cDNA Clone of β-1，3-Glucanase from Phaseolus Vulgaris and Construction of Expressed Vector for Plant

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Abstract: β-1,3-glucanase is one of the important composition involved in resist affected by plant pathogeny. We have isolated total RNA from leaves of phaseolus vulgaris, cloned cDNA of β-1,3-glucanase gene using RT-PCR in order to improve the ability of resistant pathogeny of plant. We linked this gene with pGEM-T-Easy, analysis the whole gene sequence .The result of sequence of this gene showed: cloned cDNA whole length is 1035 bp,encoded 331 amino acid, has 85% homology compared with reported sequence, conserved region of amino acid almost consistent with others .This cloned gene has been embodied by GenBank, logging number is DQ093563. We have constructed the express vector of this cloned gene for plant. [Nature and Science. 2005;4(1):37-46].

Keywords: phaseolus vulgaris; β-1，3-glucanase; cloned cDNA; sequence analysis; plant expressed vector

Introduction

β-1,3-glucanase are abundant in various plant species .Some studied showed that β-1,3-glucanase refer to many physiology process, including cereal germination, hypocotyls and coleoptile development, phloem transportation, callus movement, canaliculus tissue transportation and regulation, cell wall biology synthesis, flower development, microspore formation, pollen tube development, fruit mature, plant caducity and immobility etc. Recently, along with study deeply, that is discovered that β-1,3-glucanase play an important role in resistant disease of plant. β-1,3-glucan and Chitin are important composition in cell wall of fungi, β-1,3-glucan and Chitin exposure on surface of top end of mycelia of fungi which accept the attacked by β-1,3-glucanase and chitinase. The experimental of resistant fungi in vitro showed that β-1,3-glucanase can restrain the growth of mycelial. But then β-1,3-glucanase and chitinase work together show more distinctness about their resistant fungi than only use one.

More important thing is that oligosaccharide which released from cell wall of fungi during hydrolyzation induced whole resistance disease of plant as excitated factor in many reactions of resistant disease of plant. This aspect reports are focus on studying the work together of soybean and soybean epidemic disease.

GEBP (Glucan Elicitor Binding Protein GEBP) was found in soybean that locus at member of cytoplasm of radicle. GEBP can specific combine with oligosaccharide excitated factor which was release from β-1,3-glucanase degradation and induce defense reaction of plant.

The bioactivity of resistant disease of β-1,3-glucanase arose people think much of it. So far, at least 26 kinks of β-1,3-glucanase and their cDNA clone have been isolated. Furthermore have already transformation β-1,3-glucanase into tobacco, Chinese goosebeery, rose, tomato, cole, clover, carrot, ect, many plants, obtain expressed in different degree.

This study focus on clone cDNA of β-1,3-glucanase from Phaseolus Vulgaris and whole sequence analysis, construct expressed vector of β-1,3-glucanase for plant. Genetic transformation into plant are process of studying.

1. Materials and Methods

1.1 Plant Material

Phaseolus Vulgaris was provided by Agriculture Academic Gardening Institute of Hei Longjiang in China.

1.2 Bacteria and Plasmid

Host cell, *E. coli* JM109 from TaKaRa Bioengneering (Dalian) Co., Ltd. China, Vector, pGEM-T-Easy, from Promega Co., pMHL7133-*Gus,* from Japan.

1.3 Enzyme and Reagent

Enzymes and IPTG、X-gal、dNTP, from TaKaRa Bioengneering (DaLian) Co., Ltd, China, and Promega Co; RT-PCR Kit from Invitrogen Co., T_4DNA ligase from GIBCO Co., UNTQ-10 Kit from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd, Primer was synthesized by TaKaRa Bioengneering (DaLian) Co., ltd, China, Gene sequence analysis was done by Shang Hai Bioasia Bioengneering Ltd., Co..

1.4 Treat of PhaseolusVulgaris and Isolation Total RNA

Phaselous Vulgaris was planted in plastic shed in order to germination and growth. Two weeks later, take young leaf on the top, isolation total RNA use Guanidine Isothiocyanate method of our improved .

1.5 cDNA First Strand Synthesis of Target Gene, PCR Amplification and Clone

Template is 1 μg total RNA of Phaselous Vulgaris, primer is oligo（dT）, according to the description of ThermoScript™ RT-PCR system of Invitrogen Co., synthesize cDNA first strand.

Based on the sequence of β-1,3-D-Glucanase of Edington, B. V. (1996) reported, use Primer Premier 5.0 software to design two primers for PCR reaction:

5’CTGGATCCTCAAATCGGGGTGTGTTATG 3’

BamH Ⅰ

3’GGTGGTTTTATTCTGTCTTCTCGAGGT 5’

Sac Ⅰ

We insert the restriction enzyme sites of the BamH Ⅰ and Sac Ⅰ on the 5′ end and 3′ end in order to clone and construct expressed vector in the further.

Take 1 μl the product of RT-PCR amplication for target gene in 25 μl system.

Conditions: 94℃ 2 min, 94℃ 30s, 55℃ 50s, 72℃ 1 min， 72℃ 10 min, 4℃ hold, 30 cycles.

Use the UNTQ-10 Kit from Shang Hai Sangon Biological Engineering Technology & Services Co., to purified PCR product, T_4ligase link it with pGEM-T-Easy vector, screen positive clone on LB plate with Amp ,IPTG and X-gal.

1.6 DNA Sequence Analysis

Commission Shang Hai Bioasia Bioengneering Co., Ltd., sequence the target gene, analysis the results of target sequence.

1.7 Construction Recombinant Express Vector for Plant

Isolated plasmid of pMHL7133-*Gus*, remove phosphorylation, link the β-1,3-Glucanase gene to the vector.

2.Results and Discussion

2.1 Isolation total RNA of β-1,3-Glucanase gene from Phaselous Vulgaris and PCR amplification from Figure 1 show integrality of RNA is perferably. PCR product is 1.1 Kb, consistent with result of anticipate (Figure 2).

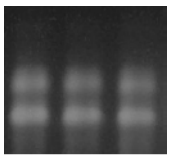


Figure 1. Isolation total RNA from phaselous vulgaris leaf

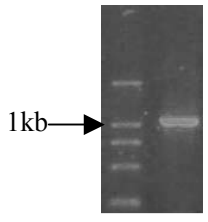
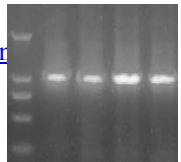
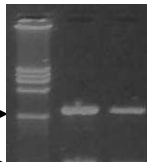


Figure 2. cDNA PCR amplification of β-1,3-Glucanase gene





2.2 cDNA Clone of β-1,3-Glucanase of Phaselous Vulgaris

Reclaim 1.1 Kb segment from gel, link with vector of pGEM-T-Easy, obtain recombinant plasmid of pGEM-T-Glu, double enzyme of BamHⅠ and SacⅠ cut it, get 1.1 Kb segment (Figure 3), PCR amplification of recombinant plasmid also get 1.1 Kb segment (Figure 4), thereout indicate cDNA clone of β-1,3-Glucanase has been insert into cloned vector.

2.3 cDNA Sequence Determine and Analysis of β-1,3-Glucanase of Phaselous Vulgaris

Sequence result shows: This cDNA whole length is 1035 bp, ORF from 3-998 bp, encod 331 amino acid, putative molecular weight 36.9 KD, isoelectric point 8.64 (Figure 5).

```
1      GCATGATGGGCAACAATCTCCCATCAGCCAATGAAGTTATAAACCTTTACAGATCAAACAAC
1        M  M  G  N  N  L  P  S  A  N  E  V  I  N  L  Y  R  S  N  N
61     ATAAGAAGAATGAGACTTTACGATCCCAATCAAGCAGCTCTGCAAGCACTCAGAAACTCA
21       I  R  R  M  R  L  Y  D  P  N  Q  A  A  L  Q  A  L  R  N  S
121    GGCATTGAACTCATTCTTGGAGTGCCAAACTCTGATCTTCAGGGTCTTGCCACCAATGCC
41       G  I  E  L  I  L  G  V  P  N  S  D  L  Q  G  L  A  T  N  A
181    GACACTGCTCGTCAATGGGTGCAAAGGAACGTGCTGAACTTTTGGCCCAGTGTTAGAATC
61       D  T  A  R  Q  W  V  Q  R  N  V  L  N  F  W  P  S  V  R  I
241    AAGTACATAGCAGTTGGCAATGAAGTGAGTCCTGTTGGAGGTTCCTCTTGGTATGCCCAA
81       K  Y  I  A  V  G  N  E  V  S  P  V  G  G  S  S  W  Y  A  Q
301    TATGTTCTACCTGCTGTCCAAAATGTATACCAAGCTATAAGGGCTCAAGGCCTCCATGAT
101      Y  V  L  P  A  V  Q  N  V  Y  Q  A  I  R  A  Q  G  L  H  D
361    CAAATCAAGGTTTCAACAGCCATTGACATGACCCTTATAGGAAACTCCTACCCTCCATCA
121      Q  I  K  V  S  T  A  I  D  M  T  L  I  G  N  S  Y  P  P  S
421    CAAGGTTCCTTCAGGGGTGATGTTAGATCATACCTAGACCCTATAATAGGGTACTTGCTA
141      Q  G  S  F  R  G  D  V  R  S  Y  L  D  P  I  I  G  Y  L  L
481    TATGCAAGTGCACCTTTGCTAGTGAATGTGTACCCTTATTTCAGTTACTCTGGCAATCCT
161      Y  A  S  A  P  L  L  V  N  V  Y  P  Y  F  S  Y  S  G  N  P
541    CGTGATATATCACTTCCCTATGCTCTTTTCACTTCACCAAATGTTGTGGTGAGGGATGGC
181      R  D  I  S  L  P  Y  A  L  F  T  S  P  N  V  V  V  R  D  G
601    CAATATGGGTACCAAAATCTGTTTGATGCTATGTTGGATTCAGTGCATGCAGCCATTGAT
201      Q  Y  G  Y  Q  N  L  F  D  A  M  L  D  S  V  H  A  A  I  D
661    AACACTAGGATTGGTTACGTGGAGGTGGTTGTGTCTGAGAGTGGGTGGCCCTCAGATGGA
221      N  T  R  I  G  Y  V  E  V  V  V  S  E  S  G  W  P  S  D  G
721    GGGTTTGGTGCCACGTATGACAACGCACGTGTGTACTTGGATAACTTGGTTCGTCGTGCT
241      G  F  G  A  T  Y  D  N  A  R  V  Y  L  D  N  L  V  R  R  A
781    GGAAGAGGAAGCCCTAGAAGGCCTTCGAAGCCTACAGAGACTTATATATTTGCCATGTTC
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261        G  R  G  S  P  R  R  P  S  K  P  T  E  T  Y  I  F  A  M  F
841        GATGAGAATCAAAAGAGTCCTGAGATAGAGAAGCATTTTGGGCTCTTTAAACCCAGCAAA
281        D  E  N  Q  K  S  P  E  I  E  K  H  F  G  L  F  K  P  S  K
901        GAGAAGAAGTACCCCTTTGGATTTGGTGCCCAAAGGGATGCAAAGATTGTGGTTGATGAG
301        E  K  K  Y  P  F  G  F  G  A  Q  R  D  A  K  I  V  V  D  E
961        TTCAATGCAACATATCCCCTTAAGAGTGACATGTAAGGTTGGAACCCTAGTTCTCAAAGT
321        F  N  A  T  Y  P  L  K  S  D  M  *
1021       CTGTTGTAATATT
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Figure 5. Glu Nucleotide Sequence of β-1,3-Glucanase Gene of Phaselous Vulgaris and Putative Amino Acid Sequence

Homologous searches 1035 bp of Glu nucleotide sequence through BLASTN software, result shows 136 items which have homologous sequence with it in database of GeneBank, more of them are β-1,3-Glucanase gene, more homology in turn are *Phaseolus vulgaris, Glycine max, Medicago sativa, Pisum sativum, Cicer arietinum, Hevea brasiliensis,etc*. Homologous rate of correspond region are almost between 84%and 99%.

Send 331 amino acids sequence of Glu encoded to the server of NCBI, use BLASTP tool to homologous searches,result show 448 items which have homologous sequence with Glu protein, all of them are β-1,3-Glucanase gene, more homology in turn are *Phaseolus vulgaris, Medicago sativa, Cicer arietinum, Pisum sativum, Hevea brasiliensis,etc.* Homologous rate of all of them are above 78%.

Compare homologous sequence of Glu gene with 3 items of β-1,3-Glucanase gene from *Phaseolus vulgaris* which were publicized in GenBank (Figure 6), result show that Glu gene has high level homology with Ga gene（caa37289）, Gb gene（P23535）， Gc gene（S13323）on correspondence amino acid sequence. Sufficiency testify that Glu gene is one of members of β-1,3-Glucanase gene family. But it has portion difference with others on amino acid sequence, putative different Phaselous Vulgaris species can cause a little diversity on amino acid sequence.

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Glu         MMGNNLPSANEVINLYRSNNIRRMRLYDPNQ    867
Gc          MMGNNLPSANEVINLYRSNNIRRMRLYDPNG     69
Ga          MMGNNLPSANEVINLYRSNNIRRMRLYDPNQ     69
Gb          MMGNNLPSANEVINLYRSNNIRRMRLYDPNQ     69
Consensus   mmgnnlpsanevinlyrsnnirrmrlydpn
Glu         AALQALRNSGIELILGVPNSDLQGLATNADT    898
Gc          AALGALRNSGIELILGVPNSDLQGLATNADT    100
Ga          AALQALRNSGIELILGVPNSDLQGLATNADT    100
Gb          AALQALRNSGIELILGVPNSDLQGLATNADT    100
Consensus   aal alrnsgielilgvpnsdlqglatnadt
Glu         ARQWVQRNVLNFWPSVRIKYIAVGNEVSPVG    929
Gc          ARQWVQRNVLNFWPSVKIKYIAVGNEVSPVG    131
Ga          ARQWVQRNVLNFWPSVKIKYIAVGNEVSPVG    131
Gb          ARQWVQRNVLNFWPSVKIKYIAVGNEVSPVG    131
Consensus   arqwvqrnvlnfwpsv ikyiavgnevspvg
Glu         GSSWYAQYVLPAVQNVYQAIRAQGLHDQIKV    960
Gc          GSSWYAQYVLPAVQNVYGAVRAQGLHDGIKV    162
Ga          GSSWYAQYVLPAVQNVYQAVRAQGLHDQIKV    162
Gb          GSSWYAQYVLPAVQNVYQAVRAQGLHDQIKV    162
Consensus   gsswyaqyvlpavqnvy a raqglhd ikv
Glu         STAIDMTLIGNSYPPSQGSFRGDVRSYLDPI    991
Gc          STAIDMTLIGNSYPPSQGSFRGDVRSYLDPI    193
Ga          STAIDMTLIGNSYPPSQGSFRGDVRSYLDPI    193
Gb          STAIDMTLIGNSYPPSQGSFRGDVRSYLDPI    193
Consensus   staidmtlignsyppsqgsfrgdvrsyldpi
Glu         IGYLLYASAPLLVNVYPYFSYSGNPRDISLP   1022
Gc          IGYLLYASAPLHVNVYPYFSYSGNPRDISLP    224
Ga          IGYLLYASAPLHVNVYPYFSYSGNPRDISLP    224
Gb          IGYLLYASAPLHVNVYPYFSYSGNPRDISLP    224
Consensus   igyllyasapl vnvypyfsysgnprdislp
Glu         YALFTSPNVVVRDGQYGYQNLFDAMLDSVHA   1053
Gc          YALFTSPNVVVRDGQYGYQNLFDAMLDSVHA    255
Ga          YALFTSPNVVVRDGQYGYQNLFDAMLDSVHA    255
Gb          YALFTSPNVVVRDGQYGYQNLFDAMLDSVHA    255
Consensus   yalftspnvvvrdgqygyqnlfdamldsvha
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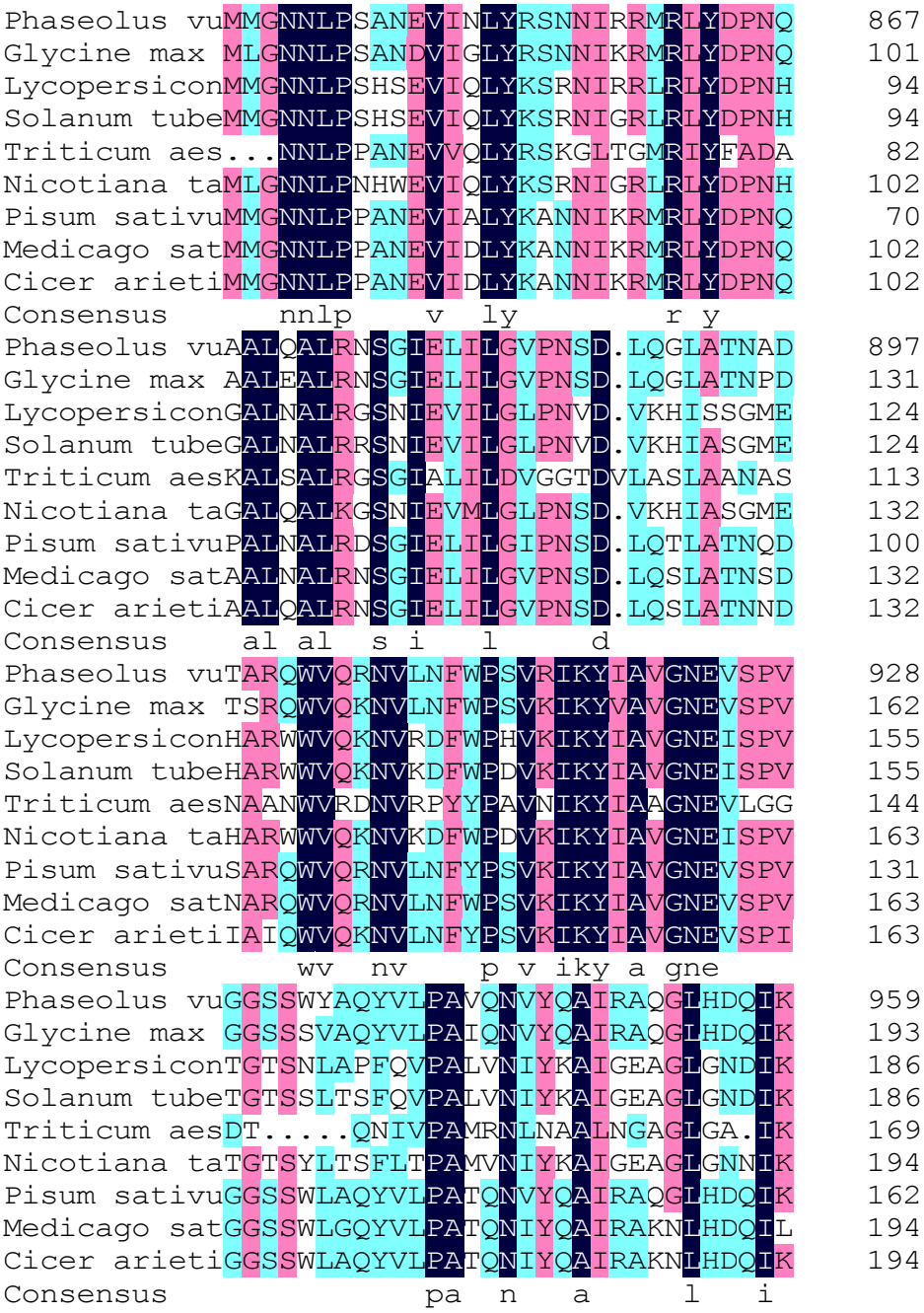
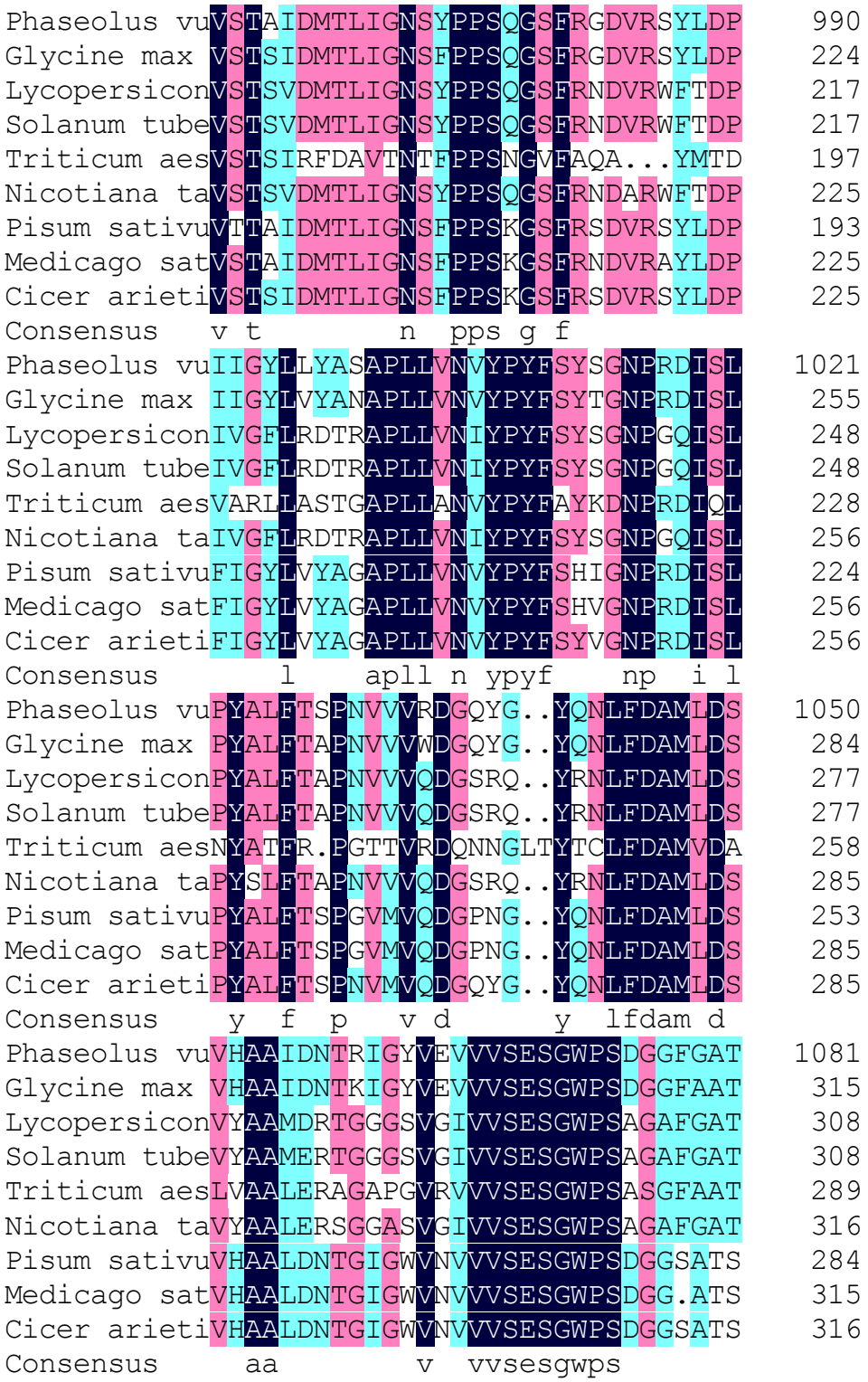
Figure 6. Compare Deduce Amino Acid Sequence of Glu Gene from Phaseolus Vulgaris with Amino Acid Sequence of β-1,3-Glucanase Genes from Other Phaseolus Vulgaris. The Embody Number of Genbank of Comparing Sequence: Ga(CAA37289); Gb(P23535); Gc(S13323).

Use DNAMAN4.0 Multiple Sequence Alignment tool compare homology of protein product of Glu gene encoded with protein product of Glycine max, Lycopersicon esculentum, Solanum tuberosum, Triticum aestivum, Nicotiana tabacum, Pisum sativum, Medicago sativa, Cicer arietinum gene encoded which were embodied in Genbank.(Figure 7), the result shows Glu gene from Phaseolus Vulgaris has portion homology with β-1,3-Glucanase gene of Glycine max, Lycopersicon esculentum, Solanum tuberosum, Triticum aestivum, Nicotiana tabacum, Pisum sativum, Medicago sativa, Cicer arietinum. Illuminate that there is different of β-1,3-Glucanase gene among different species and genus.


Phaseolus vuMMGNNLPSANEVINLYRSNNIRRMRLYDPNQ 867
Glycine max MLGNNLPSANDVIGLYRSNNIKRMRLYDPNQ 101
LycopersiconMMGNNLPSHSEVIQLYKSRNIRRLRLYDPNH 94
Solanum tubeMMGNNLPSHSEVIQLYKSRNIGRLRLYDPNH 94
Triticum aes...NNLPPANEVVQLYRSKGLTGMRIYFADA 82
Nicotiana taMLGNNLPNHWEVIQLYKSRNIGRLRLYDPNH 102
Pisum sativuMMGNNLPPANEVIALYKANNIKRMRLYDPNQ 70
Medicago satMMGNNLPPANEVIDLYKANNIKRMRLYDPNQ 102
Cicer arietiMMGNNLPPANEVIDLYKANNIKRMRLYDPNQ 102
Consensus nnlp v ly r y
Phaseolus vuAALQALRNSGIELILGVPNSD.LQGLATNAD 897
Glycine max AALEALRNSGIELILGVPNSD.LQGLATNPD 131
LycopersiconGALNALRGSNIEVILGLPNVD.VKHISSGME 124
Solanum tubeGALNALRRSNIEVILGLPNVD.VKHIASGME 124
Triticum aesKALSALRGSGIALILDVGGTDVLASLAANAS 113
Nicotiana taGALQALKGSNIEVMLGLPNSD.VKHIASGME 132
Pisum sativuPALNALRDSGIELILGIPNSD.LQTLATNQD 100
Medicago satAALNALRNSGIELILGVPNSD.LQSLATNSD 132
Cicer arietiAALQALRNSGIELILGVPNSD.LQSLATNND 132
Consensus al al s i l d
Phaseolus vuTARQWVQRNVLNFWPSVRIKYIAVGNEVSPV 928
Glycine max TSRQWVQKNVLNFWPSVKIKYVAVGNEVSPV 162
LycopersiconHARWWVQKNVRDFWPHVKIKYIAVGNEISPV 155
Solanum tubeHARWWVQKNVKDFWPDVKIKYIAVGNEISPV 155
Triticum aesNAANWVRDNVRPYYPAVNIKYIAAGNEVLGG 144
Nicotiana taHARWWVQKNVKDFWPDVKIKYIAVGNEISPV 163
Pisum sativuSARQWVQRNVLNFYPSVKIKYIAVGNEVSPV 131
Medicago satNARQWVQRNVLNFWPSVKIKYIAVGNEVSPV 163
Cicer arietiIAIQWVQKNVLNFYPSVKIKYIAVGNEVSPI 163
Consensus wv nv p v iky a gne
Phaseolus vuGGSSWYAQYVLPAVQNVYQAIRAQGLHDQIK 959
Glycine max GGSSSVAQYVLPAIQNVYQAIRAQGLHDQIK 193
LycopersiconTGTSNLAPFQVPALVNIYKAIGEAGLGNDIK 186
Solanum tubeTGTSSLTSFQVPALVNIYKAIGEAGLGNDIK 186
Triticum aesDT.....QNIVPAMRNLNAALNGAGLGA.IK 169
Nicotiana taTGTSYLTSFLTPAMVNIYKAIGEAGLGNNIK 194
Pisum sativuGGSSWLAQYVLPATQNVYQAIRAQGLHDQIK 162
Medicago satGGSSWLGQYVLPATQNIYQAIRAKNLHDQIL 194
Cicer arietiGGSSWLAQYVLPATQNIYQAIRAKNLHDQIK 194
Consensus pa n a l i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Lycopersicon VSTSVDMTLIGNSYPPSQGSFRNDVRWFTDP 217
Solanum tube VSTSVDMTLIGNSYPPSQGSFRNDVRWFTDP 217
Triticum aes VSTSIRFDAVTNTFPPSNGVFAQA...YMTD 197
Nicotiana ta VSTSVDMTLIGNSYPPSQGSFRNDARWFTDP 225
Pisum sativu VTTAIDMTLIGNSFPPSKGSFRSDVRSYLDP 193
Medicago sat VSTAIDMTLIGNSFPPSKGSFRNDVRAYLDP 225
Cicer arieti VSTSIDMTLIGNSFPPSKGSFRSDVRSYLDP 225
Consensus v t n pps g f
Phaseolus vu IIGYLLYASAPLLVNVYPYFSYSGNPRDISL 1021
Glycine max IIGYLVYANAPLLVNVYPYFSYTGNPRDISL 255
Lycopersicon IVGFLRDTRAPLLVNIYPYFSYSGNPGQISL 248
Solanum tube IVGFLRDTRAPLLVNIYPYFSYSGNPGQISL 248
Triticum aes VARLLASTGAPLLANVYPYFAYKDNPRDIQL 228
Nicotiana ta IVGFLRDTRAPLLVNIYPYFSYSGNPGQISL 256
Pisum sativu FIGYLVYAGAPLLVNVYPYFSHIGNPRDISL 224
Medicago sat FIGYLVYAGAPLLVNVYPYFSHVGNPRDISL 256
Cicer arieti FIGYLVYAGAPLLVNVYPYFSYVGNPRDISL 256
Consensus l apll n ypyf np i l
Phaseolus vu PYALFTSPNVVVRDGQYG..YQNLFDAMLDS 1050
Glycine max PYALFTAPNVVVWDGQYG..YQNLFDAMLDS 284
Lycopersicon PYALFTAPNVVVQDGSRQ..YRNLFDAMLDS 277
Solanum tube PYALFTAPNVVVQDGSRQ..YRNLFDAMLDS 277
Triticum aes NYATFR.PGTTVRDQNNGLTYTCLFDAMVDA 258
Nicotiana ta PYSLFTAPNVVVQDGSRQ..YRNLFDAMLDS 285
Pisum sativu PYALFTSPGVMVQDGPNG..YQNLFDAMLDS 253
Medicago sat PYALFTSPGVMVQDGPNG..YQNLFDAMLDS 285
Cicer arieti PYALFTSPNVMVQDGQYG..YQNLFDAMLDS 285
Consensus y f p v d y lfdam d
Phaseolus vu VHAAIDNTRIGYVEVVVSESGWPSDGGFGAT 1081
Glycine max VHAAIDNTKIGYVEVVVSESGWPSDGGFAAT 315
Lycopersicon VYAAMDRTGGGSVGIVVSESGWPSAGAFGAT 308
Solanum tube VYAAMERTGGGSVGIVVSESGWPSAGAFGAT 308
Triticum aes LVAALERAGAPGVRVVVSESGWPSASGFAAT 289
Nicotiana ta VYAALERSGGASVGIVVSESGWPSAGAFGAT 316
Pisum sativu VHAALDNTGIGWVNVVVSESGWPSDGGSATS 284
Medicago sat VHAALDNTGIGWVNVVVSESGWPSDGG.ATS 315
Cicer arieti VHAALDNTGIGWVNVVVSESGWPSDGGSATS 316
Consensus aa v vvsesgwps

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Phaseolus vuYDNARVYLDNLVRRAGRGSPRRPSKPTETY   1111
Glycine max YDNARVYLDNLVRRANRGSPRRPSKPTETY    345
LycopersiconHENAQTYLRNLIQHAKEGSPRKPG.PIETY    337
Solanum tubeQDNAATYLRNLIQHAKEGSPRKPG.PIETY    337
Triticum aesADNARAYNQGLIDHVGGGTPKRPGLL.ETY    318
Nicotiana taYDNAATYLRNLIQHAKEGSPRKPG.PIETY    345
Pisum sativuYDNARIYLDNLIRHVGKGTPRRPWA.TEAY    313
Medicago satYDNARIYLDNLIRYEGKGTPRRPWA.TETY    344
Cicer arietiYDNARIYLDNLIRHVGKGTPRRPWA.TETY    345
Consensus     na  y   l      g p  p    e y
Phaseolus vuIFAMFDENQKSPEI.EKHFGLFKPSKEKKY   1140
Glycine max IFAMFDENQKNPEI.EKHFGLFNPNKQKKY    374
LycopersiconIFAMFDENNKNPEL.EKHFGMFSPNKQPKY    366
Solanum tubeIFAMFDENNKNPEL.EKHFGLFSPNKQPKY    366
Triticum aesIFAMFNENFKTGELTEKHFGLFNPDKSPAY    348
Nicotiana taIFAMFDENNKNPEL.EKHFGLFSPNKQPKY    374
Pisum sativuLFAMFDENQKSPEL.EKHFGVFYPNKQKKY    342
Medicago satIFAMFDENQKSPEL.EKHFGVFYPNKQKKY    373
Cicer arietiIFAMFDENQKSPEL.EKHFGVFNPNKQKKY    374
Consensus    famf en k  e  ekhfg f p k   y
Phaseolus vuPFGFGAQRD.AKIVVDEFNATY.P.LKSDM   1167
Glycine max PFGFGGKRL.GKVVIDDFNATT.S.IKSDV    401
LycopersiconNLNFGVSER.VWDI...TNSTA.SSLTSEI    391
Solanum tubeNLNFGVSER.VWDISAETNSTT.SSLISEM    394
Triticum aesPIQFH                             353
Nicotiana taNLNFGVSGG.VWDSSVETNATA.S.LISEM    401
Pisum sativuPFGFGGERRDGEIVEGDFNGT.VS.LKSDM    370
Medicago satPFGFGGERMG..IVNGDFNAT.IS.LKSDM    399
Cicer arietiPFGFGGERRNGEIVNDDFNATTVS.LKSDM    403
Consensus     f
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Figure7. Compare Deduce Amino Acid Sequence of Glu Gene from Phaseolus Vulgaris with Amino Acid Sequence of β-1,3-Glucanase Genes from Other Plant.

The Embody Number of Genbank of Comparing Sequence:

Glycine max (CAA01814), *Lycopersicon esculentum* (Q01413), *Solanum tuberosum* (CAE53273), *Triticum aestivum* (AAY88778), *Nicotiana tabacum* (p27666), *Pisum sativum* (AAB24398), *Medicago sativa* (AAB41551), *Cicer arietinum*（CAA10287）

2.4 Construction Express Vector of β-1,3-Glucanase Gene of Phaselous Vulgaris for Plant

We use high effective express vector pMHL7133-*Gus* of plant which has CaMV of 35S constitutive type strong promoter that can make foreign gene express occur in all position and any development stage of transgenetic plant in order to transformation β-1,3-glucanase gene into plant and study the possibility of its anti- fungi. Furthermore, the upstream of 35S promoter on vector pMHL7133-*Gus* has E7 enhance regulated element which can enhance transcription efficiency of target gene. The downstream of 35S promoter on vector pMHL7133-*Gus* has Ω sequence which can provide the new binding site for ribosome consequently boost up translation efficiency of foreign gene.

Use double enzymes to cutted pMHL7133-*Gusv* and remove phosphorylation, link the reclaimed product from gel of Glu cDNA, obtain recombinant clone pMHL7133-Glu. Double enzymes of BamHⅠ and Sac

Ⅰ cut it, get about 1.1 Kb segment from recombinant clone (Figure 8). PCR of recombinant plasmid also get 1.1 Kb segment (Figure9), thereout testify that construction of high efficiency express vector of β-1,3-Glucanase gene for plant is successful and it is in process of applying in plant transformation.

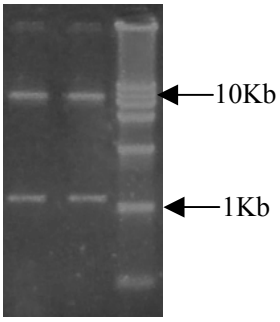

Figure8.Recombinant clone pMHL7133-Glu was cutted by BamHⅠand SacⅠ

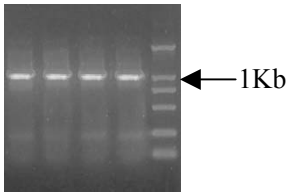

Figure 9.PCRidentification recombinant clonepMHL7133-Glu

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