

PROTEOMICS ANALYSIS FOR THE ELITE SOYBEAN JIDOU 17 AND ITS PARENTS USING ITRAQ-BASED QUANTITATIVE APPROACHES

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The elite soybean cultivar Jidou 17 was derived from Hobbit as the female parent and Zao 5241 as the male parent. It has significantly higher yield than both its parents and has a higher oil content and stronger resistance to non-biological stresses. The purpose of this study is to understand the protein expression of Jidou 17 and the correlation to the protein expression of its parental strains, and then to evaluate the parental contributions to its elite traits. The 14 days old leaves from Jidou 17 and its parental cultivars were analyzed for their differentiated protein expressions by an iTRAQ-based method. From the above three samples, a total of 1269 proteins were detected. Jidou 17 differed from its female parent Hobbit in the expression of 141 proteins, in which 57 proteins had higher expression levels, and the other 84 proteins had lower expression levels than Hobbit. Jidou 17 differed from its male parent Zao 5241 in the expression of 181 proteins, in which 61 proteins had higher expression levels, and 127 proteins had lower expression levels than Zao 5241. These differentiated proteins were classified based on the following three criteria, biological functions, biological processes, and cell components. The functional classification and the enrichment analysis revealed that the proteins fell into many functional categories, and the number of proteins varies greatly among the categories. Pathway analysis showed the enrichment of the differentiated proteins mainly to the ribosome assembly pathway. Protein expression clustering results showed that expression profiles between Jidou 17 and its female parent Hobbit had a higher similarity than between Jidou 17 and its male parent Zao 5241, and had higher similarity than the expression profiles of the two parental strains. These data indicate that the female parent Hobbit had a greater contribution to Jidou 17. This study introduced a proven technique to study the proteomics in soybean leaves, and explored the depth and breadth of soybean protein research. The results provide new data for further understanding the mechanisms of elite cultivar development.



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Proteomics Analysis for the Elite Soybean Jidou17 and Its Parents

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Outline

- **Part I**
 - **Proteomics Analysis for the Elite Soybean Jidou17 and Its Parents Using iTRAQ-based Quantitative Approaches**
- **Part II**
 - **Proteomics Analysis for the Elite soybean Jidou17 and Its Parental Using Antibody Approaches**
- **Part III**
 - **Proteomics Analysis for the Elite soybean Jidou17 and Its Parental Using Antibody of chloroplast genes Approaches**

Hige oil soybean - Jidou17 (Hobbit×Zao5241)

- **High oil : 23.42%**
- **Higher yield : Amount of increase in production 33%**
- **Stronger resistance: Non-biological stresses**
- **Wide adaptability : Spring and summer eco- region**



Hige oil soybean - Jidou17 (Hobbit×Zao5241)

- The first place in three years 2008-2010 among the participated cultivars.

- National regional average production: **3796** kg/ha(2010), **3801** kg/ha(2011), **3975** kg/ha (production testing).



Part I

Proteomics Analysis for the Elite Soybean Jidou17 and Its Parents Using iTRAQ-based Quantitative Approaches

Materials and Methods

- **Jidou17, Hobbit, and Zao5241**
- **The first fully developed trifoliolate leafs from evenly growth seedlings at 14th day**
- **iTRAQ method**

Result and Analysis

- **Protein expression profiling**
- **Protein analysis and classification in function groups**
- **Protein expression pattern analysis**
- **Protein enrichment analysis**
- **Pathway analysis**

The Profiling of Protein Expression for the Elite Soybean Jidou17 and Its Parents

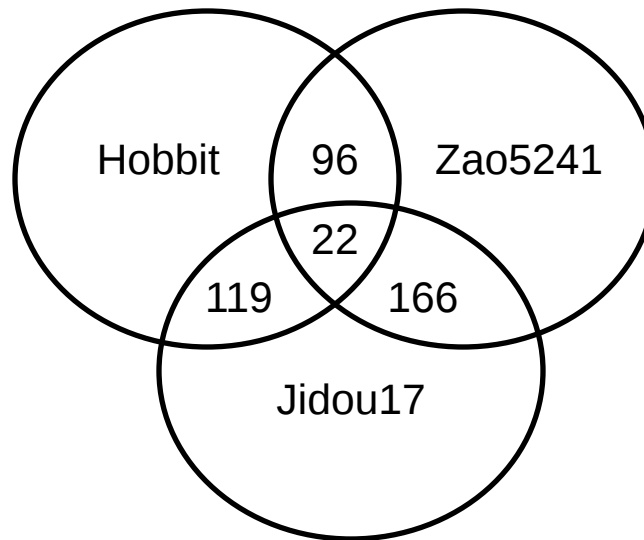


Fig.1 Venn diagram showing differentially expressed proteins in Jidou17 and its parents

Protein analysis and classification in function groups

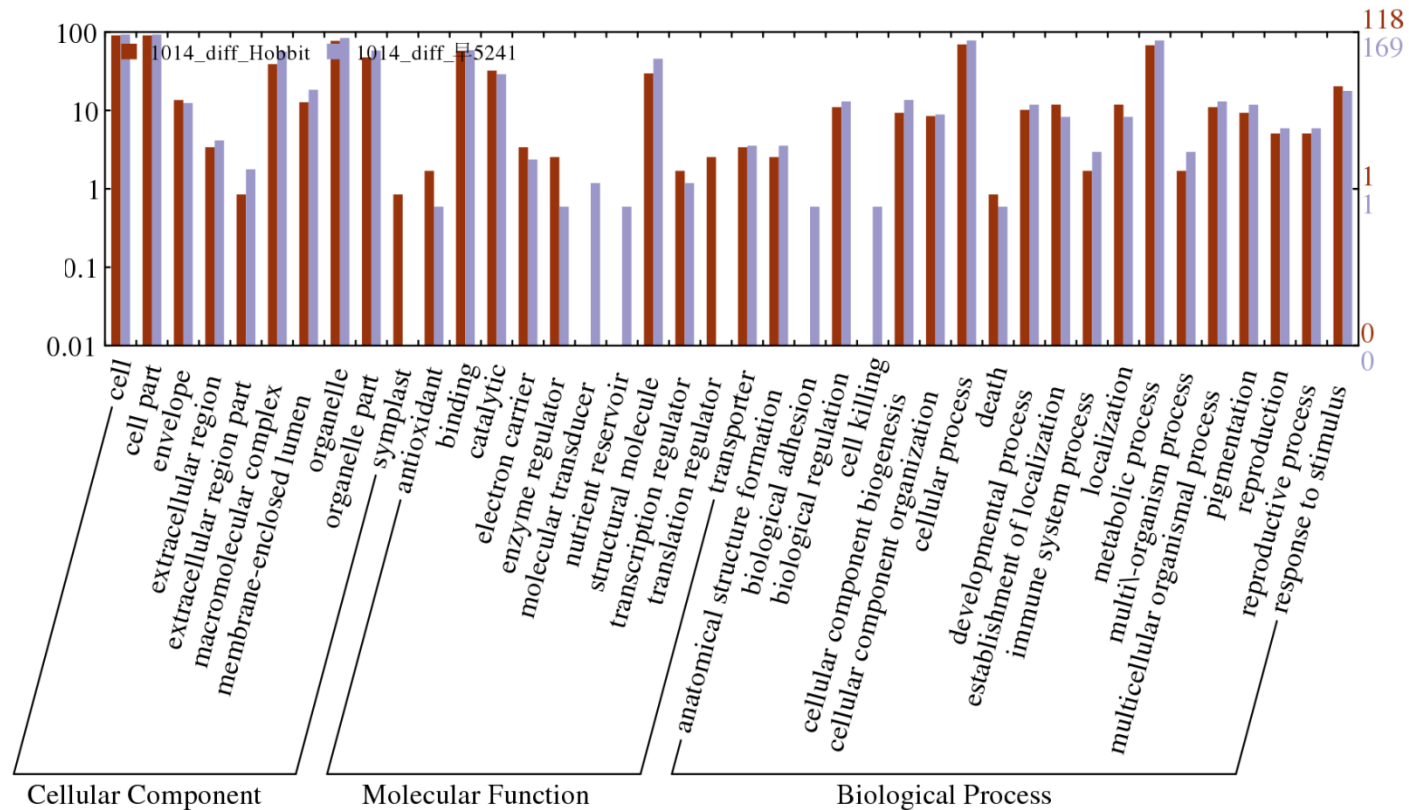


Fig. 2 Functional categorization of the differentially expressed proteins among Jidou17 and its parental strains.

Protein expression pattern analysis

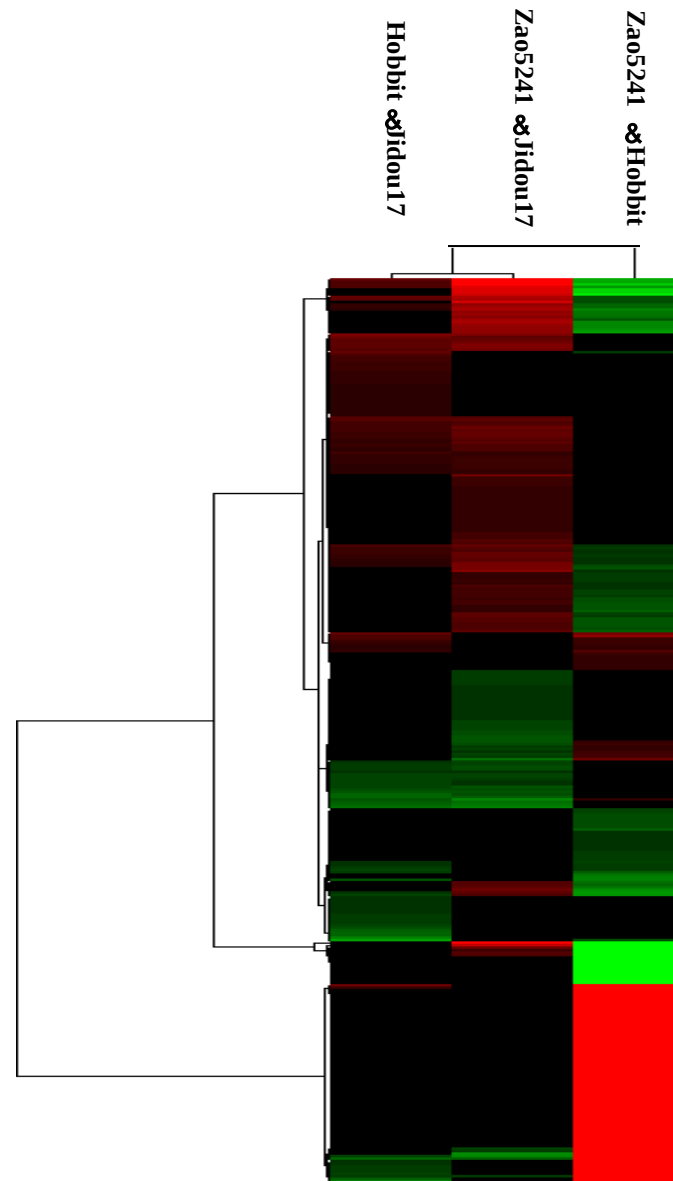


Fig. 3 Cluster map of the comparison of protein expression for Jidou17, Zao5241 and Hobbit. Red indicates higher expression, green indicates lower expression, and black indicates the same expression levels in two strains.

Protein enrichment analysis

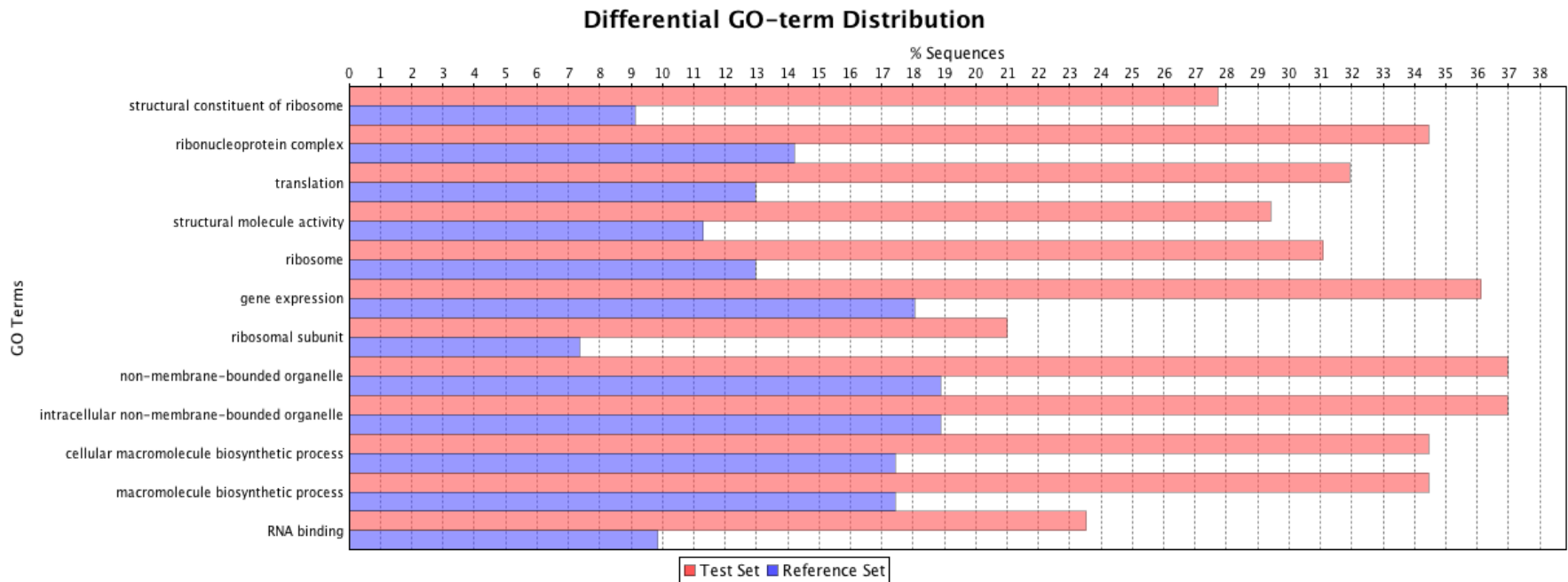


Fig. 4 GO terms distribution of the enriched differential expressed proteins between Jidou17 and hobbit.

Protein enrichment analysis

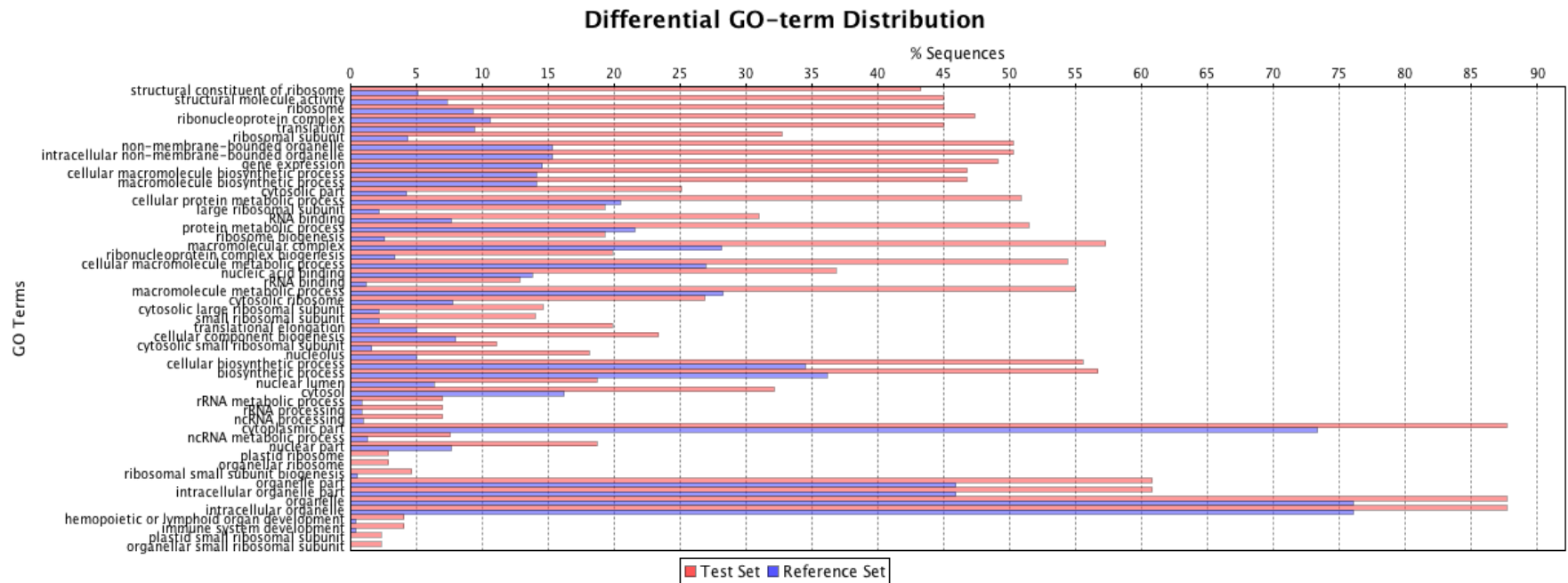


Fig. 5 GO terms distribution of the enriched differential expressed proteins between Jidou17 and Zao5241.

Pathway analysis

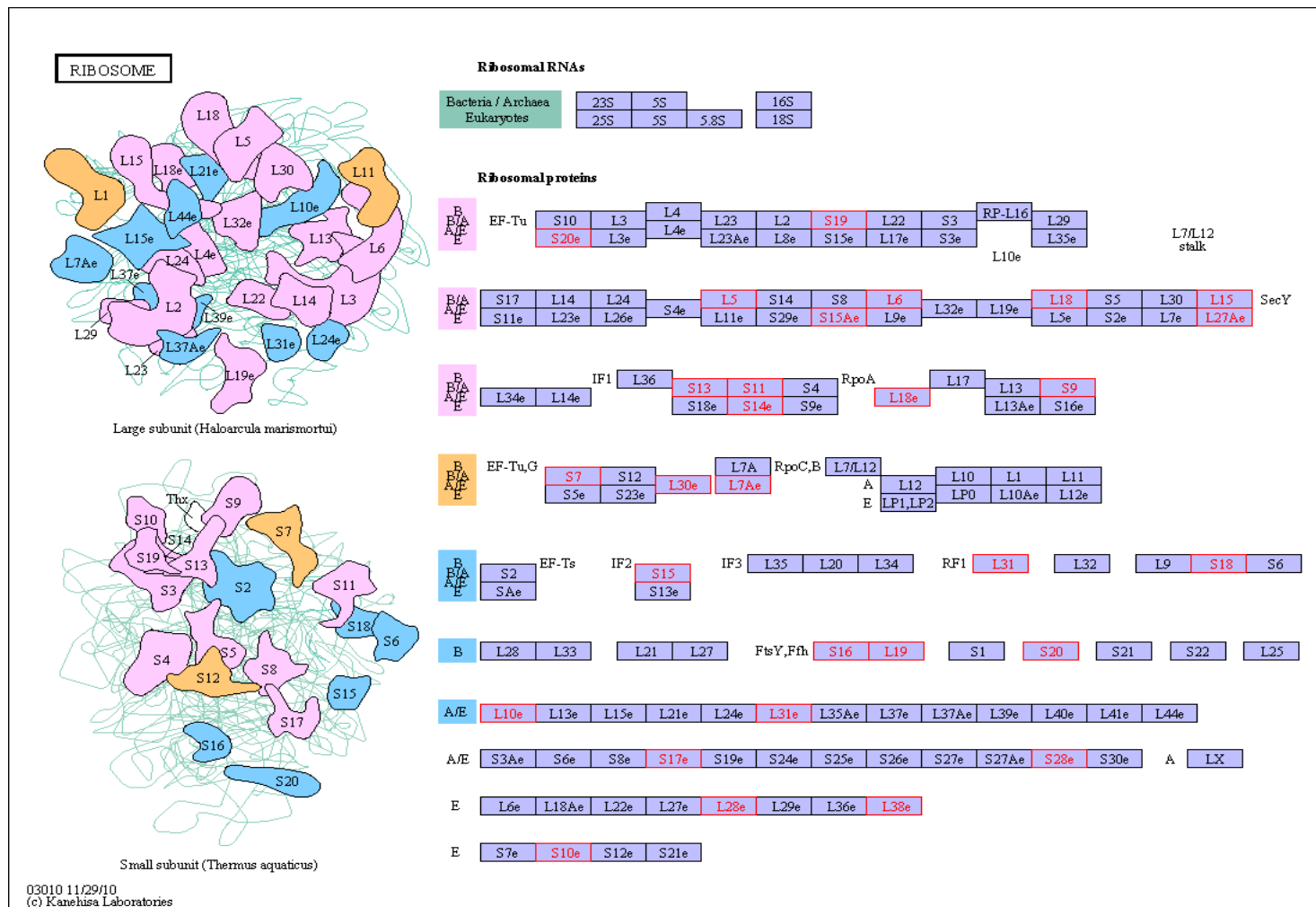


Fig. 6 The differentially expressed proteins between Jidou17 and hobbit enriched in the ribosomal assembly pathway. Red indicates differentially expressed proteins.

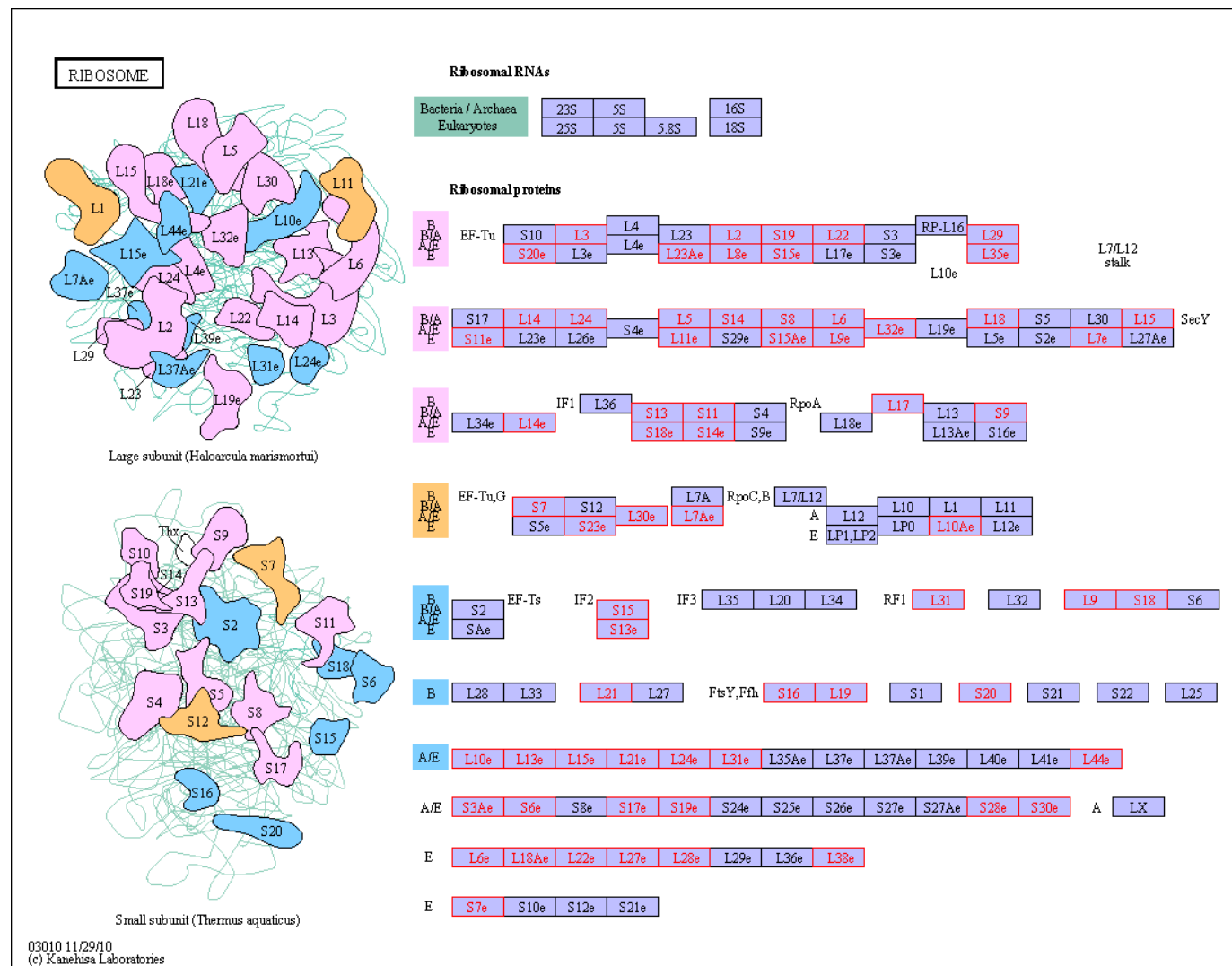
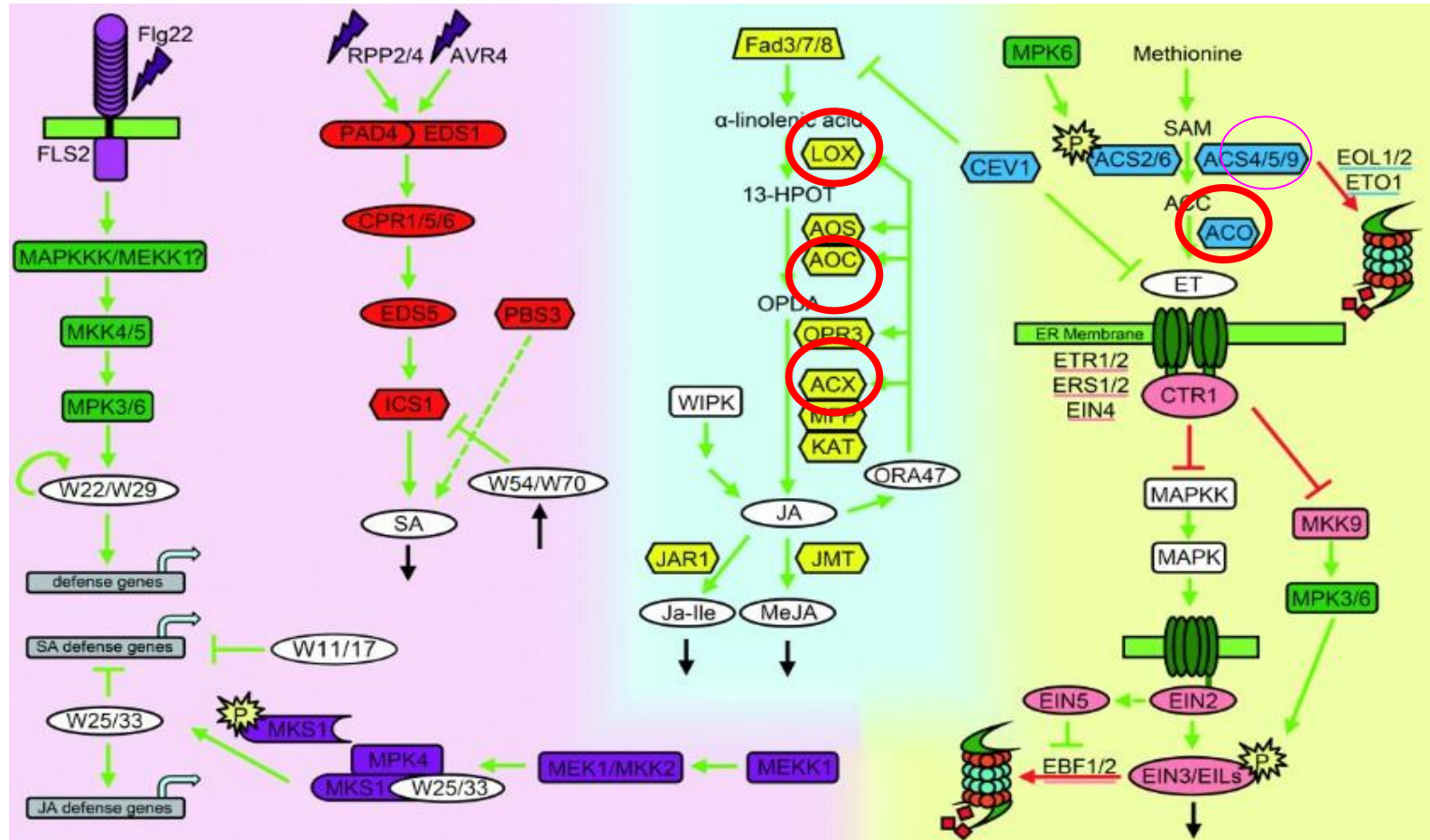


Fig. 7 The differentially expressed proteins between Jidou17 and Zao5241 enriched in the ribosomal assembly pathway. Red indicates differential expressed proteins expression.

Part II

Proteomics Analysis for the Jidou17 and Its Parental Inbreds Using Antibody Approaches

The Biosynthetic pathway of MeJA



LOX, AOC, ACX, ACO

Antibody-based soybean proteins

Protein ID	Protein name	pepA	pepB
C6SWE8	SOD	PDDLKGKGHELSKTTGNAC	FNPNNKEHGAPEDVNRHAC
P39657	RUAP	RDYDGNNSTNGKFIPYWC	KNKQFPFTIGSDSNSEAWC
Q38JD2	Q38JD2	ASFPSRNQPKDGENTRC	RKTPQSDPPPEEEGPC
E6YBW4	PR10	TLSEEELTANKAKIQGLVKC	NGGPGTIKKITTIEGDKTKC
C6ZRY8	LRR	DLSNNQLCGEIPEDFGNLC	LSRNGFEGSIPDVFGSHC
Q43446	LOX	LIEKNNDETLRNRYGPAC	TGRGKTRKDPNSEKPGDC
Q71UA1	ISOD	KKEGPKVNAKFELKPPPYC	GENAANPPSPDEDNKLC
C6ZQJ7	GST	LEEQLGDKTYFGGDDLGC	VWNDRNPLLPSDPYQC
O49856	FTRC	PSDKSVEIMRKFSEQYARKS GTC	CRHYDDKAAEVAQGFWN
P29756	CATA1	ENNFKQPGERYRSWPSDRQ ERC	ENEDRLDFDPLDVTKTWPEDC
C6T930	C6T930	NLKAPRSSPRYSPIC	TENVEREYLEYDDPKTVC
C6SWC4	C6SWC4	PTTTNSQTTTPQSQNAC	THKGSPQVKSLGIQEAKC
C6SVX7	C6SVX7	AEARVPKAKESEGGRRC	FPENFTGCQDLAKQKKC

13 soybean protein antibodies were generated

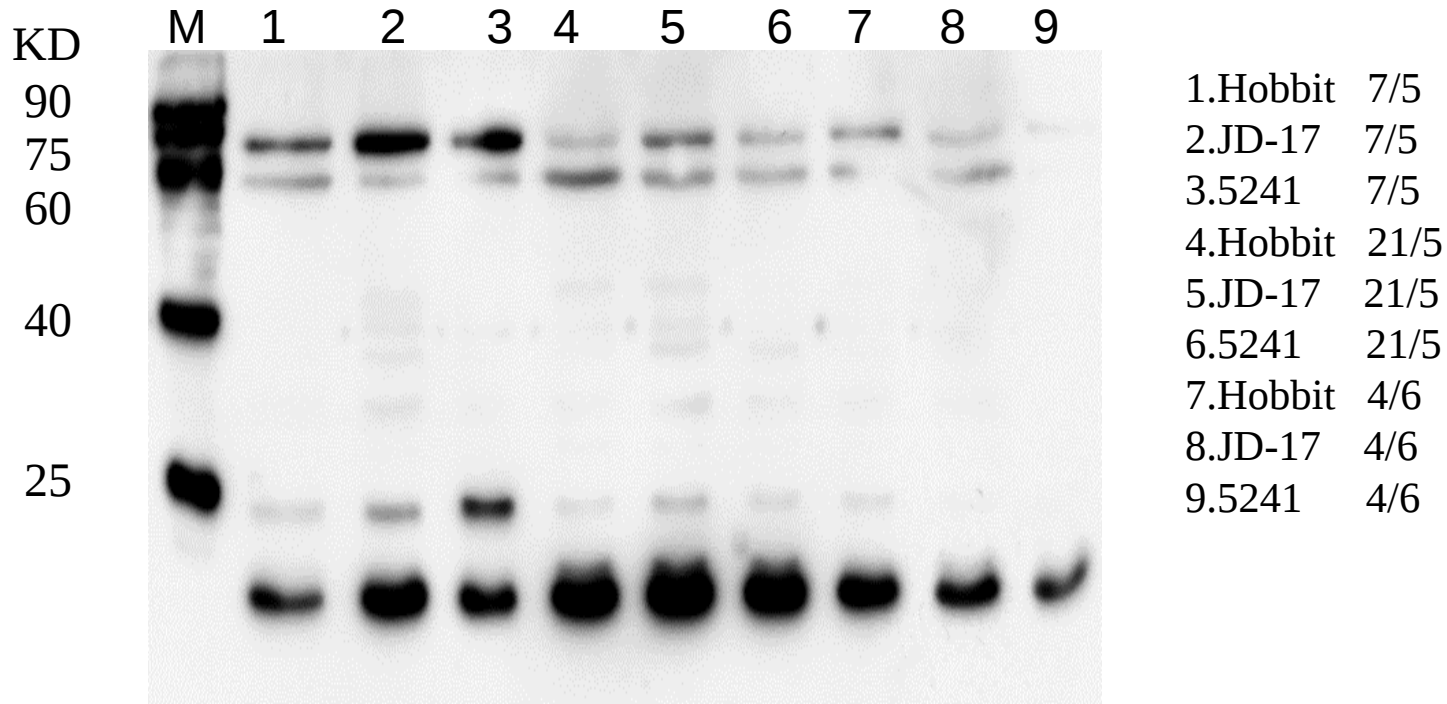


Fig. 1 The Western blotting detection of SOD

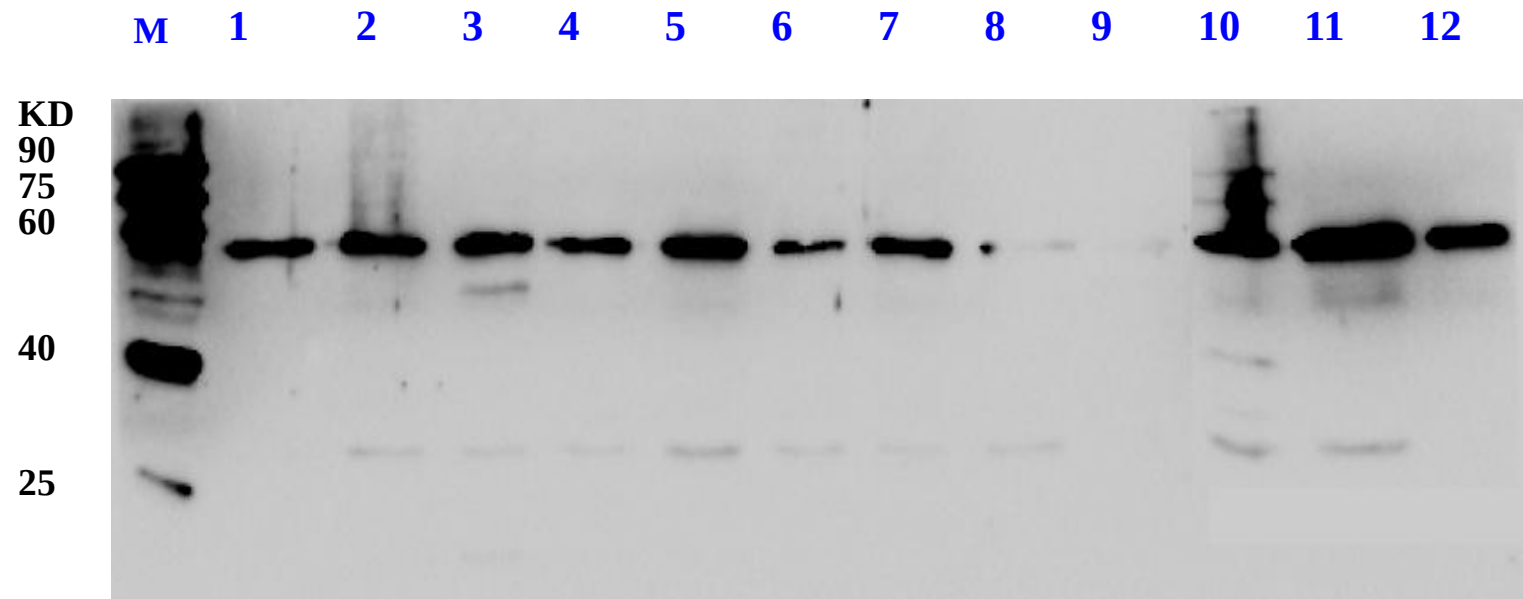


Fig. 2 The Western blotting detection of CAT

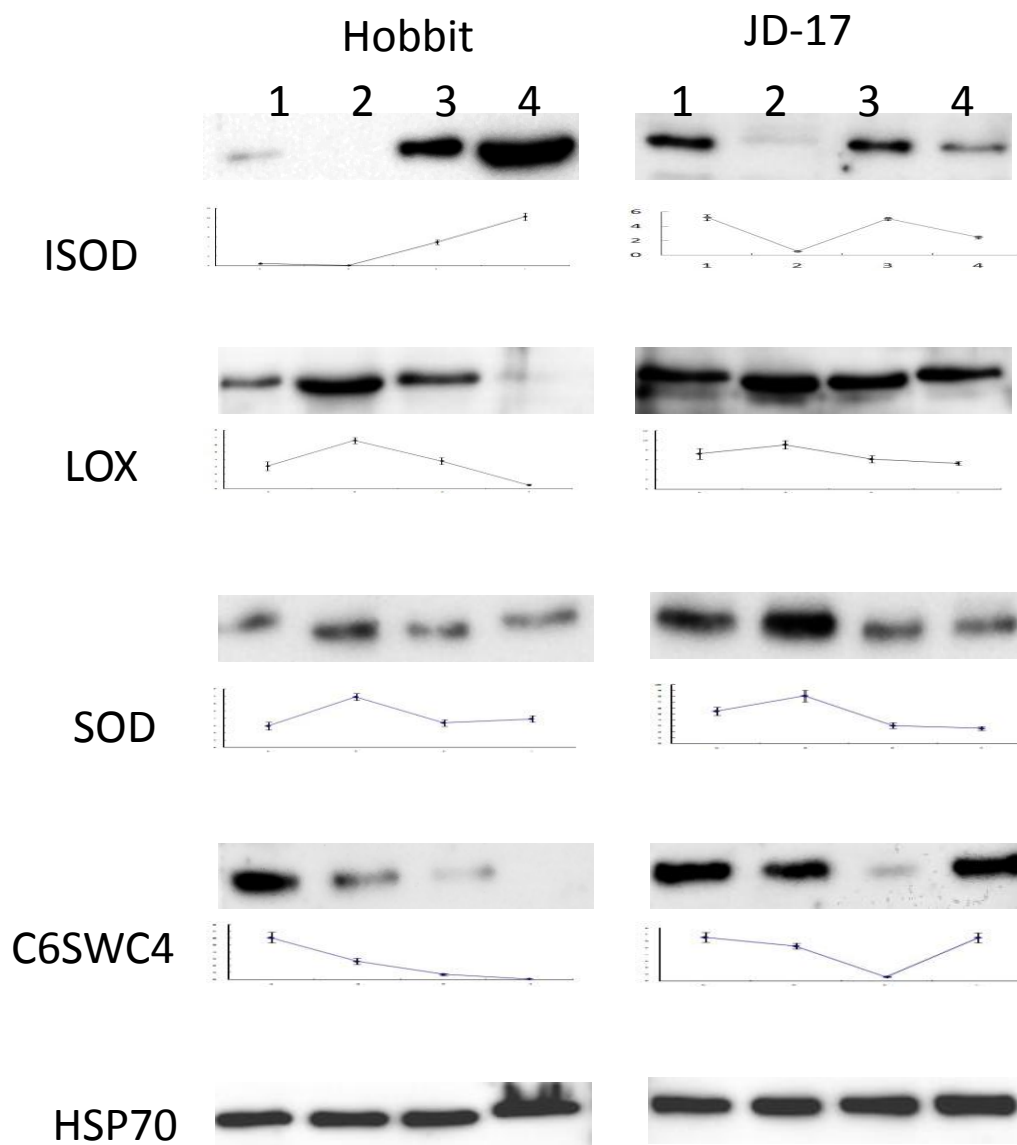


Fig. 3 The Western blotting detection of ISOD LOX SOD C6SWC4 HSP70

Part III

**Proteomics Analysis for the Jidou17 and Its
Parental Inbreds Using Antibody of chloroplast
genes Approaches**

Fig. 1

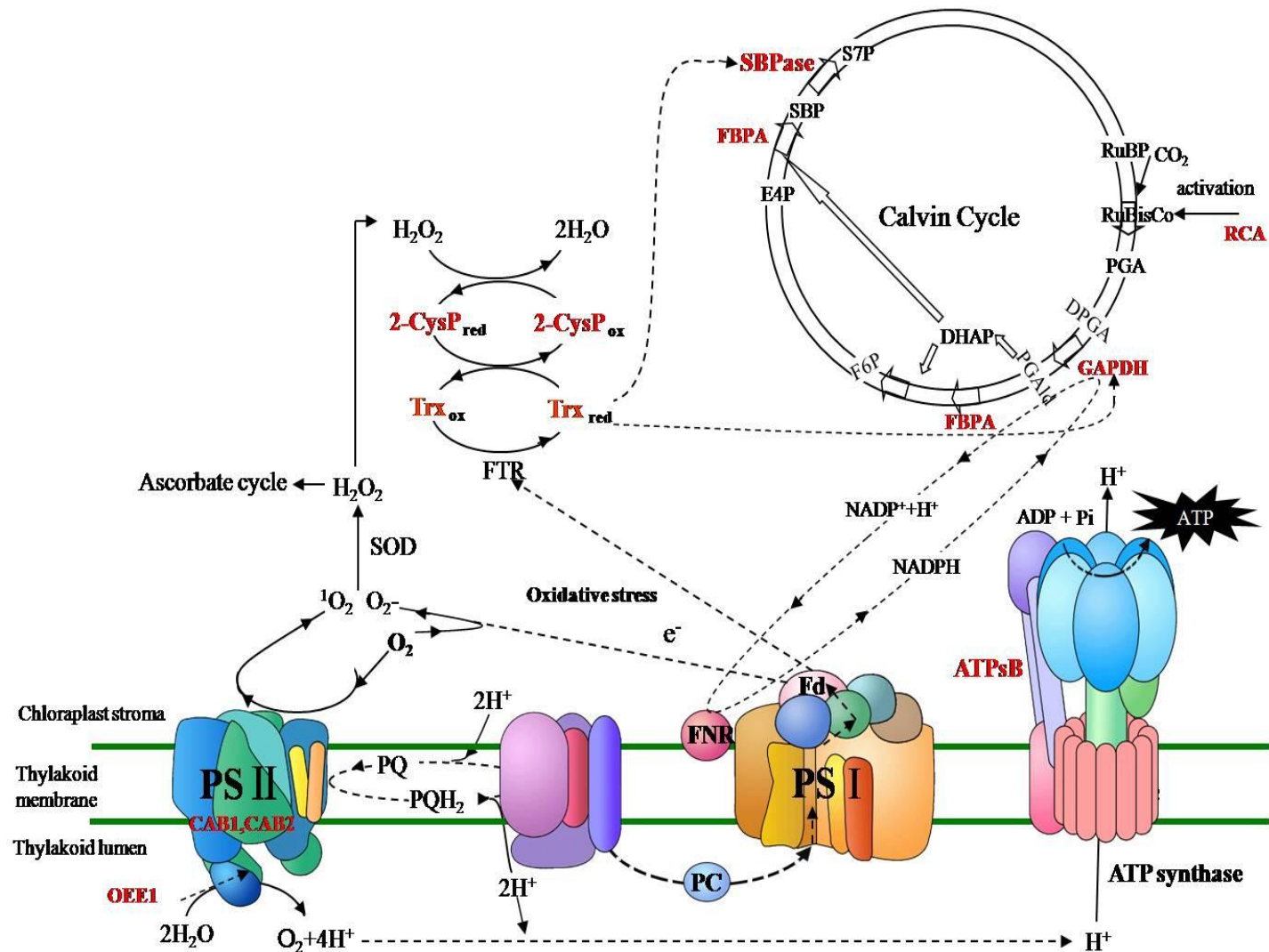


Fig 1 The locations of target chloroplast proteins in photosynthesis and Ros-scavenging pathway

List of chloroplast genes and related information

Gene name	Locus number	Gene annotation	M/ku	Antigen	Fragment/Peptide
CAB1	Os09g17740.1	Chlorophyll A-B binding protein	28	Expressed protein	140AA(1~140)
CAB2	Os03g39610.1	Chlorophyll A-B binding protein	28	Synthesized peptide	SIWYGPDRLPKYLGPFE
OEE1	Os01g31690	Oxygen-evolving enhancer protein 1	35	Expressed protein	333AA(1~333)
ATPsB	Os03g17070	ATP synthase B chain	23	Expressed protein	211AA(1~211)
RCA	Os11g47970.4	AAA-type ATPase family protein	40	Expressed protein	178AA(61~238)
GAPDH	Os03g03720.1	Glyceraldehyde-3-phosphate dehydrogenase	47	Expressed protein	444AA(1~444)
FBPA	Os11g07020	Fructose-bisphosphate aldolase isozyme	42	Expressed protein	388AA(1~388)
SBPase	Os04g16680	Fructose-1,6-bisphosphatase	42	Expressed protein	180AA(21~201)
2-CysP	Os02g33450	2-Cys peroxiredoxin	28	Expressed protein	162AA(101~262)
Trx	Os07g29410	Thioredoxin	32	Synthesized peptide	GSGRSKTARFLRRRRRGAV

The expression profiles of photosynthesis-related protein OEE1

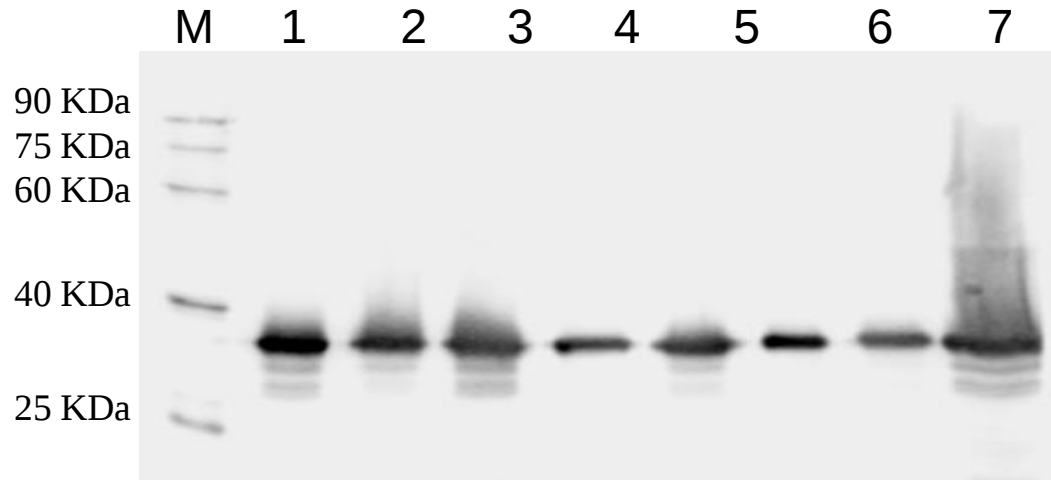


Fig. 2 The Western blotting detection of OEE1

Conclusion

- From the above three samples, a total of 1269 proteins had been detected.
- Pathway analysis showed the differentiated proteins mainly classified to the ribosome assembly pathway.
- Protein expression clustering result showed that expression profiles between Jidou17 and its female parent had higher similarity than those between Jidou17 and its male parent, and had higher similarity than the expression profiles of the two parental strains.

Conclusion

- The female parent Hobbit contributed more to Jidou17.
- The differentially expressed proteins between Jidou17 and its parents might play important role for the development of the elite trait of Jidou17.

Acknowledgment

This work was supported by National Natural Science Foundation of China (31100880), key Project of Natural Science Foundation of Hebei Province (C2012301020)

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Thanks a lot !



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1977 — 1981: B. Ag. Sci (Agronomy) – Hebei Agricultural University

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1999 — 2000: Visiting Scholar, University of Illinois, USA

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Mengchen Zhang - Professional services in the scientific community

- **Director of National Soybean Improvement Center
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