

苹果斑点落叶病菌的寄主应答反应蛋白质水平的研究

汇报人：肖龙

组员：肖龙、王惠梅、王婷、毕真真

小组：G05 2013.12.1

- 1 背景介绍
- 2 材料与amp;方法
- 3 分析与讨论
- 4 展望

1 背景介绍

- 苹果苹果斑点落叶病是苹果产业中较为严重的真菌病害，目前关于苹果对这种病害的抗病系统却并不清楚。
- 本试验主要目的是通过对于苹果与斑点落叶病原菌互作过程中，苹果在蛋白质水平的响应进行研究，获得了苹果对这种病害的抗性反应相关信息。

2 材料与amp;方法

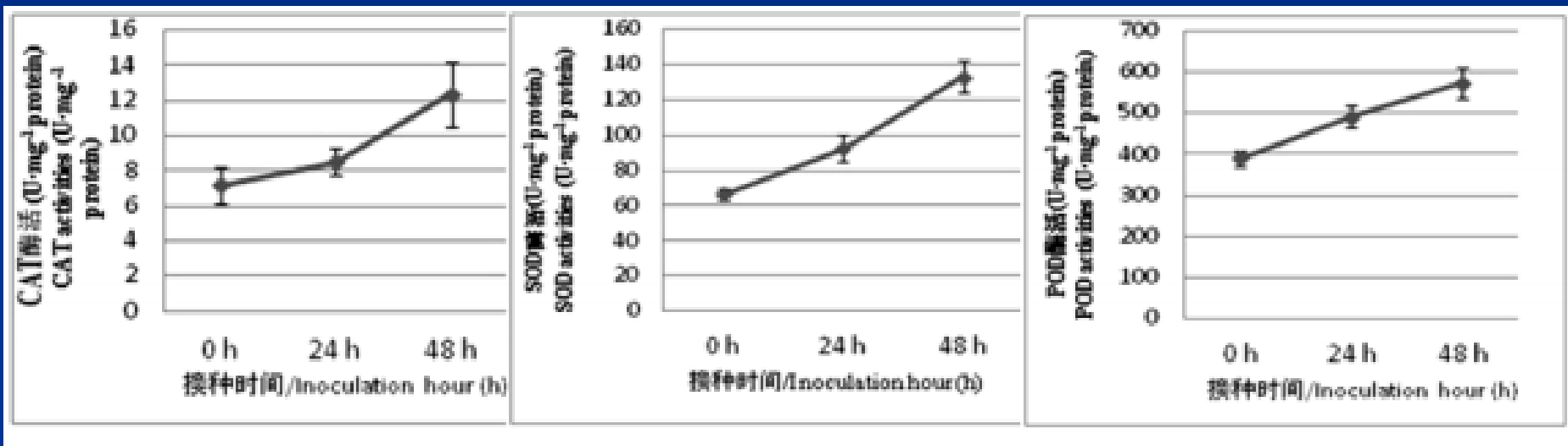
- 供试病原菌株来自苹果育种中心，编号AML0801，由中国农业科学院果树研究所植保中心惠赠。
- 苹果叶片试材取自国家苹果育种中心资源圃内，以8年生苹果杂交组合（‘华脆’×‘金冠’）F1代杂种实苗中选育的抗病植株为研究对象。

2 材料与amp;方法

- 通过对抗病品种在病菌侵染后蛋白质组学水平的一系列研究，获得的结果；
- 过氧化氢酶、过氧化物酶及超氧化物歧化酶活性测定；
- 苹果叶片总蛋白提取及其双向电泳分析；
- 苹果叶片应答斑点落叶病菌胁迫的蛋白质组学分析；
- 差异蛋白点酶解处理及质谱分析；

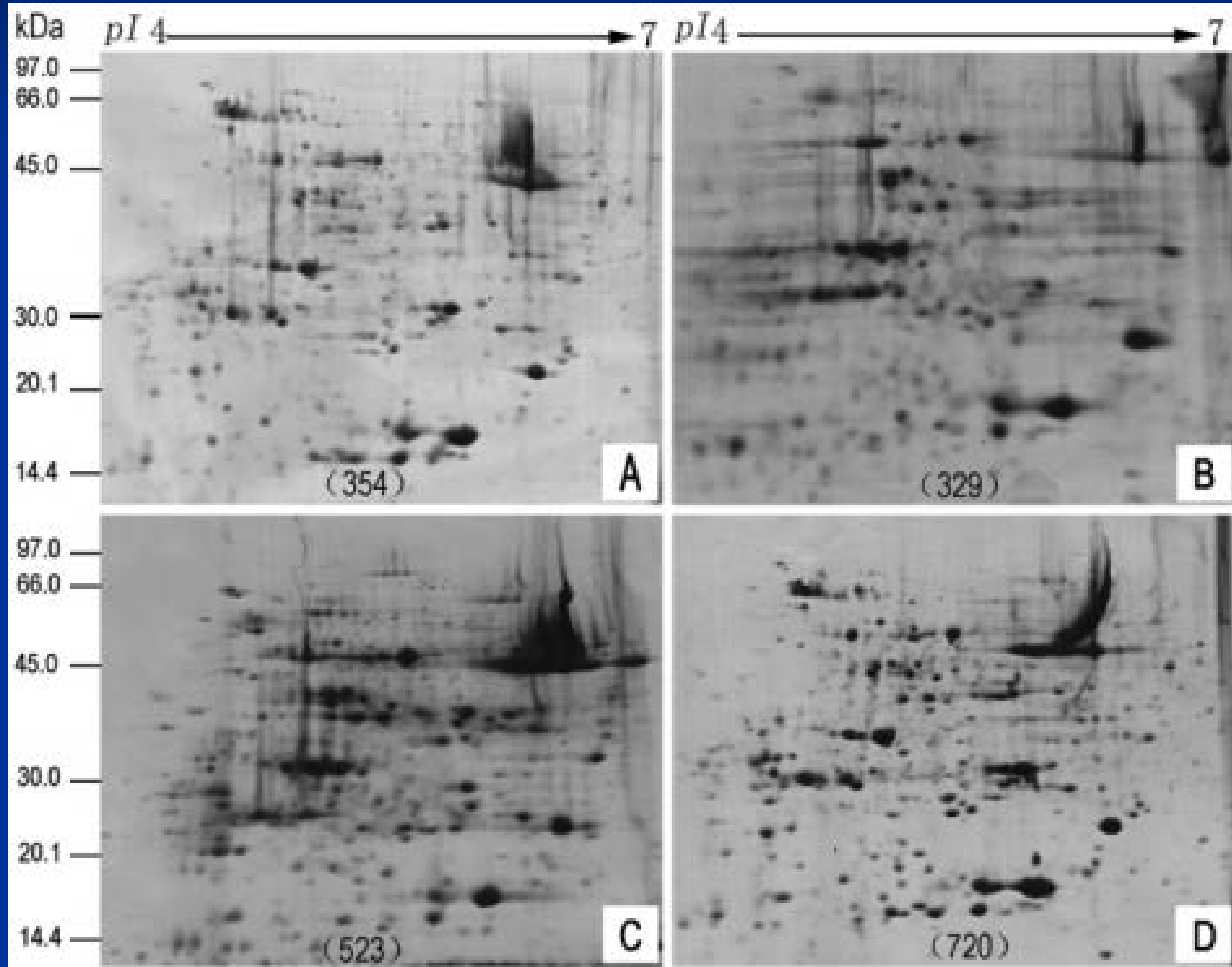
3.分析与讨论

1. 苹果叶片抗氧化酶活性的变化



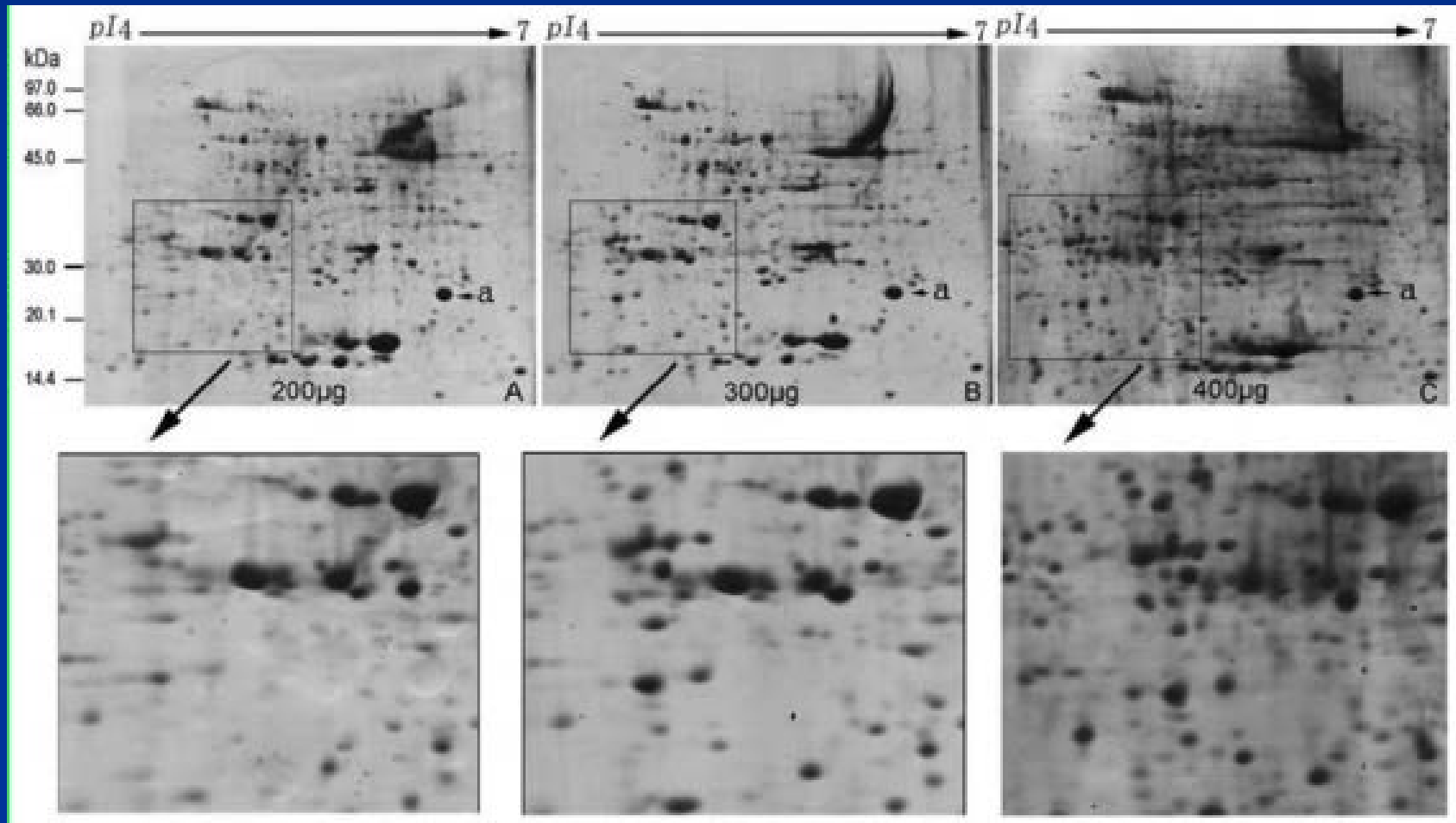
3. 分析与讨论

■ 2. 4种苹果蛋白质提取方法的比较



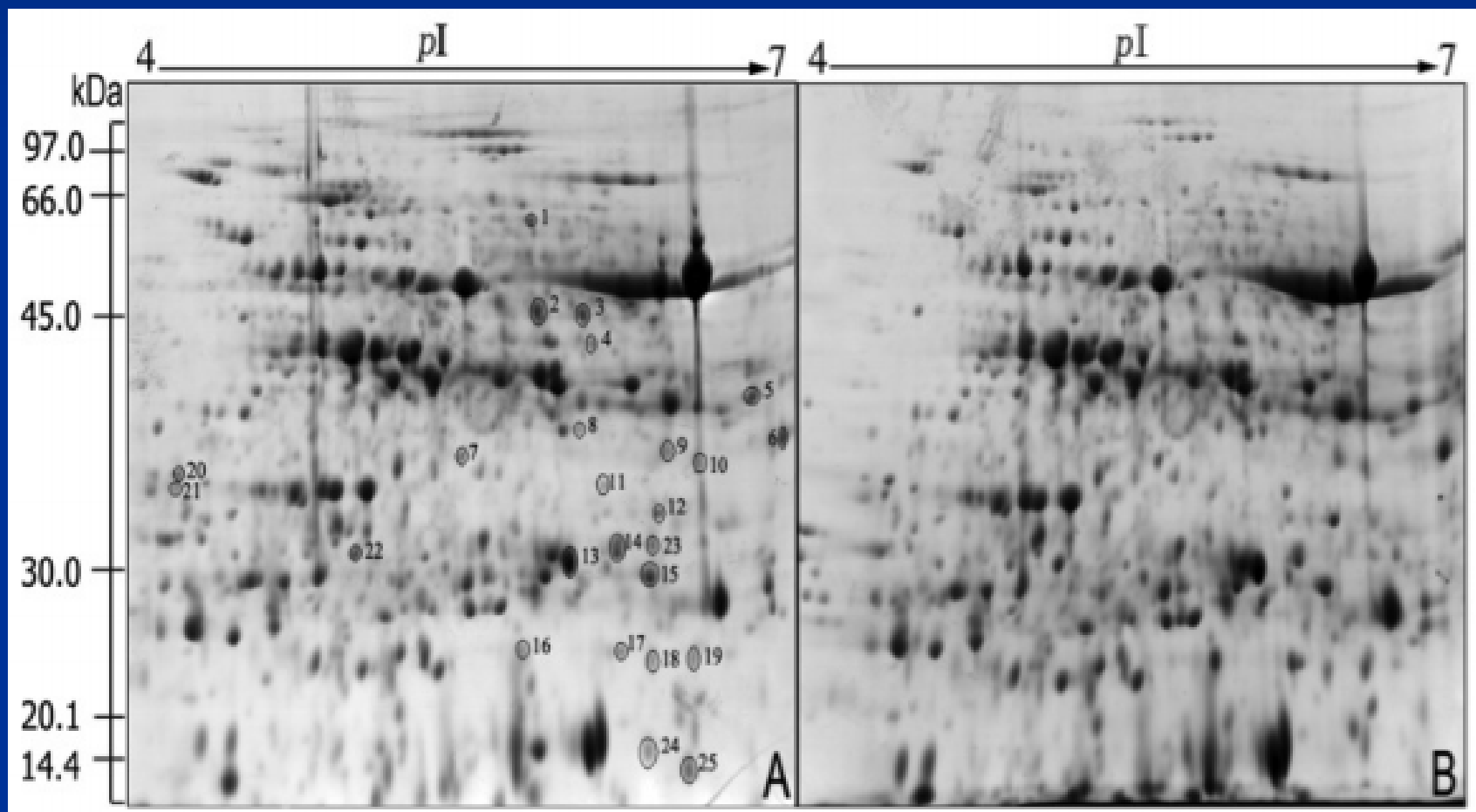
3.分析与讨论

■ 3. 蛋白质样品上样量优化:



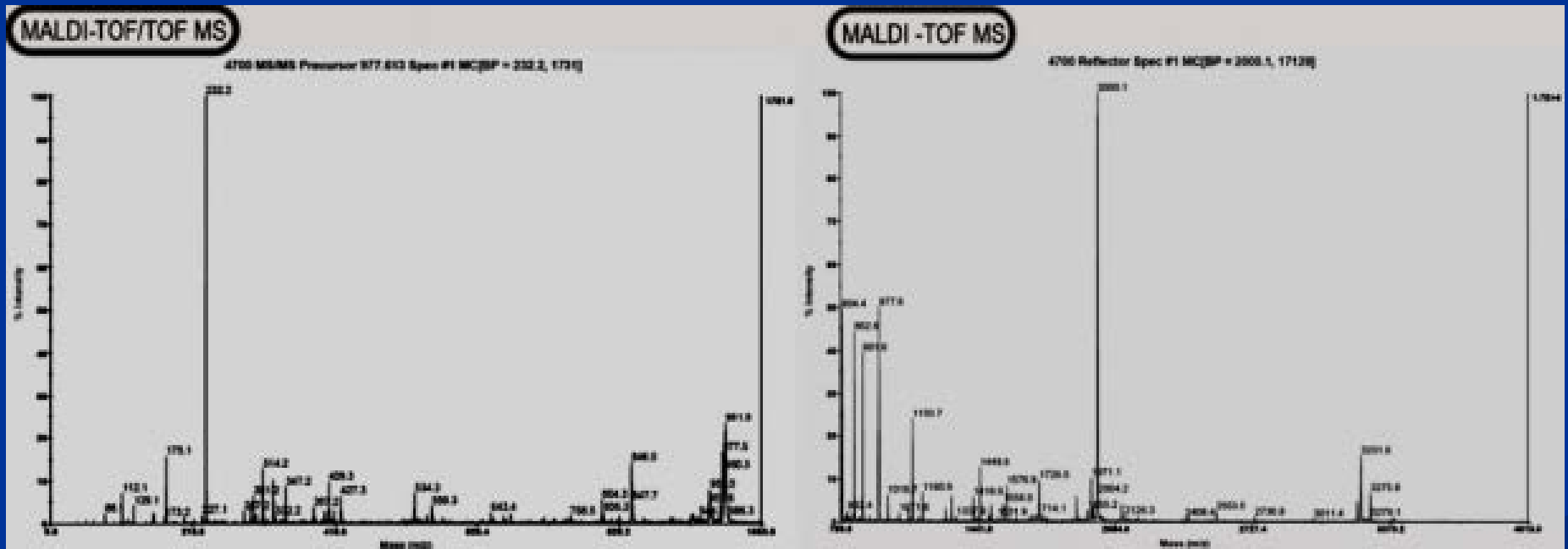
3.分析与讨论

■ 4. 苹果叶片应答病原菌胁迫的双向电泳图谱分析



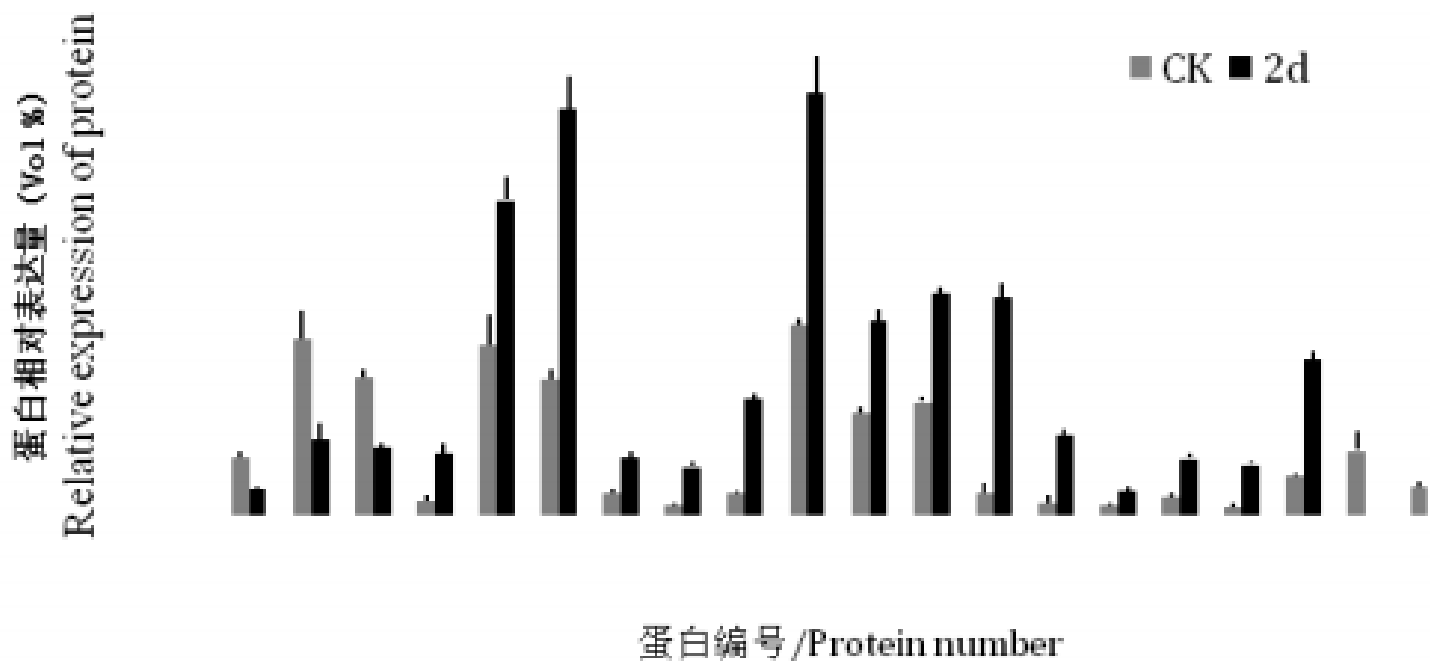
3.分析与讨论

- 5.差异蛋白质点质谱分析
(以24号蛋白点为例，其PMF图谱数据)



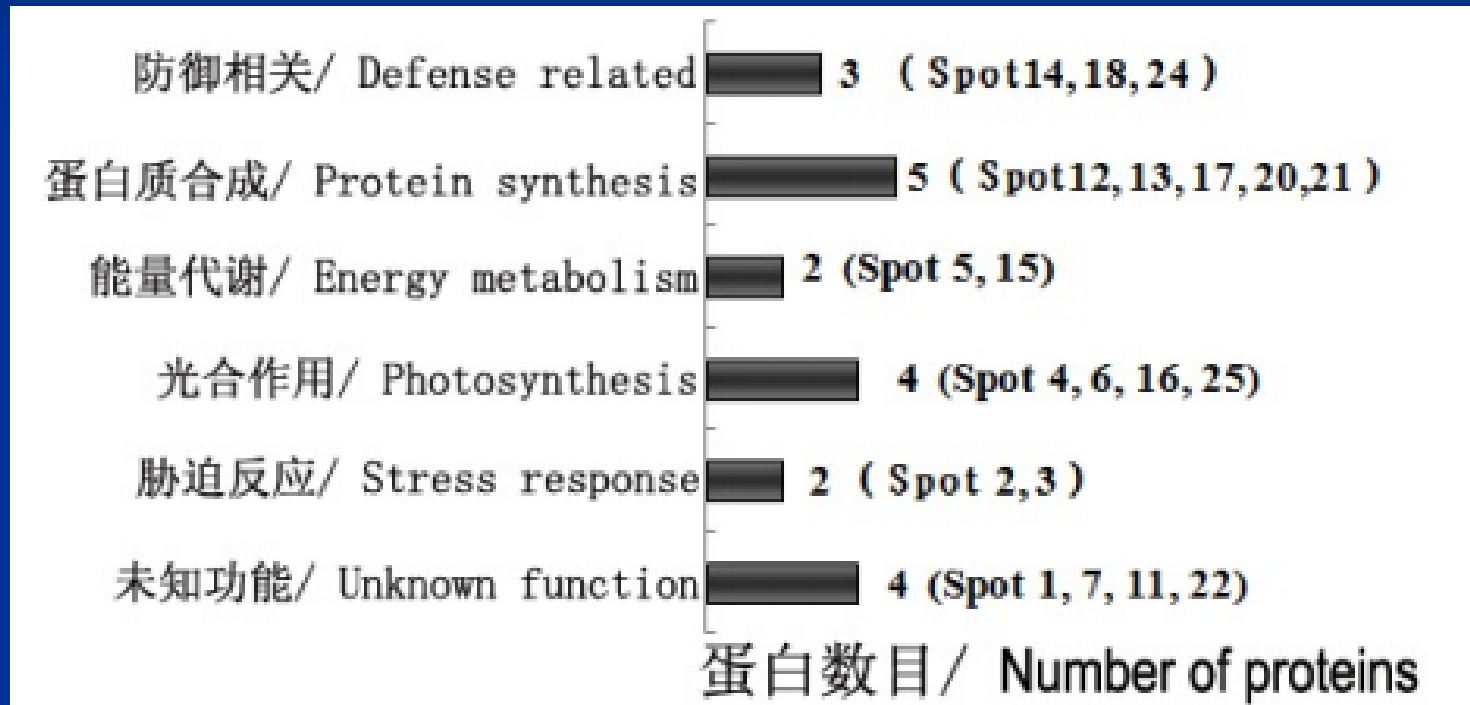
3.分析与讨论

6. 苹果叶片发病前后差异表达蛋白的丰度变化



3.分析与讨论

■ 7. 蛋白功能鉴定



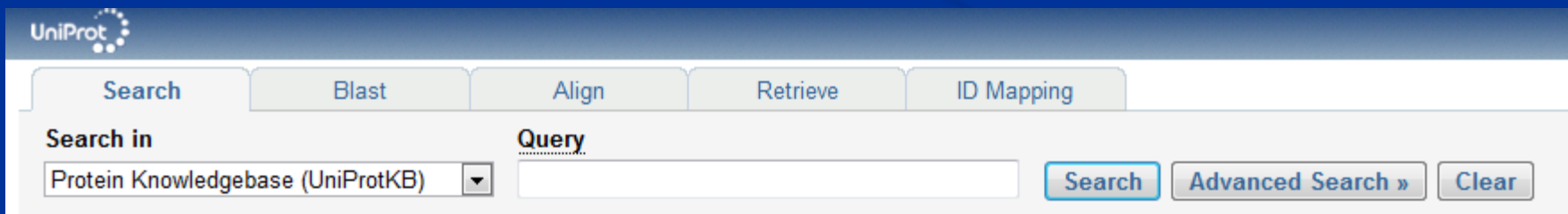
3.分析与讨论

- 8.讨论
- 8.1. 确定应用改良的HB-酚抽提方法提取苹果叶片的总蛋白，优化了苹果叶片总蛋白的提取方法、上样量、等程序。
- 8.2. 推测3个防御反应相关的PR类蛋白(APX、GPX、Mal d1) 是苹果叶片应答病原菌胁迫的关键蛋白。
- 8.3. 苹果叶片应答斑点落叶病菌侵染后抗氧化酶系(SOD、CAT、POD) 活性均显著提高，其中的POD活性上调表达与差异蛋白质组研究中APX、GPX上调表达结果一致，辅助验证APX、GPX参与苹果叶片抗病反应。

4. 展望--分析APX蛋白的性质、功能等

■ 1. 同源蛋白序列的获取

分析APX蛋白的基本构成、性质等
未取得苹果中APX的序列，因此借助
Uniprot网页查找拟南芥中同源的APX
的序列



The image shows the UniProt search interface. At the top left is the UniProt logo. Below it are five navigation tabs: Search, Blast, Align, Retrieve, and ID Mapping. The 'Search' tab is active. Underneath the tabs, there is a 'Search in' dropdown menu set to 'Protein Knowledgebase (UniProtKB)'. To the right of this is a 'Query' input field. Below the input field are three buttons: 'Search', 'Advanced Search »', and 'Clear'.

4. 展望

Results [Customize](#)

Entry	Entry name	Status	Protein names	Gene names	Organism	Length
<input type="checkbox"/> Q01613	SHRM1_XENLA	★	Protein Shroom1	shroom1 apx	Xenopus laevis (African clawed frog)	1,420
<input type="checkbox"/> Q1PER6	APX2_ARATH	★	L-ascorbate peroxidase 2, cytosolic	APX2 APX1B At3g09640 F11F8_23	Arabidopsis thaliana (Mouse-ear cress)	251
<input type="checkbox"/> P55130	RTX31_ACTPL	★	RTX-III toxin determinant A from serotype 2	apxIIIA clyIIIA ptxA rtxA	Actinobacillus pleuropneumoniae (Haemophilus pleuropneumoniae)	1,049
<input type="checkbox"/> P55131	RTX32_ACTPL	★	RTX-III toxin determinant A from serotype 8	apxIIIA clyIIIA ptxA rtxA	Actinobacillus pleuropneumoniae (Haemophilus pleuropneumoniae)	1,052
<input type="checkbox"/> Q04473	RTX3B_ACTPL	★	Toxin RTX-III translocation ATP-binding prote...	apxIIIB clyIIIB rtxB	Actinobacillus pleuropneumoniae (Haemophilus pleuropneumoniae)	711
<input type="checkbox"/> P55128	RTX11_ACTPL	★	RTX-I toxin determinant A from serotypes 1/9	apxIA clyIA hlyIA	Actinobacillus pleuropneumoniae (Haemophilus pleuropneumoniae)	1,023
<input type="checkbox"/> P55129	RTX12_ACTPL	★	RTX-I toxin determinant A from serotypes 5/10	apxIA clyIA hlyIA	Actinobacillus pleuropneumoniae (Haemophilus pleuropneumoniae)	1,023
<input type="checkbox"/> P26760	RTX1B_ACTPL	★	Toxin RTX-I translocation ATP-binding protein	apxIB appB clyIB hlyIB	Actinobacillus pleuropneumoniae (Haemophilus pleuropneumoniae)	707
<input type="checkbox"/> P26761	RTX1D_ACTPL	★	RTX-I toxin determinant D	apxID appD clyID hlyID	Actinobacillus pleuropneumoniae (Haemophilus pleuropneumoniae)	477
<input type="checkbox"/> P15377	RTX2A_ACTPL	★	RTX-II toxin determinant A	apxIIA appA clyIIA cytC hlyIIA	Actinobacillus pleuropneumoniae (Haemophilus pleuropneumoniae)	956
<input type="checkbox"/> Q08633	RTX3D_ACTPL	★	RTX-III toxin determinant D	apxIIID clyIIID rtxD	Actinobacillus pleuropneumoniae (Haemophilus pleuropneumoniae)	477
<input type="checkbox"/> P0A3I3	RTX2C_ACTPL	★	RTX-II toxin-activating lysine-acyltransferas...	apxIIC appC ashC clyIIC cytC hlyC	Actinobacillus pleuropneumoniae (Haemophilus pleuropneumoniae)	160
<input type="checkbox"/> Q04474	RTX3C_ACTPL	★	RTX-III toxin-activating lysine-acyltransfera...	apxIIIC clyIIIC rtxC	Actinobacillus pleuropneumoniae (Haemophilus pleuropneumoniae)	173
<input type="checkbox"/> P55132	RTX1C_ACTPL	★	RTX-I toxin-activating lysine-	apxIC clyIC hlyIC	Actinobacillus pleuropneumoniae	172

4. 展望

获取APX同源蛋白—拟南芥中的APX2蛋白

<input type="checkbox"/>	Q1PER6	APX2_ARATH	★	L-ascorbate peroxidase 2, cytosolic	APX2 APX1B At3g09640 F11F8_23	Arabidopsis thaliana (Mouse-ear cress)	251
--------------------------	--------	------------	---	-------------------------------------	----------------------------------	--	-----

Names and origin

Protein names	<i>Recommended name:</i> L-ascorbate peroxidase 2, cytosolic EC=1.11.1.11 <i>Alternative name(s):</i> L-ascorbate peroxidase 1b Short name=APX1b Short name=AtAPx02
Gene names	Name: APX2 Synonyms: APX1B Ordered Locus Names: At3g09640 ORF Names: F11F8_23
Organism	Arabidopsis thaliana (Mouse-ear cress) [Reference proteome]
Taxonomic identifier	3702 [NCBI]

4. 展望

Protein attributes

Sequence length	251 AA.
Sequence status	Complete.
Sequence processing	The displayed sequence is further processed into a mature form.
Protein existence	Evidence at transcript level

General annotation (Comments)

Function	Plays a key role in hydrogen peroxide removal By similarity .
Catalytic activity	$2 \text{ L-ascorbate} + \text{H}_2\text{O}_2 + 2 \text{ H}^+ = \text{L-ascorbate} + \text{L-dehydroascorbate} + 2 \text{ H}_2\text{O}$.
Cofactor	Binds 1 heme B (iron-protoporphyrin IX) group per subunit.
Subcellular location	Cytoplasm By similarity .
Tissue specificity	Detected in bundle sheath cells, the photosynthetic cells that surround the phloem and xylem. Ref.9
Induction	By excess light treatment, by wounding and by heat-shock stress. Ref.2 Ref.7 Ref.8 Ref.9
Miscellaneous	Binds one cation per subunit; probably K^+ , but might also be Ca^{2+} By similarity .
Sequence similarities	Belongs to the peroxidase family . Ascorbate peroxidase subfamily .
Sequence caution	The sequence AAF23294.1 differs from that shown. Reason: Erroneous gene model prediction.

4. 展望

Ontologies

Keywords

Biological process	Hydrogen peroxide
Cellular component	Cytoplasm
Ligand	Calcium Heme Iron Metal-binding Potassium
Molecular function	Oxidoreductase Peroxidase
Technical term	Complete proteome Reference proteome

4. 展望

Gene Ontology (GO)

Biological_process

[hydrogen peroxide catabolic process](#)

Inferred from electronic annotation. Source: UniProtKB-KW

[response to oxidative stress](#)

Inferred from direct assay [PubMed 9306897](#). Source: TAIR

Cellular_component

[cytosol](#)

Traceable author statement [Ref.2](#). Source: TAIR

Molecular_function

[L-ascorbate peroxidase activity](#)

Traceable author statement [Ref.2](#). Source: TAIR

[heme binding](#)














Inferred from electronic annotation. Source: InterPro

[metal ion binding](#)

Inferred from electronic annotation. Source: UniProtKB-KW

4. 展望

Sequence annotation (Features)

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	
Molecule processing						
<input type="checkbox"/>	Initiator methionine	1	1	Removed By similarity		
<input checked="" type="checkbox"/>	Chain	2 – 251	250	L-ascorbate peroxidase 2, cytosolic		PRO_0000261322
Sites						
<input type="checkbox"/>	Active site	43	1	Proton acceptor By similarity		
<input type="checkbox"/>	Metal binding	163	1	Iron (heme axial ligand) By similarity		
<input type="checkbox"/>	Metal binding	164	1	Potassium or calcium By similarity		
<input type="checkbox"/>	Metal binding	180	1	Potassium or calcium By similarity		
<input type="checkbox"/>	Metal binding	182	1	Potassium or calcium By similarity		
<input type="checkbox"/>	Metal binding	185	1	Potassium or calcium; via carbonyl oxygen By similarity		
<input type="checkbox"/>	Metal binding	187	1	Potassium or calcium By similarity		
<input type="checkbox"/>	Site	39	1	Transition state stabilizer By similarity		
Experimental info						
<input type="checkbox"/>	Sequence conflict	6	1	Y → F in ABE65932 . Ref.0		
<input type="checkbox"/>	Sequence conflict	231	1	F → S in CAA66925 . Ref.1		
<input type="checkbox"/>	Sequence conflict	231	1	F → S in CAA56340 . Ref.2		

4. 展望

Sequences

Sequence	Length	Mass (Da)	Tools	
<input type="checkbox"/> Q1PER6 [UniParc].	FASTA	251	28,006	Blast <input type="button" value="go"/>

Last modified January 23, 2007. Version 3.

Checksum: FFC7F6D82A4EF3E0

```
      10      20      30      40      50      60
MVRKSYPEVK EYKKAQVRC KRKLRGLIAE KHCAPIVLRL AWHSAGTFDV KTKTGGPFGT

      70      80      90     100     110     120
IRHPQELAHD ANGLDIAVR LLDPIKELFP ILSYADFYQL AGVVAVEITG GPEIPFHPGR

     130     140     150     160     170     180
LDKVEPPPEG RLPQATKGV D HLRDVFGRMG LNDKDIVALS GGHTLGRCHK ERSGFEGAWT

     190     200     210     220     230     240
FNPLIFDNSY FKEILSGEKE GLLQLPTDKA LLDDPLFLPF VERYAADEDA FFEDYTEAHL

     250
KLSELGFADK E
```

4. 展望

进化树---与其他同源序列的比对

Guide tree



4. 展望

2.在Uniprot网站中获取拟南芥APX2蛋白的序列后，使用ExPASy网站中的相关软件，分析拟南芥APX蛋白的性质；

4. 展望

使用ExPASy网站中的ProtParam软件检索蛋白质序列理化参数；

ProtParam

User-provided sequence:

```

      10           20           30           40           50           60
MVKKSYPEVK EYKKAQRC KRKLRGLIAE KHCAPIVLRL AWHSAGTFDV KTKTGGPFGT

      70           80           90           100          110          120
IRHPQELAHD ANGLDIAVR LLDPIKELFP ILSYADFYQL AGVVAVEITG GPEIPFHPGR

      130          140          150          160          170          180
LDKVEPPPEG RLPQATKGV D HLRDVFGRMG LNDKDIVALS GGHTLGRCHK ERSGFEGAWT

      190          200          210          220          230          240
PNPLIFDNSY FKEILSGEKE GLLQLPTDKA LLDDPLFLPF VEKYAADEDA FFEDYTEAHL

      250
KLSELGFADK E
```


4. 展望

Number of amino acids: 251

Molecular weight: 28006.0

Theoretical pI: 5.87

Atomic composition:

Carbon	C	1272
Hydrogen	H	1978
Nitrogen	N	338
Oxygen	O	365
Sulfur	S	5

Formula: $C_{1272}H_{1978}N_{338}O_{365}S_5$

Total number of atoms: 3958

Amino acid composition:

Ala (A)	20	8.0%
Arg (R)	12	4.8%
Asn (N)	5	2.0%
Asp (D)	18	7.2%
Cys (C)	3	1.2%
Gln (Q)	5	2.0%
Glu (E)	21	8.4%
Gly (G)	22	8.8%
His (H)	9	3.6%
Ile (I)	11	4.4%
Leu (L)	29	11.6%
Lys (K)	21	8.4%
Met (M)	2	0.8%
Phe (F)	14	5.6%
Pro (P)	18	7.2%
Ser (S)	8	3.2%
Thr (T)	10	4.0%
Trp (W)	2	0.8%
Tyr (Y)	7	2.8%
Val (V)	14	5.6%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%
(B)	0	0.0%
(Z)	0	0.0%
(X)	0	0.0%

4. 展望

```
Total number of negatively charged residues (Asp + Glu): 39  
Total number of positively charged residues (Arg + Lys): 33
```

```
Estimated half-life:
```

```
The N-terminal of the sequence considered is M (Met).
```

```
The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).  
                           >20 hours (yeast, in vivo).  
                           >10 hours (Escherichia coli, in vivo).
```

4. 展望

使用ExPASy网站中的ProtScale软件分析APX蛋白质不同区域疏水和亲水等性质

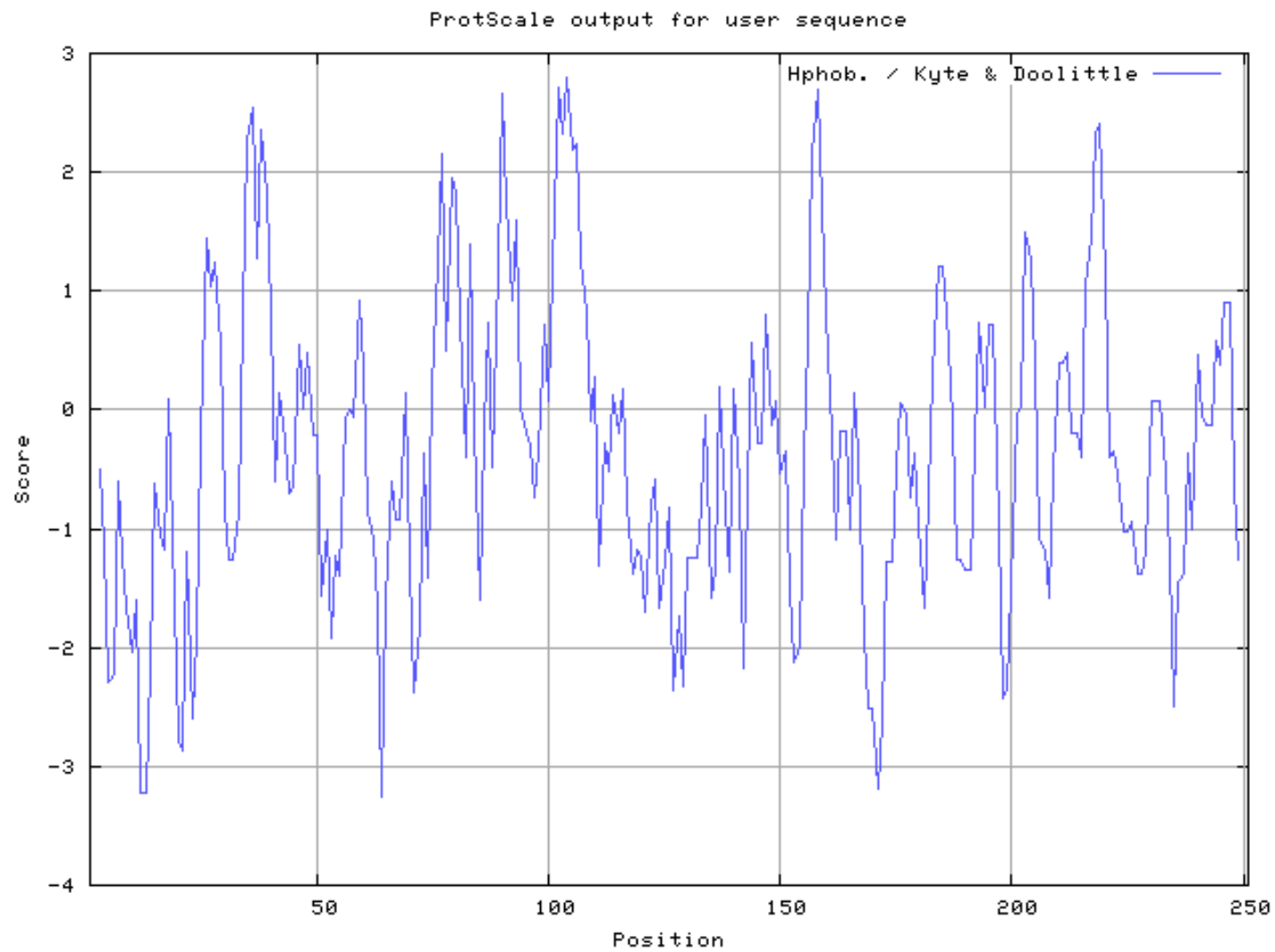


ProtScale

4. 展望

- Molecular weight
- Bulkiness
- Polarity / Grantham
- Recognition factors
- Hphob. OMH / Sweet et al.
- Hphob. / Kyte & Doolittle
- Hphob. / Abraham & Leo
- Hphob. / Bull & Breese
- Hphob. / Guy
- Hphob. / Miyazawa et al.
- Hphob. / Roseman
- Hphob. / Wolfenden et al.
- Hphob. HPLC / Wilson & al
- Hphob. HPLC pH3.4 / Cowan
- Hphob. / Rf mobility
- HPLC / TFA retention
- HPLC / retention pH 2.1
- % buried residues
- Hphob. / Chothia
- Ratio hetero end/side
- Average flexibility
- beta-sheet / Chou & Fasman
- alpha-helix / Deleage & Roux
- beta-turn / Deleage & Roux
- alpha-helix / Levitt
- beta-turn / Levitt
- Antiparallel beta-strand
- A.A. composition
- Relative mutability
- Number of codon(s)
- Polarity / Zimmerman
- Refractivity
- Hphob. / Eisenberg et al.
- Hphob. / Hopp & Woods
- Hphob. / Manavalan et al.
- Hphob. / Black
- Hphob. / Fauchere et al.
- Hphob. / Janin
- Hphob. / Rao & Argos
- Hphob. / Tanford
- Hphob. / Welling & al
- Hphob. HPLC / Parker & al
- Hphob. HPLC pH7.5 / Cowan
- HPLC / HFBA retention
- Transmembrane tendency
- HPLC / retention pH 7.4
- % accessible residues
- Hphob. / Rose & al
- Average area buried
- alpha-helix / Chou & Fasman
- beta-turn / Chou & Fasman
- beta-sheet / Deleage & Roux
- Coil / Deleage & Roux
- beta-sheet / Levitt
- Total beta-strand
- Parallel beta-strand
- A.A. comp. in Swiss-Prot

4. 展望



感谢罗静初老师的悉心指导！
感谢中国农业科学院提供
的学习机会！

感谢丛佩华老师的耐心指导！

感谢中国农业科学院果树研究所
提供的试验平台！



希望大家多提宝贵意见！
谢谢大家！