SOYBEAN IMPROVEMENT FOR HUMAN CONSUMPTION

ZHU D, YUAN F, YU X, ZHU Z, FU X AND DONG D

Hangzhou Sub-center of National Soybean Improvement, Zhejiang Academy of Agricultural Sciences, Hangzhou, 310021, China
E-mail: danhua163@hotmail.com

The benefits of soybean for human health consumption are not only related to its rich protein content, high levels of essential fatty acids, but also to numerous vitamins, minerals, isoflavones, fiber and other functional phytochemicals. Soybean cultivars with higher concentration of these components are beneficial to consumers and provide a value-added product for the farmers.

Seed α-tocopherol content of 376 soybean lines ranged between 0.6 and 8.2 mg/100g and two of them were higher than 8.0 mg/100g. Seed total vitamin E content of these germplasm ranged between 12.3 and 45.5 mg/100g and the highest one was Hefeng No. 37.

The amount and composition of isoflavones are affected by environmental conditions. The mean isoflavone concentration was between 2000-4000 μg/g. Five soybean germplasm accessions with isoflavone concentrations above 5000 μg/g were found among 2003 germplasm lines. Xiangshuiheidou from Guangxi province had highest isoflavone concentration of 6021 μg/g.

The inorganic phosphorus content of the mutant Gm-lpa-TW-1 was about 7-8 times higher than its wild-type parent Taiwan 75, while that of Gm-lpa-ZC-2 was 2-4 times higher than its parent Zhechun No. 3. The phytate content of these two mutants was reduced by more than 50% as compared with their wild type parents. The reduction of phytic acid P (PA-P) in mutant Gm-lpa-ZC-2 was higher than the increase of inorganic phosphorus. It was found that the PA-P reduction was also accompanied by an increase of lower inositol phosphorus.

A vegetable soybean variety Taiwan75 was treated by 150 Gy gamma ray. Among 2500 M2 individuals, one high sugar and low raffinose mutant, 96H005, was identified. Compared to its wild type parent Taiwan75, it had significantly higher concentrations of sucrose (+47.4-86.1%) and reduced concentrations of raffinose and stachyose.

A high protein soybean variety ‘3618’ was mutated with irradiation. A lectin-free line, ‘R3618-58’ was identified, which inherited the characteristic of high protein content, but has a deficiency of lectins.

Vegetable soybean has become a very important crop in some areas of China, such as Zhejiang province. About 50 varieties have been approved and released since 2000, which have shown significant improvement in yield, stability, adaptability and disease resistance than introduced varieties from Japan.
Soybean Improvement for Human Healthy Consumption

Danhua ZHU, Fengjie YUAN, Xiaomin YU, Shenlong ZHU, Xujun FU, and Dekun DONG

National Sub-center of Soybean Improvement
Zhejiang Academy of Agricultural Sciences

198, Shiqiao Rd., Hangzhou, 310021
Zhejiang, P.R. China,
dhzhu@mail.zaas.ac.cn; 86-571-8640-4248
http://www.zaas.ac.cn/
GENERAL INTRODUCTION:

HANGZHOU SUB-CENTER OF INTERNATIONAL SOYBEAN IMPROVEMENT
Hangzhou City
E 118°21’-120°30’,
N 29°11’-30°33’.

198, Shiqiao Rd.,
Hangzhou, 310021
Zhejiang, P.R. China,
86-571-8640-4248
dhzhu@mail.zaas.ac.cn;
http://www.zaas.ac.cn/
China had historically been a net soybean exporter; the highest soybean export was more than one million tons a year. About 3.9 million tons soybean meal as a byproduct.
Soybean demand

• China's annual demand for oil consumption averages approximately 80 million tons,
• The demand for soybean meal for feed averages about 30 million tons.
• There is a huge gap between the production capacity and demand in China, resulting in annual imports of soybeans or oil to make up
• Since 1996, China's soybean import has been increasing from 294,000 tons in 1995 to 58.38 million tons in 2012.
Disadvantage in soybean price

Soybean has lower price when compared with other local crops, especially corn and rice.

- The corn-soybean price ratio is only 1 to 1.85, (the normal ratio is 1 to 2.4)
- The rice-soybean price ratio is only 1 to 1.5, much less than the normal ratio of 1 to 2.5
Farmers in China planted less soybean acreage this year, especially in northeast provinces because corn and rice yields and prices are better than soybeans.

Soybean production is predicted at 13.0 million tons in 2012; 1.8 million tons down from 2011 due to lower acreage.
Advantage of soybean production

◆ As a legume crop, soybean is a low input, soil enriching, and sustainable crop that could help farmers minimize insect and disease build up as well.

◆ Soybean remains a very interested crop for local farmers because of its advantages in crop rotation systems.
Soybean with value-added traits

• Development of improved soybean cultivars with value-added traits will offer a potential for maintaining the advantage of domestic soybean production for the local markets.

• The farmers, particularly some traditional food producers will still choose soybean as a profitable rotation crop.
• The benefits of soybean are related to its rich protein content, high levels of essential fatty acids, numerous vitamins and minerals, isoflavones, fiber and other functional phytochemicals which are associated with human health.
• Because of these, we have devoted our research efforts to develop soybean with more healthy composition which may make us directly consume more soybean and soybean will become more value-added.
Section II

EXPLORATION OF HEALTHY SOYBEAN GERMLASM
Exploration of healthy soybean germplasm

Collection of Germplasms

Screening for specific lines

Utilizing in breeding

Value added varieties
**2003 germplasm**

- 1086 from Zhejiang province
- 859 from other province
- 58 from foreign countries

- 840 spring soybean
- 692 summer soybean
- 412 autumn soybean
High protein quality germplasm

• We have analyzed 605 soybean germplasms using SDS-PAGE (Jiang et al., 2010). The ratio of 11S to 7S globulins was between 1.270 and 4.008.

• Among these germplasm screened, we found four germplasm lines Pandou, Zeiwuyao, Baimaojian, and Qingdadoou that had a ratio of 11S to 7S greater than 3.8.
High vitamin E germplasm

- Vitamin E is a group of fat-soluble compounds that include four forms of tocopherols, α-, β-, γ-, and δ-tocopherol.

- It is a good antioxidant that stops the production of reactive oxygen species (ROS) formed when fat undergoes oxidation.
• The average of α-tocopherol and total Vitamin E contents of lines from Northeast China were 2.9 and 25.4 mg/100g respectively;

• The average of α-tocopherol and total Vitamin E contents of North American lines were 1.7 and 19.8 mg/100g.
• Seed α-tocopherol content of 376 soybean germplasm lines ranged between 0.6 and 8.2 mg/100g and two of them were higher than 8.0 mg/100g.

• Seed total vitamin E content of these germplasm ranged between 12.3 and 45.5 mg/100g and the highest one was Hefeng No.37.
High isoflavone germplasms

- Antiestrogenic effect
- Inhibit the enzymes involved in cancer growth
- Inhibit blood vessels development
- Prevention heart disease and cancer

Fig. 1 Structure of main lupin isoflavones genistin (1), licoisoflavone A (2), wighteone (3), licoisoflavone B (4), genistein (5), 2’-hydroxygenistein (6), lupinalbin A (7), and as internal standard daidzein (IS).
• The amount and composition of isoflavones are different according to the year, location, and the overall growing environment.

• The mean isoflavones content of Chinese soybean germplasm was about 2000-4000 ug/g.

• Five soybean germplasms with isoflavone content high than 5000ug/g had been found among 789 germplasm lines.

• Xiangshuiheidou from Guangxi province had highest isoflavone content of 6021 ug/g.
Creation of mutant

- Experimental mutagenesis is an important resource to produce mutation in higher frequencies in soybean.
- Radiation has been an effective way for selecting desirable valued-added varieties for human healthy consumption.

National Sub-center of Soybean Improvement, The Institute of Crops and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Sciences.
Low phytic acid mutants

• Phytic acid is a nearly ubiquitous component and is usually the most abundant form of phosphate in plant seeds, account for more than 60% of the total seed phosphate in many cases.

• In soybean seeds, phytic acid, as a strong anti-nutritional chelator, can form salt compounds with mineral nutrients, i.e., Fe and Zn, which are deposited in protein bodies.

• These phytates, however, are largely indigestible by humans and other non-ruminant animal.
In our group, two mutants, **Gm-lpa-TW-1** and **Gm-lpa-ZC-2**, were developed from two commercial soybean varieties Taiwan75 and Zhechun No.3.

The inorganic phosphorus (Pi) content of **Gm-lpa-TW-1** was about 7-8 times higher than its wild-type parent Taiwan 75, while that of **Gm-lpa-ZC-2** was only 2-4 times higher than its parent Zhechun No 3.

The **phytate content** of these two mutants was reduced by more than 50% as compared with their wild type parents.
### Phosphorus concentration of various fractions in soybean seeds of two *lpa* mutant line and their parents (mg/g)

<table>
<thead>
<tr>
<th></th>
<th>TP</th>
<th>Pi</th>
<th>PA-P</th>
<th>L-Ins- P</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhechun No.3</td>
<td>7.46 ±0.07</td>
<td>0.56±0.01**</td>
<td>3.73±0.63**</td>
<td>1.15±0.20**</td>
<td>4.88±0.40</td>
</tr>
<tr>
<td>Gm-lpa-ZC-2</td>
<td>7.69±0.15</td>
<td>1.48±0.01</td>
<td>1.87±0.15</td>
<td>2.52±0.10</td>
<td>4.39±0.27</td>
</tr>
<tr>
<td>Taiwan 75</td>
<td>7.67±0.15</td>
<td>0.58±0.01**</td>
<td>4.07±0.24**</td>
<td>1.08±0.20**</td>
<td>5.15±0.02**</td>
</tr>
<tr>
<td>Gm-lpa-TW-1</td>
<td>7.94±0.13</td>
<td>4.01±0.16</td>
<td>1.24±0.06</td>
<td>0.26±0.02</td>
<td>1.50±0.01</td>
</tr>
</tbody>
</table>

**P level of the parental variety was significantly different from that of its mutant (p=0.01)**
IP6: phytic acid (myo-Inositol-1,2,3,4,5,6-hexakisphosphate);
IP5: myo-inositol-pentakisphosphate;
IP4: Myo-inositol-tetrakisphosphate
IP3: Myo-inositol-trisphosphate

Lower ester myo-inositol phosphate
Genetic analysis of two mutants

◆ The two *lpa* mutations were both inherited in a single recessive gene model.

◆ Two genes from different mutants are non-allelic.

◆ The *lpa* mutation in *Gm-lpa-ZC-2* was mapped on LG B2, linked with microsatellite loci satt416 and satt168. The soybean IPK1 orthologue of a low phytic acid mutant reveals an exon-excluding splice-site mutation.

◆ *Gm-lpa-TW-1 lpa* mutation resulted from a 2bp deletion in the soybean *MIPS1* gene.
High sugar and low raffinose mutant

• Soybean seed contain about 33% carbohydrates and up to 16.6% of the carbohydrates are soluble sugars including glucose, fructose, sucrose, raffinose and stachyose.

• Soluble sugars especially sucrose can help improve the taste and flavor of soyfood and vegetable soybean.

• But raffinose and stachyose can not be digested by humans and non-ruminant animals, and led to flatulence or diarrhea.
• Taiwan 75 was treated by 150Gy gamma ray
• M1 harvested in bulk
• M2 harvested in individuals
• M2:3 seeds to screen *high sugar and low stachyose* phenotype
• One mutation line 96H005 was generated
Compared to its wild type parent Taiwan75, 96H005 had significantly higher concentrations of sucrose (+47.4-86.1%) and reduced concentrations of raffinose and stachyose. The raffinose content of the mutant was only about 20% that of the parent, while stachyose content was almost diminished in the mutant.
Genetic analysis of the mutant

◆ The high sugar mutation was inherited in a single recessive gene model.

◆ The high sugar mutation in 96H005 was mapped to LG G and positioned on the upside of SSR markers satt570 and satt115 at the genetic distance of 5.5 and 15.2cM.
Lectin free soybean mutant

- Soybean lectins are a part of soybean protein subunits, which take up 5% to 10% of total protein.

- Soybean lectins are able to disrupt small intestinal metabolism and damage small intestinal villi, thus cause lesion and abnormal development and affect digestion and absorption of nutrient.
One individual named 3618-58 was found with lectin free phenotype.
• This trait was stably expressed during M3, M4, M5, and M6 generation.

• Compared to the parental line, ‘3618’, ‘R3618-58’ inherited the characteristic of high protein content but has a deficiency of lectins, thus serving as a lectin-free and high-protein soybean variety.
The protein contents of soybean germplasms collected were between 30.8% and 48.9%.

• Zheqiudou No.3 is another high protein soybean variety that also has high yield and lodging resistance. The seed protein content is up to 50.05%.
High protein soybean variety Zheqiudou No.3

Tedali × Xiangqiudou No.1

Pedigree selection

Zheqiudou No.3
( released by Zhejiang province in 2003 )

High yield

High protein content

The crude protein content of Zheqiudou No.3 was about 50.05%
Zheqiu No.3 and High protein lines 3618
VEGETABLE SOYBEAN PRODUCTION IN CHINA
Vegetable soybean

Maodou

Turag

Edamame

(Glycine max (L.) Merr.)
Green bean cooking
The development of vegetable soybean in early years was restricted to the Yangtze River basin.

The policy of the Economic Reform and Opening-up has driven the development of vegetable soybeans to a new era.

Since vegetable soybeans can offer large markets and good benefits, they have become a very important crop in some areas of Zhejiang and Fujian.
Breeding of vegetable soybean

• In 1996
  - ‘Aijiaomaodou’ was released in Zhejiang province.
  - It was the first soybean variety named as ‘vegetable soybean’ in China.

• In 2002
  - Ministry of Agriculture of China organized the first vegetable soybean regional experiment.
Breeding of vegetable soybean

• In 2005
  ‘Zhediandou No.2’, a variety developed by our team, was formally released as the first vegetable soybean variety at National level.
Breeding of vegetable soybean

• From 2006 to 2010
  ➢ ‘Huning96-10’, ‘Qixing No.1’, ‘Zhongdou37’, ‘Zhexiandou No.4’, No.5, ‘Jiaoda02-89’ and more were subsequently released.

● Today
  ➢ About fifty vegetable soybean varieties were planted in more than 11 provinces in China.
Quality Improvement

• Appearance
  • Color, length, width, weight ...

• Nutritional qualities
  • Carbohydrate, protein, fat, amino acids, vitamins, mineral elements, isoflavones, lecithin ...

• Edible qualities
  • Sweetness, texture, flavor, aroma...
Appearance

• Appearance of pods is the most important commercial characteristic.

• Recognized standards:
  ✓ Emerald green pod with less gray hairs
  ✓ Pod longer than 4.5 cm, wider than 1.3 cm
  ✓ Pod filled with more than 2 well-developed seeds
  ✓ Fresh weight per hundred pods exceed 285 g
  ✓ Fresh weight per hundred seeds exceed 75 g
## Appearance quality variation of vegetable soybeans

<table>
<thead>
<tr>
<th>Index</th>
<th>Mean value</th>
<th>Change range</th>
<th>Coefficient of variation(%)</th>
<th>Number of superior varieties (total 55 varieties)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pod length (cm)</td>
<td>5.47</td>
<td>4.50-6.60</td>
<td>9.01</td>
<td>26 varieties &gt; 5.50cm</td>
</tr>
<tr>
<td>Pod width (cm)</td>
<td>1.35</td>
<td>1.20-1.5</td>
<td>6.73</td>
<td>18 varieties &gt; 1.40cm</td>
</tr>
<tr>
<td>100-pod fresh weight (g)</td>
<td>262.12</td>
<td>142.8-340.3</td>
<td>14.22</td>
<td>11 varieties &gt; 285g</td>
</tr>
<tr>
<td>100-seed fresh weight (g)</td>
<td>70.74</td>
<td>56.00-99.00</td>
<td>11.81</td>
<td>14 varieties &gt; 75g</td>
</tr>
<tr>
<td>Standard pod rate (%)</td>
<td>66.95</td>
<td>60.16-77.00</td>
<td>6.35</td>
<td>11 varieties ≥ 70%</td>
</tr>
</tbody>
</table>
## Edible qualities

### Textures analysis of vegetable soybean seeds

<table>
<thead>
<tr>
<th>Lines</th>
<th>Ecotype</th>
<th>Taste</th>
<th>Rigidity (kg)</th>
<th>Rigidity was measured using a TA.XT. Plus texture analyzer.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZDD6202</td>
<td>Summer</td>
<td>soft</td>
<td>8.39</td>
<td></td>
</tr>
<tr>
<td>Danbohei</td>
<td>Summer</td>
<td>soft</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>A1761</td>
<td>Summer</td>
<td>mid-hard</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>ZDD6056</td>
<td>Summer</td>
<td>mid-hard</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>A1759</td>
<td>Autumn</td>
<td>hard</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>ZDD6246</td>
<td>Summer</td>
<td>hard</td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td>Jiuyueba</td>
<td>Autumn</td>
<td>hard</td>
<td>17.5</td>
<td></td>
</tr>
<tr>
<td>4203</td>
<td>Summer</td>
<td>hard</td>
<td>18.1</td>
<td></td>
</tr>
<tr>
<td>4201</td>
<td>Summer</td>
<td>hard</td>
<td>18.4</td>
<td></td>
</tr>
</tbody>
</table>
Edible qualities

Seed rigidity of different vegetable soybean lines before and after cooking
Vegetable Soybean Production

- Now, the production area has exceeded 300,000 hm², in which Zhejiang province contributes about 1/4 of the total areas followed by Jiangsu, Fujian, Anhui and Shanxi provinces.

- The production areas of vegetable soybean in southern China account for more than 80 percent of total production areas.

- Fresh vegetable soybean can be supplied about 7 months a year in Yangtze River Basin.
Production area of vegetable soybean

1. Zhejiang 84,000 hm²
2. Jiangsu 67,000 hm²
3. Fujian 30,000 hm²
4. Anhui 30,000 hm²
5. Shanxi 20,000 hm²

SP Liaoning 8,000 hm²
(Seed Production)
Type 1
Raise seedling & Transplant in greenhouse
Double plastic film culture
Double plastic film culture
Double plastic film culture
Type 3
Single plastic film culture
Early stage
No-plastic film culture
Harvest (1)
Freshen soybean pod
Local Marketing
Selecting by machine
Transportation
SOYBEAN PROCESSING AND UTILIZATION
Tofu
Dried bean curd
Fried bean curd
Thanks for your attention!
Welcome to visit our institute