High Oleic Acid Peanut Breeding and Application in China

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1. Peanut growth and consumption in China

1.1 Peanut growth area and production in China

- Total growth area: 5 million ha; total production: 15 million tons
- Two major cultivation models: the spring sowing model and summer sowing model after wheat or oil seed rape. Peanut intercrop with maize, sorghum, cotton, sugarcane, fruit tree......
1.2 Peanut growth in different provinces

Three major growth area:

(1) Yellow River/Huai River
(2) Yangtze River
(3) South/South East China
1.3 Major peanut utilization in China

- More than 50% of the peanut in China is used for oil extraction.
- Oil companies: Luhua, Jinlongyu, Hujihua, Changshouhua, Longda.
- Peanut oil is one of the most popular oils in the north part of China.
- Peanut butter, roast peanut, sweet coated peanut and fried peanut are also available in the market.
1.3 Major peanut utilization in China
1.4 Major concerns of peanut breeding

- Yield: the most important factor
- Disease resistance and food safety
- Quality: oil content and composition
Peanut food and oil rancidity

- **Rancidity is a big issue for peanut food and oil.** Lipid rancidity includes a series of reactions and generates large number of small molecules, affecting quality, nutrition and shelf-life of food and oil.
- **Lipid rancidity is affected by the fatty acid saturation level, temperature, light, oxygen, metal ions, water activity and anti-oxidants.**

![Diagram of Peanut food and oil rancidity](image)

- **Hydrolysis Rancidity**
  - Enzymes, pH value, Temperature
  - Glycerol, Fatty acids, Monoglyceride, Diacylglycerol ester

- **Oxidative Rancidity**
  - Auto-oxidation, Enzyme cat oxidation, Light oxidation
  - Acids, aldehyde, ketone, Alcohol substances, Hundreds of other molecules
### Oxidation rate and lipid saturation level

<table>
<thead>
<tr>
<th>Days</th>
<th>POV meqO2/kg</th>
<th>AV mgKOH/g</th>
<th>SV mgKOH/g</th>
<th>TBARS mgMDA/ka</th>
<th>IV gI/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.88</td>
<td>0.17</td>
<td>191.1</td>
<td>0.22</td>
<td>129.38</td>
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<tr>
<td>26</td>
<td>114.48</td>
<td>0.22</td>
<td>200.49</td>
<td>3.94</td>
<td>122.18</td>
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<tr>
<td>32</td>
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<td>202.75</td>
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<td>37</td>
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<td>212.47</td>
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<td>42</td>
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<td>0.83</td>
<td>216.44</td>
<td>4.81</td>
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<td>46</td>
<td>209.26</td>
<td>0.97</td>
<td>226.12</td>
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<tr>
<td>51</td>
<td>255.63</td>
<td>1.18</td>
<td>233.49</td>
<td>5.64</td>
<td>97.3</td>
</tr>
</tbody>
</table>

### Fish oil oxidation

<table>
<thead>
<tr>
<th>H</th>
<th>POV meqO2/kg</th>
<th>AV mgKOH/g</th>
<th>SV mgKOH/g</th>
<th>TBARS mgMDA/ka</th>
<th>IV gI/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.48</td>
<td>1.22</td>
<td>184.3</td>
<td>16.5</td>
<td>136.6</td>
</tr>
<tr>
<td>10</td>
<td>37.4</td>
<td>1.41</td>
<td>201.6</td>
<td>152.0</td>
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<tr>
<td>20</td>
<td>359.9</td>
<td>1.91</td>
<td>206.0</td>
<td>542.3</td>
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<tr>
<td>25</td>
<td>1026.0</td>
<td>5.38</td>
<td>212.8</td>
<td>1002.6</td>
<td>128.17</td>
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<tr>
<td>30</td>
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<td>8.42</td>
<td>234.2</td>
<td>1332.7</td>
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<tr>
<td>35</td>
<td>1758.2</td>
<td>9.31</td>
<td>239.5</td>
<td>1288.5</td>
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<tr>
<td>44</td>
<td>1991.2</td>
<td>14.37</td>
<td>242.1</td>
<td>1317.7</td>
<td></td>
</tr>
</tbody>
</table>

- **AV**: 酸价
- **POV**: 过氧化物价
- **SV**: 皂化价
- **TBARS**: 硫代巴比妥酸反应物
- **IV**: 碘价

Yuan et al., 2007
Malondialdehyde (MDA) is a major product of lipid oxidation. It is generated from oxidation of unsaturated fatty acids. MDA levels are closely related to lipid unsaturation level.

<table>
<thead>
<tr>
<th>Acid</th>
<th>MDA Contents (nmole/mg)</th>
</tr>
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<tbody>
<tr>
<td>Oleic acid</td>
<td>0.1</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>3.2</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>20.1</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>64.0</td>
</tr>
</tbody>
</table>

The oxidation rates of oleic acid, linoleic acid, linolenic acid, and arachidonic acid are 1:10:20:40.

Reduce polyunsaturated lipid is an efficient way to decrease oxidation.
### Peanut major fatty acid contents

<table>
<thead>
<tr>
<th></th>
<th>A. aequatoriana</th>
<th>A. vulgaris</th>
<th>A. hypogaea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid</td>
<td>9.6-12.9</td>
<td>8.8-13.5</td>
<td>7.9-12.5</td>
</tr>
<tr>
<td>Steric acid</td>
<td>1.8-3.7</td>
<td>2.4-3.9</td>
<td>1.8-3.3</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>37.8-46.4</td>
<td>37.0-54.5</td>
<td><strong>38.2-64.7</strong></td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>33.6-40.1</td>
<td>27.2-40.4</td>
<td><strong>18.0-39.4</strong></td>
</tr>
<tr>
<td>Arachidic acid</td>
<td>1.1-1.7</td>
<td>1.3-1.7</td>
<td><strong>1.1-1.8</strong></td>
</tr>
<tr>
<td>Eicosenoic acid</td>
<td>0.8-1.4</td>
<td>0.8-1.5</td>
<td>0.9-1.6</td>
</tr>
<tr>
<td>Behenic acid</td>
<td>2.7-3.3</td>
<td>2.3-3.5</td>
<td>2.3-3.9</td>
</tr>
</tbody>
</table>

Lower linoleic acid content is important due to its large proportion in peanut oil.

Jianwei Lv et al., 2010
Yueyi Tang et al., 2013
1.5 Fatty acid biosynthesis pathway

- Fatty acid synthesis takes place in the cytosol and starts from Acetyl-CoA.
- Acetyl-CoA generated from pyruvate by the action of pyruvate dehydrogenase (PDH) and by β-oxidation of fatty acids in the mitochondria.
- Acetyl-CoA has to be transported from mitochondria to cytoplasm by a shuttle system called the Citrate Shuttle.
- TAG is stored in oil body in seed
1.5 Fatty acid biosynthesis pathway

**ACCases:** Acetyl-CoA Carboxylase, the first enzyme in the fatty acid biosynthesis pathway.

Catalyze Acetyl-CoA to form Malonyl-CoA which used in the following steps of fatty acid synthesis.

Two types of ACCase: heteromeric form in plastid, homomeric form in cytosol.
1.5 Fatty acid biosynthesis pathway

**FAS:** enzymes of fatty acid synthesis are packaged together in a complex called fatty acid synthase.

- In plant and bacteria, FASII, a multi-subunit complex, is responsible for de novo fatty acid synthesis.
- In animals, some fungi, FA synthesis is carried out by FASI, a dimeric protein with all enzymatic activities for FA synthesis.
- Fatty acid biosynthesis is a four-step repeating cycle, extension by 2-carbons/cycle.
  1. Condensation;
  2. Reduction;
  3. Dehydration;
  4. Reduction.

FASII comprising 6 enzymes and 1 non-enzymic protein: acyl carrier protein (ACP)

Biochemistry & Molecular Biology of Plants, 2000
1.5 Fatty acid biosynthesis pathway
1.5 Fatty acid biosynthesis pathway

- Primary products of the FAS II are palmitic acid (16:0) and stearic acid (18:0).
- Modifications of this primary FA lead to other longer FA and unsaturated FA.
- FAD2 catalyzes the formation of linoleic acid from oleic acid. Decrease in FAD2 activity causes the accumulation of oleic acid, decrease of linoleic acid.
1.6 The discovery and breeding of HO-peanut

- Norden et al (1987) examined fatty acid of 500 peanut lines and identified 2 lines with higher oleic acid than other lines.
- 11 NT difference between FAD2A and FAD2B, one SNP at 432. HO-peanut has a “A” insertion at 442 in FAD2B, and a G to A transition at 448 in FAD2A.

<table>
<thead>
<tr>
<th>Lines</th>
<th>Oleic %</th>
<th>Linoleic %</th>
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</thead>
<tbody>
<tr>
<td>81206</td>
<td>63.21</td>
<td>15.28</td>
</tr>
<tr>
<td>76-4a-3-4</td>
<td>36.78</td>
<td>43.14</td>
</tr>
<tr>
<td>435-2-1</td>
<td>79.91</td>
<td>2.14</td>
</tr>
<tr>
<td>435-2-2</td>
<td>79.71</td>
<td>2.29</td>
</tr>
<tr>
<td>PI342664</td>
<td>79.0</td>
<td>-</td>
</tr>
<tr>
<td>PI342666</td>
<td>79.3</td>
<td>-</td>
</tr>
</tbody>
</table>

\[
\begin{array}{c|c|c|c}
\text{Lines} & \text{Oleic \%} & \text{Linoleic \%} \\
\hline
81206 & 63.21 & 15.28 \\
76-4a-3-4 & 36.78 & 43.14 \\
435-2-1 & 79.91 & 2.14 \\
435-2-2 & 79.71 & 2.29 \\
PI342664 & 79.0 & - \\
PI342666 & 79.3 & - \\
\end{array}
\]
HO-peanut breeding in USA and Australia

- After discovery of HO-peanut mutants, breeding programs carried out in USA, Australia and other countries.
- The first HO-peanut **SunOleic 95R** was registered in 1995, and now >50 HO-peanuts registered in USA.

- USDA Florida
- University of Florida
- Golden Peanut Company, LLC
- University of Georgia Research Foundation, Inc.
- Texas Agricultural Experiment Station
- Florida Agricultural Experiment Station
- North Carolina State University
- Florida Foundation Seed Producers, Inc.
- Texas AgriLife Research
- AgResearch Consultants, Inc.
- Kim M. Moore’s company
2. High-Oleic acid peanut in China

2.1 Discovery of high oleic acid peanut in China

- The earliest report on HO-peanut was in 1992 in China
- Cultivars name: Shiyouhong (♀ Shitouqi X Youguo ♂); Oleic acid: 75%
- Two parents of Shiyouhong are all conventional peanut cultivars

- 100 pod weight: 171 g
- 100 seed weight: 74 g
- Growth period: 120 days

Ren et al. (2011) Screening 576 core germplasms didn’t find high oleic peanut. Lines with 60%-67% oleic acid were identified.

Yu (2008), Hou (2016), Zhuang (2016) found FAD2 mutations in their mutant populations.


Wang (2015) found natural mutants from Jihua 4; the offsprings of a cross: rihua 1 x huayu 9618.
2.1 Discovery of high oleic acid peanut in China

When the first HO-peanut was identified before 1989, the importance of HO-peanut is not fully recognized.

- The major breeding goal is yield, not quality at that time.
- High oleic is better, or high linoleic is better? No answer at that time.
- High oleic trait was found not stable in different places.

### Oleic acid contents in Guangdong

<table>
<thead>
<tr>
<th>Year</th>
<th>Oleic acid</th>
<th>Linoleic acid</th>
<th>棕榈酸</th>
<th>硬脂酸</th>
<th>山嵛酸</th>
<th>花生酸</th>
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<tbody>
<tr>
<td>1989</td>
<td>74.04</td>
<td>10.91</td>
<td>7.16</td>
<td>1.96</td>
<td>3.01</td>
<td>1.48</td>
</tr>
<tr>
<td>1991</td>
<td>76.21</td>
<td>8.06</td>
<td>6.28</td>
<td>3.69</td>
<td>2.18</td>
<td>1.74</td>
</tr>
</tbody>
</table>

### Oleic acid contents in 3 other provinces

<table>
<thead>
<tr>
<th>Year</th>
<th>Wuhan Oleic acid</th>
<th>Wuhan Linoleic acid</th>
<th>Nanning Oleic acid</th>
<th>Nanning Linoleic acid</th>
<th>Laixi Oleic acid</th>
<th>Laixi Linoleic acid</th>
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<tbody>
<tr>
<td>1993</td>
<td>57.95</td>
<td>25.46</td>
<td>66.56</td>
<td>17.33</td>
<td>54.83</td>
<td>27.42</td>
</tr>
<tr>
<td>1994</td>
<td>64.68</td>
<td>18.60</td>
<td>69.24</td>
<td>14.10</td>
<td>65.46</td>
<td>17.79</td>
</tr>
</tbody>
</table>
2.2 Breeding programs and HO-peanut varieties

- HO-peanut breeding programs were started in 2000. Liaoning Jinzhou, Henan Kaifeng are the first institutes to design HO-Peanut breeding.

a) Introduction of OH-line (AT-201) and directly grow in China.

b) Development of HO-peanut by conventional methods, cross of two lines and phenotype selection.

Jinyin-01  

KN H03-3
2.2 Breeding programs and HO-peanut varieties

c) Marker Assisted Selection: Cross of two lines, genotype selection by MAS, validated by phenotype selection by measuring oleic acid content.
2.2 Breeding programs and HO-peanut varieties

Example of back cross and MAS breeding

Five research groups from universities and institutes carry out collaboration on OH-peanut breeding from 2013, supported by MARS.
Selection of male and female parent lines

The purpose of this program is to develop HO-peanut for different regions.

**Kainong 176**: a large seeded HO-peanut.
**DF12**: a medium seeded HO-peanut.
**Sunoeic 95R**: a small seeded HO-peanut.
They are with the same genotype as F435.

Female parents are elite cultivars from different provinces. For SAAS we used 5 varieties:

① Luhua 14 (100 pod wt: 274 g)
② Fenghua 1 (100 pod wt: 241 g)
③ Huayu 19 (100 pod wt: 251 g)
④ Huayu 23 (100 pod wt: 153 g)
⑤ Huayu 31 (100 pod wt: 220 g)
Maker development and genotyping

The similarity of $FAD2A$ and $FAD2B$ coding regions in peanut are 99% in nucleotide sequence. There are only 11 nucleotide difference between these two genes including one SNP at 432. High oleic peanut has a “A” insertion at position 442 in $FAD2B$. There is a G to A transition at position 448 in $FAD2A$. 

![Diagram showing the comparison of nucleotide sequences between AhFAD2A WT, AhFAD2A mutant, AhFAD2B WT, and AhFAD2B mutant]
Genotyping by CAPS maker

CAPS (Cleaved amplified polymorphic sequence): The insertion of A (441_442 insA) and a 448G>A transition resulted in changes of restriction sites, and different restrict pattern could be observed between WT and mutants.

- Seed DNA was extraction for PCR amplification.
- PCR products were digested using Hpy188I or Hpy991 and separated on agrose gel.
- Genotype analysis based on gel patterns.
Genotyping by AS-PCR marker

AS-PCR (Allele-specific PCR): specific primers were designed based on the mutations. One pair of primers could only amplify one genotype, can’t amplify the other. Two AS-PCR amplifications were required to distinguish the mutations.

DNA extraction ➔ AS-PCR ➔ M AABB Aa Bb aa bb

- 300bp PCR control
- 200bp WT primer
- 300bp PCR control
- 200bp G-A primer
- 300bp PCR control
- 200bp InsA primer
Genotyping FAD2 genes by KASP and other methods

- Genotyping FAD2 genes by KASP (Kompetitive Allele Specific PCR)
- Genotyping by direct sequencing.
- Genotyping by real time PCR.

The scatter plot with axes x and y represents allelic discrimination of FAD2A or FAD2B genotypes. The red, green and blue dots represent the mutant homozygous, heterozygous and wild-type homozygous.
Back cross breeding program

One year two generations strategy is followed for the breeding program:

Generation 1:
The beginning of May to end of September, in Shandong province;

Generation 2:
Beginning of November to end of March, in Sanya.

Phase I of the program is from 2013 to 2017.
Regular season cross and genotyping in Shandong Province

- Plant growth and pollination.
- Harvest the putative F1 seeds.
- Cut top of the cotyledons.
- DNA extraction and PCR amplification.
- Genotyping using different methods.
CAPS genotyping of KN176xHY31 putative F1 seeds

(a) Hpy99I digestion of PCR products genotyping FAD2A.
(b) Hpy188I digestion of PCR products genotyping FAD2B.
M: DL5000 DNA Marker.
Lane 1 and Lane 2 showed the genotypes of Huayu31 and Kainong176.
Lanes 3–22 showed the genotypes of 20 F1 putative seeds.

CAPS genotyping for small number of seeds
## CAPS Genotyping of different cross combinations in 2013

<table>
<thead>
<tr>
<th>Name of crosses</th>
<th>Cross combination</th>
<th>Seed harvested</th>
<th>Real F1 hybrids</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAAS-1</td>
<td>LH14 x KN176</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>SAAS-2</td>
<td>HY19 x KN176</td>
<td>27</td>
<td>3</td>
</tr>
<tr>
<td>SAAS-3</td>
<td>HY23 x DF12</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>SAAS-4</td>
<td>HY31 x DF12</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>SAAS-5</td>
<td>HY23 x KN176</td>
<td>27</td>
<td>15</td>
</tr>
<tr>
<td>SAAS-6</td>
<td>HY31 x KN176</td>
<td>38</td>
<td>15</td>
</tr>
<tr>
<td>SAAS-7</td>
<td>HY23 x Sunoleic</td>
<td>26</td>
<td>8</td>
</tr>
<tr>
<td>SAAS-8</td>
<td>HY31 x K17-15</td>
<td>40</td>
<td>29</td>
</tr>
<tr>
<td>SAAS-9</td>
<td>FH001 x KN17-15</td>
<td>30</td>
<td>11</td>
</tr>
<tr>
<td>SAAS-10</td>
<td>YH15 x KN176</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>SAAS-11</td>
<td>HY23 x KN17-15</td>
<td>24</td>
<td>7</td>
</tr>
</tbody>
</table>

F1 genotyping results in 2013
Off season back cross and genotyping in Sanya
Genotyping results of back cross seeds

检测基因型的结果，选出的材料的考种
Progress in 2017

BC$_4$F$_4$ lines planted in Sanya for BC$_4$F$_5$ seeds

<table>
<thead>
<tr>
<th>Cross combination</th>
<th>BC$_4$F$_4$ lines</th>
<th>Seeds number</th>
</tr>
</thead>
<tbody>
<tr>
<td>S3 (HY23 x DF12)</td>
<td>S3-1</td>
<td>2084</td>
</tr>
<tr>
<td></td>
<td>S3-3</td>
<td>1917</td>
</tr>
<tr>
<td></td>
<td>S3-5</td>
<td>608</td>
</tr>
<tr>
<td>S6(HY31 x KN176)</td>
<td>S6-17</td>
<td>1600</td>
</tr>
<tr>
<td></td>
<td>S6-11</td>
<td>1200</td>
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<td>S6-23</td>
<td>2000</td>
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<td>S6-63</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>S6-64</td>
<td>1000</td>
</tr>
<tr>
<td>S11 (HY23 x KN1715)</td>
<td>S11-103</td>
<td>1000</td>
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<td></td>
<td>S11-91</td>
<td>2000</td>
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</tbody>
</table>
Phenotyping by near infrared spectrophotometer

<table>
<thead>
<tr>
<th>Serial Number</th>
<th>Pod Number/500g</th>
<th>100-pods weight (g)</th>
<th>100-kernels weight (g)</th>
<th>Kernel production rate (%)</th>
<th>Plot pod yield (kg)</th>
<th>Oleic acid content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F7 S8-1-1-42</td>
<td>378</td>
<td>168.31</td>
<td>62.82</td>
<td>73.454</td>
<td>5.4</td>
<td>80.67</td>
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<tr>
<td>BC2F6 S6-41-100</td>
<td>368</td>
<td>173.24</td>
<td>64.99</td>
<td>68.888</td>
<td>6.72</td>
<td>76.59</td>
</tr>
<tr>
<td>F7 S5-27-21</td>
<td>300</td>
<td>210.29</td>
<td>76.37</td>
<td>66</td>
<td>5.34</td>
<td>75.14</td>
</tr>
<tr>
<td>BC2F6 S3-179-61</td>
<td>341</td>
<td>184.99</td>
<td>69.88</td>
<td>65.246</td>
<td>5.38</td>
<td>79.62</td>
</tr>
<tr>
<td>F7 S6-23-40</td>
<td>402</td>
<td>157.94</td>
<td>65.78</td>
<td>66.448</td>
<td>5.7</td>
<td>75.83</td>
</tr>
<tr>
<td>F7 S5-3-7</td>
<td>425</td>
<td>147.03</td>
<td>62.11</td>
<td>76.102</td>
<td>4.94</td>
<td>79.35</td>
</tr>
<tr>
<td>BC2F6 S3-117-40</td>
<td>406</td>
<td>169.22</td>
<td>58.91</td>
<td>64.134</td>
<td>5.22</td>
<td>76.92</td>
</tr>
<tr>
<td>BC2F6 S6-50-111</td>
<td>313</td>
<td>214.12</td>
<td>82.43</td>
<td>64.254</td>
<td>5.76</td>
<td>76.58</td>
</tr>
<tr>
<td>BC2F6 S3-183-64</td>
<td>336</td>
<td>192.3</td>
<td>77.13</td>
<td>68.12</td>
<td>5.2</td>
<td>82.87</td>
</tr>
<tr>
<td>F7 S5-22-53</td>
<td>353</td>
<td>191.44</td>
<td>67.08</td>
<td>69.394</td>
<td>5.14</td>
<td>79.37</td>
</tr>
<tr>
<td>BC2F6 S3-36-16</td>
<td>382</td>
<td>163.42</td>
<td>64.08</td>
<td>66.9</td>
<td>5.12</td>
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<tr>
<td>BC2F6 S3-182-63</td>
<td>311</td>
<td>189.81</td>
<td>78.27</td>
<td>67.684</td>
<td>4.9</td>
<td>76.6</td>
</tr>
<tr>
<td>BC2F6 S6-43-102</td>
<td>307</td>
<td>218.12</td>
<td>86.66</td>
<td>64.866</td>
<td>6.86</td>
<td>79.78</td>
</tr>
<tr>
<td>BC2F6 S6-52-2-115</td>
<td>324</td>
<td>210.54</td>
<td>92.85</td>
<td>67.85</td>
<td>6.02</td>
<td>79.45</td>
</tr>
<tr>
<td>F7 S6-22-37</td>
<td>356</td>
<td>196.13</td>
<td>81.42</td>
<td>65.23</td>
<td>5.48</td>
<td>87.83</td>
</tr>
<tr>
<td>BC2F6 S3-187-68</td>
<td>319</td>
<td>196.41</td>
<td>81.42</td>
<td>70.342</td>
<td>5.48</td>
<td>83.63</td>
</tr>
<tr>
<td>BC2F6 S3-179-60</td>
<td>321</td>
<td>193.3</td>
<td>78.51</td>
<td>67.41</td>
<td>5.14</td>
<td>85.86</td>
</tr>
<tr>
<td>BC2F6 S6-47-106</td>
<td>277</td>
<td>225.66</td>
<td>91.05</td>
<td>67.81</td>
<td>6.02</td>
<td>78.53</td>
</tr>
<tr>
<td>BC2F6 S6-36-98</td>
<td>304</td>
<td>213.54</td>
<td>84.14</td>
<td>65.114</td>
<td>5.78</td>
<td>80.92</td>
</tr>
<tr>
<td>UH14-17 2-2</td>
<td>390</td>
<td>149.4</td>
<td>62.66</td>
<td>70.47</td>
<td>5.3</td>
<td>78.84</td>
</tr>
</tbody>
</table>
Summary of the MAS breeding project

- Backcross 4 times to get BC4F1 (AaBb).
- BC4F1 selfing to get BC4F2--BC4F6 with aabb and AaBb.
- aabb plants are selected for seed multiplication phenotyping.
- 2013-2017: >20 lines were selected with >75% oleic acid. The other traits are similar to female parents. Some lines will join the registration program in 2019.
2.2 Breeding programs and HO-peanut varieties

There are about 40 HO-peanut varieties have been registered from different provinces in China. Shandong, Henan and Hebei registered more varieties than other provinces. In 2015, more varieties were registered than other years.
2.2 Breeding programs and HO-peanut varieties

The 100 pod weight of these HO-peanuts developed in China is >150 g (150-250 g). The 100 seed weight >60 (60-110 g). On average seeds of USA HO-peanut are smaller.
2.2 Breeding programs and HO-peanut varieties

- The maturation periods of HO-peanut developed in China are around 120-130 days.
- The HO-parents of the varieties are mostly from Kaixuan 016 and CTWE.

From 《High Oleic Acid Peanut》 by CT Wang, 2017
2.3 The growth of HO-peanut in China

- Breeders grow their varieties in different regions.
- Peanut processing companies grow HO-peanut in their production stations.
- The government directed growing and application of HO-peanut.
2.3 The growth of HO-peanut in China

Companies grow HO-Peanuts
Luhua Group grows HO-peanut from 2013.
2016, 30,000 Mu;  2017, 40,000 Mu;  2018, 60,000 Mu
2019, 120,000 Mu

- Luhua grows HO-peanut in Shandong, Henan, Hebei provinces through small companies and farmers.
- HO-peanut is given higher price than common peanut, 0.5 yuan/kg.
- Luhua has a processing company for HO oil production.
2.3 The growth of HO-peanut in China

Government directed growth and application of HO-peanuts

- A group for demonstration and growth of HO-peanuts.
- This group started in 2016.
- It includes 36 research institutions, universities, companies and local government agents.
Government directed growth and application of HO-peanuts

<table>
<thead>
<tr>
<th>Provinces</th>
<th>Regions</th>
<th>Regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hebei Province</td>
<td>Shi Jia Zhuang</td>
<td>Hebei Agr. University</td>
</tr>
<tr>
<td></td>
<td>Ruan Xian</td>
<td>Baixin Peanuy Growing Company</td>
</tr>
<tr>
<td>Shandong</td>
<td>Yi Nan</td>
<td>Wolong Seed Company</td>
</tr>
<tr>
<td>Province</td>
<td>Lai Xi</td>
<td>Shandong Peanut Research Institute</td>
</tr>
<tr>
<td>Henan Province</td>
<td>Zheng Yang</td>
<td>Zhumadian Agr. Research Institute</td>
</tr>
<tr>
<td></td>
<td>Kai Feng</td>
<td>Kaifeng Agr. For. Academy</td>
</tr>
<tr>
<td>Liaoning</td>
<td>Xing Cheng</td>
<td>Xincheng Agr. Tech Extension</td>
</tr>
<tr>
<td>Province</td>
<td>Fu Xin</td>
<td>Institute of Liaoning Agri. Academy</td>
</tr>
</tbody>
</table>

2016, 18 varieties  
2017, 24 varieties  
2018, 22 varieties  
Each variety grown in these 8 places for two years.
## 2.3 The growth of HO-peanut in China

<table>
<thead>
<tr>
<th>Province</th>
<th>Total Area (Mu/667 m²)</th>
<th>HO-peanut varieties</th>
<th>Yield (kg)</th>
<th>Major regions</th>
<th>Company involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hebei</td>
<td>200,000</td>
<td>Jihua11,13,16,18,19, Jinonghua 6,8,10</td>
<td></td>
<td>Tangshan Baoding Shijiazhuang Xingtai, Handan, Ruanxian</td>
<td>Baixin cooperation; Tianshen Liangyou;</td>
</tr>
<tr>
<td>Liaoning</td>
<td>10,000</td>
<td>Huayu 51, 52, 661, 662; Jihua 11, 16</td>
<td>244-388</td>
<td>Fuxin, Huludao Dalian, Shenyang, Xingcheng</td>
<td></td>
</tr>
<tr>
<td>Shandong</td>
<td>170,000</td>
<td>Huayu 662, 51, 962, 951, Jihua 13, 16</td>
<td></td>
<td>Yantai, Linyi, Weifang, Rizhao, Liaocheng, Laixi</td>
<td>Luhua Group Jinsheng Liangyou</td>
</tr>
<tr>
<td>Henan</td>
<td>200,000</td>
<td>Kainong series Yuhua series</td>
<td>384</td>
<td>19 demonstration area</td>
<td>Qihua Oil Company</td>
</tr>
<tr>
<td>Sichuan</td>
<td>1000</td>
<td>Shandong, Henan, Hebei varieties</td>
<td></td>
<td>Mianyang Nanchun Chengdu, Yibin</td>
<td></td>
</tr>
</tbody>
</table>

To the end of 2017, total HO-peanut growth area is 2.2 million Mu.
2.4 HO-peanut products in China

The only product of HO-peanut available in the market is high oleic oil. From several peanut processing companies in Henan and Shandong provinces.

The general price of HO-peanut oil is much higher than the common peanut oil. HO-peanut oil is 118 yuan/L, while common peanut oil is about 26-29 yuan/L.
### 3. Advantages and Concerns

#### 3.1 HO-peanut oil is more stable

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Common peanut %</th>
<th>HO-peanut %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid (棕榈酸)</td>
<td>9.98</td>
<td>6.01</td>
</tr>
<tr>
<td>stearic acid （硬脂酸）</td>
<td>4.40</td>
<td>3.13</td>
</tr>
<tr>
<td>Oleic acid （油酸）</td>
<td>53.81</td>
<td>81.84</td>
</tr>
<tr>
<td>Linoleic acid （亚油酸）</td>
<td>25.01</td>
<td>4.10</td>
</tr>
<tr>
<td>arachidic acid （花生酸）</td>
<td>2.04</td>
<td>1.00</td>
</tr>
<tr>
<td>Peanut monoenoic acid （花生一烯酸）</td>
<td>1.14</td>
<td>1.39</td>
</tr>
<tr>
<td>Behenic Acid （山嵛酸）</td>
<td>3.61</td>
<td>2.54</td>
</tr>
<tr>
<td>O/L</td>
<td>2.15</td>
<td>19.96</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Different peanut</th>
<th>氧化诱导期（h）</th>
<th>Shelf-life （y）</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common peanut</td>
<td>11.7</td>
<td>0.89</td>
</tr>
<tr>
<td>HO-peanut</td>
<td>38.97</td>
<td>4.98</td>
</tr>
</tbody>
</table>

Chang Zheng, 2014
Oxidative stability was found to be 3.4 to 14.5 times greater for high-O/L peanut oil.

Lipid oxidation is potentially a significant problem as peanuts contain 50% oil.

Approximately 95% of this oil is in the form of TAG. The eight major FA constituting the TAG in peanut oil are palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), arachidic (20:0), eicosenoic (20:1), behenic (22:0), and lignoceric (24:0)
3.2 The disease resistance and cold resistance

- Disease seriously affect peanut production worldwide. For example, leaf spot disease causes premature leaf dropping, reduce photosynthesis, and eventually reduce yield.
- HO-peanuts developed from the mutants and other common peanuts by back cross or traditional selection. No novel disease resistant genes added to HO-peanut varieties.
- Improve disease resistant of HO-peanut is highly desirable.

- Cold resistance of HO-peanut is another concern. Low temperature during sowing and harvest time may cause low germination rate.
3.3 Yield and purity of HO-peonuts

Low yield is a concern for HO-peonuts

● During regional testing of 12 HO-peonut varieties, yield ranged from 194-242 kg/mu (Wang et al., 2016).


How to keep seed purity?

● Grow in an isolated place, only HO-peonuts are grow.

● Grown by experienced seed companies.

● Strict management during sowing, harvest and storage.
3.4 Essential Fatty Acids

Mammals lack the enzymes to introduce double bonds at the carbon atoms beyond C9. All fatty acids containing a double bond at positions beyond C9 have to be supplied in the diet: Essential Fatty Acids (EFA).

Linoleic acid (18:2; C9,12) (亚油酸)
Linolenic acid (18:3; C9,12,15) (亚麻酸)

Arachidonic acid (20:4; C5,8,11,14) (花生酸四烯酸)

Eicosanoids (类花生酸，类二十烷酸)
Prostaglandins (前列腺素类)
Thromboxanes (凝血噁烷)
Leukotriens (白三烯)

Two EFA derived from plant diet.

More unsturated fatty acid from Linoleic acid and Linolenic acid

Eicosanoids are derivatives of arachidonic acid and have hormonal and signaling properties, showing key functions in human reproductive, cardiovascular, respiratory and neural system.
Acknowledgement

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East Carolina University
University of California
Indiana University Bloomington
Thank you very much!
1. Background
   (1) China peanut growth area, yield, quality, the traditional peanut O/L ratio is low to compare with USA.
   (2) Fatty acid biosynthesis: places, enzymes and regulation.
   (3) FAD2A and FAD2B is the key enzymes for oleic acid accumulation.
   (4) The mutation of these two genes increase the oleic acid content in peanut.
   (5) The studies of high oleic acid in other crops
   (6) High oleic acid in USA and Australia

2. High oleic acid peanut breeding in China
   (1) the early stage of high oleic acid peanut development and awards
   (2) Strong interest for high oleic acid peanut development—many institutions involved in and Mars’ promotion.
   (3) Marker assisted breeding: method and different marker development
   (4) large number of high oleic acid peanut have been registered
   (5) promotion of the high oleic acid peanut by Ministry of Agriculture

3. Concerns
   (1) linolenic acid is the Essential Fatty Acids
   (2) the cold resistance
   (3) the price