克隆及序列分析

GenBank α-
P1 5’-ATG AAG ACC TTT CTC ATC CTT G-3’ P2 5’-TCA GTT RGT ACC RAA GAT GCC-3’ http://web.expasy.org/translate/ MEGA 5.0
DNAMAN CD
[8]

1.4 原核表达与纯化

ORF
P3 5’-GCA GTT AGA GTT CCA GTG CCA-3’ P4 5’-TCA GTT RGT ACC RAA GAT GCC-3’
pEASY-E1 Trans1-T1
DNA BL21(DE3)
PCR [13,14]

2 结果与分析

2.1 α-醇溶蛋白的克隆及序列分析

P1 P2 DNA 1000 bp
1 7 Genbank

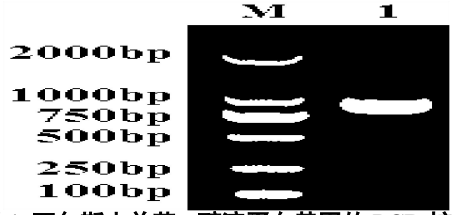

图 1 西尔斯山羊草α-醇溶蛋白基因的 PCR 扩增
Fig.1 PCR amplification of the α-gliadin gene from *Aegilops searsii*
M: DL2000; 1: Y2131

α-

α- α- Genbank KC421084-KC421090 849-954 bp 1 282 317 α- 2 α- [15] 6 SNPs

表 1 西尔斯山羊草α-醇溶蛋白的特征分析

Table 1 Characterization of 7 novel α-gliadin from *Aegilops searsii*

Genbank No.	(bp) Length of gene (bp)	Deduced amino acids	KD Molecular weight (KD)	pI pI
KC421084	849	282	32.40	7.64
KC421085	954	317	36.57	8.56
KC421086	909	302	34.82	7.65
KC421087	900	299	34.33	7.61
KC421088	936	311	35.56	8.24
KC421089	849	282	32.41	7.64
KC421090	924	307	35.07	8.24

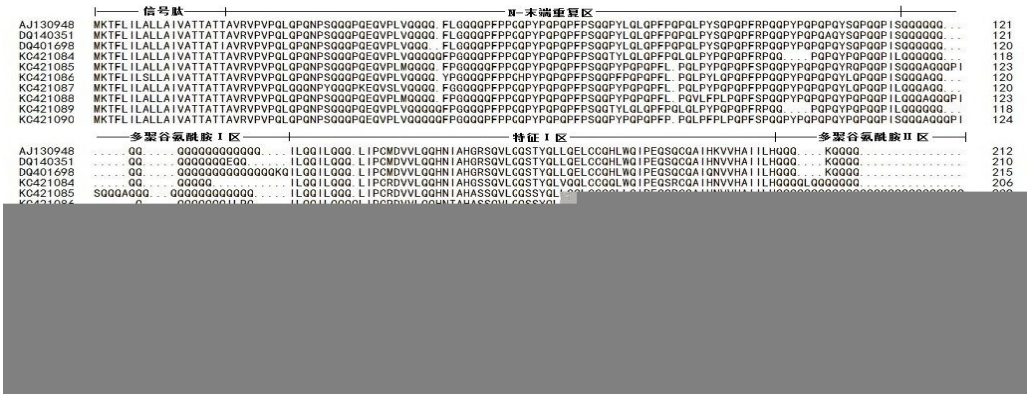

图 2 西尔斯山羊草α-醇溶蛋白基因序列与斯卑尔脱小麦的 AJ130948，圆锥小麦的 DQ140351，栽培一粒小麦的 DQ401698 的推导氨基酸序列比较

注:△表示保守的半胱氨酸残基，方框中的序列为 glia-α 毒性多肽

Fig.2 The deduced amino acid sequences of *Aegilops searsii* α-gliadin genes compared with *Triticum spelta* derived sequence AJ130948; *Triticum turgidum* derived sequence DQ140351 and *Triticum monococcum* derived sequence DQ401698

Note: represents conserved cysteine residues. The boxed letters are glia-α toxic epitope

2.2 CD 毒性多肽的识别与分析

α- 4 glia-α9 glia-α20 glia-α glia-α2 A α- glia-α9 glia-α20 B α- glia-α D α- 4 [6] α- glia-α 2 B α-

表 2 西尔斯山羊草α-醇溶蛋白具有的 T 细胞抗原表位数量及类型

Table 2 Number and type of the four T cell stimulatory toxic epitopes in the α-gliadin from *Aegilops searsii*

Genbank No.	glia-α	glia-α2	glia-α9	glia-α20
KC421084	1	0	0	0
KC421085	1	0	0	0
KC421086	1	0	0	0
KC421087	1	0	0	0
KC421088	1	0	0	0
KC421089	1	0	0	0
KC421090	1	0	0	0

2.3 α-醇溶蛋白聚类分析

Genbank α- Neighbor-joining 3 4 A D S^s B α- S^s D S^s α- B α-

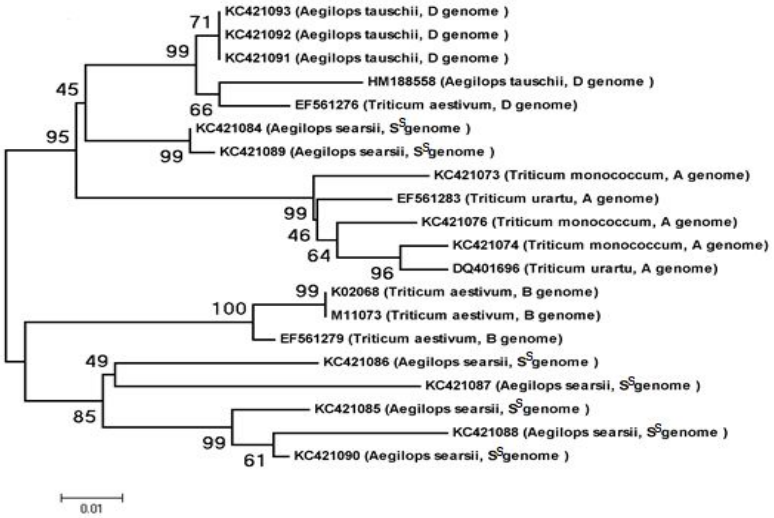

图 3 α-醇溶蛋白基因的系统进化分析

Fig.3 Phylogenetic analysis of α-gliadin genes.

2.4 基因的诱导表达与检测

KC421089 BL21(DE3)

OD_{600} 0.6 IPTG 37 °C 10 h SDS-PAGE 18.8 35KD

4 α-

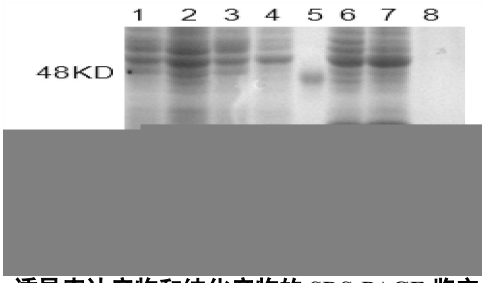

图 4 诱导表达产物和纯化产物的 SDS-PAGE 鉴定分析

注: 1:未诱导的 BL21(DE3);2:诱导后的 BL21(DE3);3:未诱导的转化空载体的菌株;4:诱导的转化空载体的菌株;5:Marker;6:转化重组质粒后未经诱导的菌株;7:转化重组质粒经诱导的菌株;8:纯化的目的蛋白

Fig.4 Analysis of expressed and purified proteins by SDS-PAGE

Note: 1: Protein of BL21(DE3) without induction; 2: Protein of BL21(DE3) induced by IPTG; 3: BL21(DE3) with pEASY-E1 without induction; 4: BL21(DE3) with pEASY-E1 induced by IPTG; 5: Protein molecular weight marker; 6: Protein of recombinant plasmid without induction; 7: Protein of recombinant plasmid induced by IPTG; 8: Purified protein.

3 讨 论

T

CD

CD CD

[16] Van Herpen B α-

[6] S^s glia-α α-

S^s B

α-

A D [17]

B

B [15]

D α- B α-
3 S^s
B
[11]

4 结 论

4.1 α-醇溶蛋白基因的克隆和序列分析

7 α-
KC421084-KC421090 7 α-
6 2
D α-
B α-

4.2 α-醇溶蛋白基因的原核表达

KC421089 α-

α-

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