ZAAS, the Vegetable Institute, and the Study on Cowpea Orphan Genes

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Part I.

An introduction to
Zhejiang Academy of Agricultural Sciences, the
Institute of Vegetables & Our Team
ZAAS Location

Beijing

Shanghai

Hangzhou (Zhejiang Province)
Hangzhou-Capital of Zhejiang Province

- Located in East China, 1h to Shanghai by bullet train
- Population: ~ 6,000,000
- Famous for tourism (West Lake) and business (Alibaba)
Overview of ZAAS

- Established in 1908
- A comprehensive non-profit agricultural research institution, biggest in Zhejiang
- Basic and applied research covering all agricultural areas except for tea and fishery
Chairman Mao visited ZAAS

Jan 5, 1958
Premier Zhou visited ZAAS

Dec 17, 1957
Overview of ZAAS

- ZAAS has a faculty number of 1031:
  - 895 research personnel
  - 446 senior professionals
  - 368 holding a PhD degree
16 Institutes of ZAAS

- Institute of Crops and Utilization of Radiation
- Institute of Animal Husbandry and Veterinary Science
- Institute of Vegetables
- Institute of Plant Protection and Microbiology
- Institute of Environmental Resources, Soils and Fertilizers
- Institute of Horticulture
- Institute of Sericulture
- Institute of Rural Development and Information
- Institute of Food Processing
- Institute of Virology and Biotechnology
- Institute of Quality Standards for Agricultural Products
- Institute of Digital Agriculture
- Institute of Flowers
- Institute of Maize
- Institute of Sub-tropical Crops
- Institute of Citriculture
The Institute of Vegetables

Our old building

Our new building
History

• In 1960:
  Vegetable research laboratory, ZAAS

• In 2001:
  Former Institute of Horticulture
    - Facility cultivation laboratory
    - Vegetable breeding laboratory
  Former Introduction and Development Center of New Varieties

Institute of Vegetables
Overview of Institute of Vegetables

- 61 staffs
  - 13 professors
  - 32 Ph.D researchers
  - 1 elected to the National Program for the Support of Top-notch Young Professionals

- Departments:
  - Administration Office
  - 12 research groups
  - 1 state key lab (part of it)
  - 1 national consortium for broccoli improvement
  - 1 provincial key engineering center
  - 1 seed company
  - 2 international cooperation centers
Each group focuses on 1-2 vegetable crops

- Tomato and Pepper
- Eggplant and Radish
- Cowpea and Bottle gourd
- Chinese Cabbage and Head Cabbage
- Cauliflower and Broccoli
- Vegetable soybean
- Cucumber and Bitter gourd
- Melon
- Watermelon
Vegetable Breeding Research

Research areas

- Germplasm collection, identification & utilization
- Genetics & genomics
- Genes controlling key traits
- New breeding techniques
- Breeding of new varieties
- High efficient cultivation techniques
Vegetable Breeding Research

Breeding techniques

- Molecular marker development and MAS
- Microspore culture
- Transfer and utilization of male sterility
- Gene modification
- Somatic cell fusion
- Precise phenotype identification
Vegetable Breeding Research

Main breeding targets

- Better flavor/appearance
- Multiple disease resistance
- Better storage capacity
- High content of functional ingredients
- Abiotic stress resistance
- High yield
Selected released varieties

浙粉202
浙蒲6号
浙秀1号
翠雪5号
浙茄1号
浙农6号
双耐
浙农松花50天
Genomic & Genetic Research

Study on population genomics of the bottle gourd

(Xu et al. 2014, The Plant Journal)
Study on cowpea domestication

Xu et al. 2017, Plant Biotechnol J
Physiological & Molecular Research

A review on evolution of sucrose metabolism

Wan et al. 2018.
Trends in Plant Science
Breeding Technology Research

Study on mature microspore culture
Protected Horticulture Research

- Hydroponics and matrix culture
- Greenhouse environmental control
- Industrial seedling
- Vertical agriculture
- Special vegetables cultivation
Laboratory

Molecular lab

Phytotron

qPCR cycler

Tissue culture lab

Fluorescent microscopy

Microplate spectrophotometer
Experiment station

- 50000 m² tunnel house
- 10000 m² glasshouse
- 20ha open field
Total research funding in 2002-2017 (ten thousand RMB)

~ 40% of the funding is from the national level
Total income in 2002-2017 (ten thousand RMB) from R&D achievements transfer
International Cooperation

China-Korea collaboration on protected horticulture

Partner:
National Horticultural Research Institute, RDA, Korea

Joint research area:

• Breeding for rootstocks resistance to soil-born disease
• Automatic grafting technique for vegetables.
International Cooperation

ZAAS-UCR collaboration on cowpea research

Partner:
UC-Riverside, USA

Joint research area:
• Genetics & genomics of cowpea
• Molecular breeding for climate-resilient cowpea varieties

Genome resources for climate-resilient cowpea, an essential crop for food security

María Muñoz-Amatriain, Hamid Mirebrahim, Pei Xu, Steve I. Wanamaker, MingCheng Luo, Hind Alhakami, Matthew Alpert, Ibrahim Atokple, Benoit J. Batienen, ... See all authors
International Cooperation

ZAAS-HUJI collaboration on drought resistance

Partner:
Hebrew University of Jerusalem, Israel

Joint research area:
• Phenomics-based selection of drought tolerance
• Molecular mechanisms controlling whole plant water relations
International Cooperation

We are looking forward to establishing official collaboration with ICRISAT

- Genomics of legume and other grain crops
- Molecular breeding theories and applications
- Phenomics
- ...

Dr. Rajeev Varshney visited ZAAS in 2017
The Cowpea & Bottle Gourd Research Team

Two Professors

- Director of the Institute of Vegetables
- PI of the Vegetable Breeding Consortium in Zhejiang Province
- PI of the National Consortium for Broccoli Improvement
- Member of the Chinese Society of Horticulture

Guojing Li
Ph D, Professor
The Cowpea & Bottle Gourd Research Team

Two Professors

- Executive council member of the Young Section for the Chinese Society of Horticulture
- Elected to the National Program for the Support of Top-notch Young Professionals
- Editor of the Indian-based international journal Legume Research

Pei Xu
Ph D, Professor
The Cowpea & Bottle Gourd Research Team

Three Senior Breeders

Xiaohua Wu
Assoc. Professor
Traditional breeding

Baogen Wang
Assoc. Professor
Traditional breeding

Zhongfu Lu
Senior Agronomist
Seed technology
The Cowpea & Bottle Gourd Research Team

Two PhD Researchers

Ying Wang
Assis. Professor

Xinyi Wu
Assis. Professor

Genetics & molecular breeding
The Cowpea & Bottle Gourd Research Team

- 4 technicians
- 3 graduate students and visitors
- We won the Pioneer Worker Award last year
Part II.

Orphan genes are involved in drought adaptations and ecoclimatic-oriented selections in domesticated cowpea

Seminar
• Darwin's Theory of Evolution:
  - all life is related and has descended from a **common ancestor**
  - the diversity of life is a product of “modifications of populations by natural selection”

**Ancestor DNA-mutation**

**-selection-new species**
Orphan genes: or lineage specific genes, are genes that are restricted to a single species or a particular taxonomic group.
Orphan genes remain largely enigmatic

Facts we know:

- OGs constitute $1\%$ - $1/3$ of the total gene number in a genome
- Total number of OGs > NOGs (ecological perspective)
- Essential in morphological speciation or environmental adaptation
- Can remain functional when introduced into other species, even far-related

*Khalturin et al. 2009, Trends Genet*
Orphan genes remain largely enigmatic

We don’t yet know:

- Origin and turn over of the OGs
- Function of most of the OGs
- Subspecies-level signatures
- ......

DNA sequences of OGs appear suddenly and fully functional without any trace of evolutionary ancestry

One gene may carry a triple function (regulatory DNA, regulatory RNA, and protein)
Cowpea (Vigna. unguiculata L. Walp)

- A member of the Phaseoleae tribe
- Native to Africa, cultivated worldwide
- 2n=2x=22, genome=630 Mb

Two main cultivation groups

- Domesticated in humid Southeast Asia
  - ssp. sesquipedalis

- Domesticated in dry West Africa
  - ssp. unguiculata

Fabaceae Family

Chio et al. 2004  Xu et al. 2012. Heredity
Characteristic adaptive trait:

• Excellent drought tolerance

More tolerant to soil drought than Sorghum and Pearl millet (Ewansiha & Singh. 2006. J Food Agric Environ)

Q: are cowpea orphan genes related to drought tolerance?

(Photo credit: Jeff Ehlers)
Identification of expressed OGs

- **Source data: the EST-derived unigenes**
  - 29,728 unigenes, 26,248 annotated \( (Muchero\ et\ al.,\ 2009,\ PNAS) \)
  - **3480 unigenes with no annotation** used as the input data
Identification of expressed OGs

- A microarray representing all unigenes (Xu et al., 2015, *fpls*)
- DNA of cowpea and adzuki bean as probes

- Statistic method
  - 95% percentile (=2.1) of the cowpea to adzuki bean signal ratio of known NOGs taken as threshold

a. DNA microarray hybridization
b. Traditional Southern hybridizations
   (Anamthawat-Jonsson & Heslop-Harrison, 1992)

578 unigenes determined as OGs
Characterization of Cowpea OGs

Sequence characteristics

- Shorter length
- Lower GC-content

- Coding-Non-Coding Index (CNCI) estimation

~ 3/4 OGs are predicted to be non-coding
Characterization of Cowpea OGs

Organ expression patterns: generally **low or very low expressions**


Flower & seed expression patterns (microarray data, Agilent platform, unpublished)
Drought-responsive expression patterns:

- A high rate of OGs (20.7%) were drought-responsive in **roots**, with the vast majority being **up-regulated**.
- Only 3.8% of the OGs were differentially expressed in **leaves**.

**Transcriptomic signatures**

Possible roles in drought sensing and signal regulation

Agreeing with the non-coding nature of the majority of the OGs.
Transcriptomic signatures

Drought associated gene co-expression patterns:

- Three significant co-expression modules, 85 OGs involved
- Enriched in GO terms including “response to stimulus”

Cowpea OGs
Genes involved in response to stimulus
Genes involved in oxidoreductase activity
Genes involved in both GO terms

(properoxidase precursor)
Validation of a drought-inducible OG

Hairy-root transformation

- **UP12_8740, 7.4-fold** up-regulated in roots upon drought stress
- Over-expression driven by 35S promoter

PEG induced osmotic stresses
Validation of a drought-inducible OG

Hairy-root transformation

- *UP12_8740*, 7.4-fold up-regulated in roots upon drought stress
- Over-expression driven by 35S promoter

Better performance and survival rate of the UP12_8740-OE lines than the CK lines under progressive soil drought

*Unpublished data*
SNP discovery & Genotyping

Strategy to catch polymorphisms in the OGs

- Capture-Seq
- A SureSelect Target Enrichment Capture Assay (572 OGs)

The SNP discovery panel: 8 genotypes selected from 223 accessions

*Xu et al. 2017. Plant Biotechnol J*
SNP discovery & Genotyping

Capture-Seq results

- Rate of on-target reads: \(\sim 45\%\) for each library
- Average on-target sequencing coverage: 7.5 \(\times\)
- 1560 SNPs detected from transcribed regions in 390 of the 572 OGs

SureSelect in-solution targeted enrichment of OG fragments
SNPs validation

- PCR of 20 randomly selected OGs containing 78 SNPs
- 77 SNPs confirmed by Sanger sequencing

UP12_12216: SNPs at positions 282, 301, 327, 390, 409, 471.

A high accuracy (98.7%) of SNP discovery was confirmed.
**SNP discovery & Genotyping**

**Conversion of SNPs to KASP markers**

- 1 SNP each OG selected for conversion
- 379 ultimately succeeded
- 223 accessions genotyped
- 354 (93.4%) technically successful

OGs vs NOGs

- **331** SNPs in OGs, **48,216** published SNPs in NOGs
- Higher **heterozygosity** (*He*), polymorphism information content (PIC), nucleotide diversity (*π*) in OGs than in NOGs
- **Tajima's D** value: 3 times **greater** in OGs than in NOGs
OGs: Subpopulation 1 vs Subpopulation 2

- Tajima's D values were in opposite directions in the two subpopulations.
- In subpop1, more OGs showed empirically large positive Tajima's D value.
- In subpop2, more OGs appear to be under no or purifying selection.
- UP12_8740 had a considerable Tajima's D (1.503) in subpop 1.

Unpublished data
**Implications**

- The more dominant balancing selection, known to be beneficial for plant adaptation by maintaining genetic diversity, is consistent with the importance of OGs in drought adaptation in grain cowpea.

- In vegetable cowpea, the higher level of purifying selection appears to be a reflection of artificial selection toward balancing the adaptive and agronomical traits in the less drought-prone regions.

- Some mutations in OGs beneficial for drought tolerance may harm the quality for vegetable use, and might have been removed during domestication.
Summary

• Expressed OGs account for 2% of the total number of unigenes
• OGs may exert functions through drought sensing and signaling in the roots, as well as participating in conserved stress-responsive pathways
• OGs are a valuable resource for identifying new genes related to environmental adaptations
• Our results fosters a new insight that artificial selections on OGs might have contributed to balancing the adaptive and agronomical traits in domesticated crops in various eco-climatic conditions
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