The Rapeseed Industry and Research Focus in China

Presentation on the Canola Industry Meeting at Saskatoon, Canada, Dec 5, 2017

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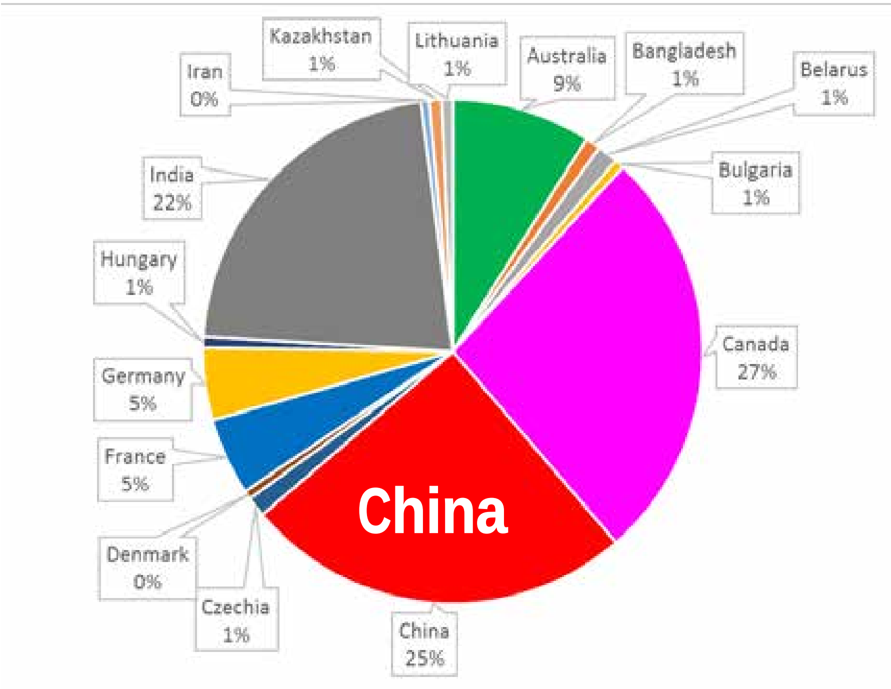
The proportion of China rapeseed production of the world

Data source: FAO, UN (http://www.fao.org/faostat/en/)

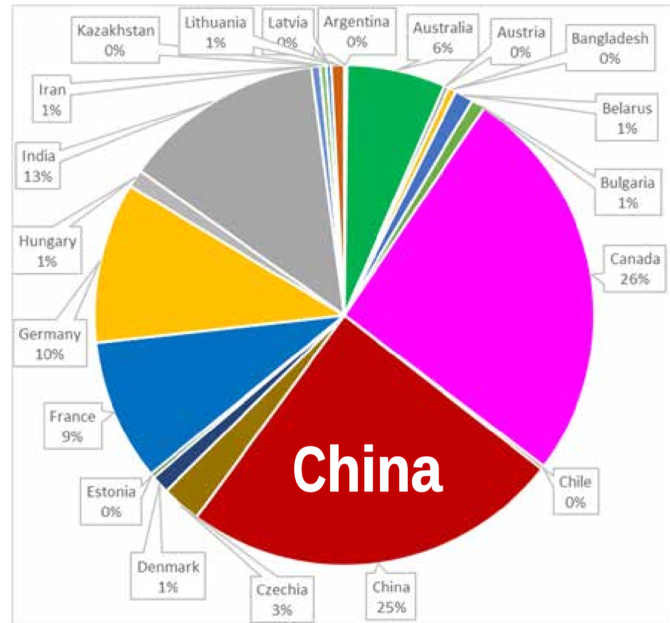
Harvested Area 2015



Canada 27%, China 25%, India 22%,

Australia 9%, Germany 5%, France 5%

Total production 2015



Canada 26%, China 25%, India 13%,

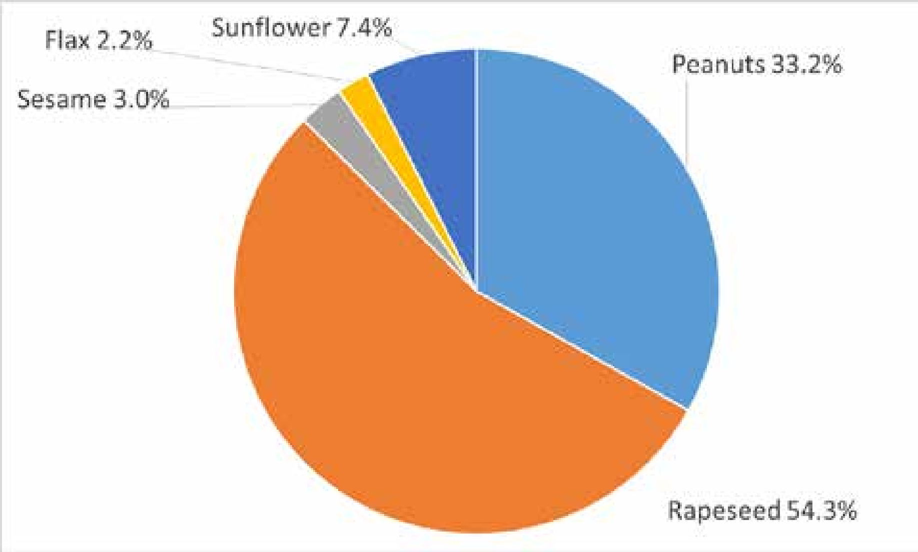
Australia 6%, Germany 10%, France 9%

China is one of the most important countries for rapeseed production. If we look at the data 2015, China accounts for 25% of the world rapeseed harvested area as well as the total rapeseed production. EU, Canada and China together accounts for more than 75% of the world total rapeseed production. Australia, India and other countries share the rest 25%.

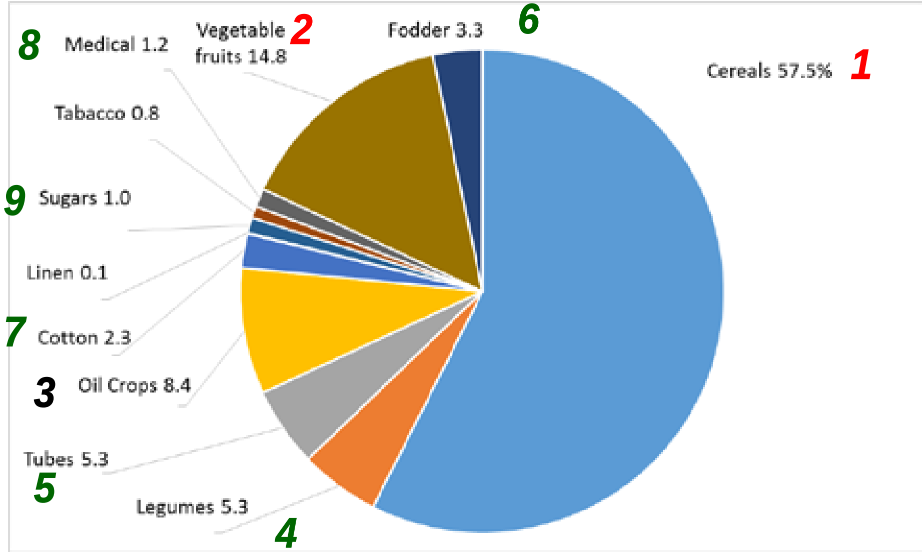
The ratio of rapeseed planting area in China

Data source: http://www.Chinabgao.gov.cn

The ratio of rapeseed in oil crops



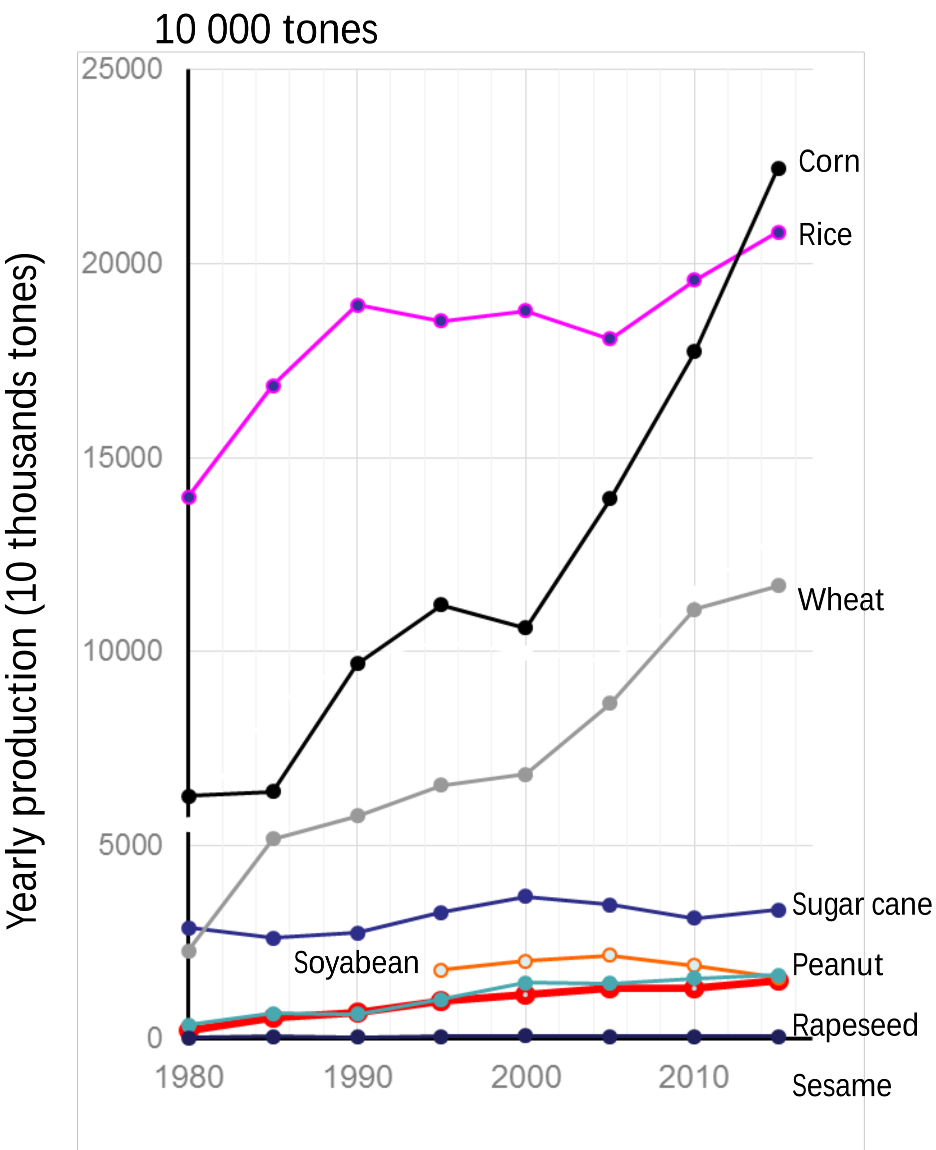
The ratio of oil crops in all major field crops

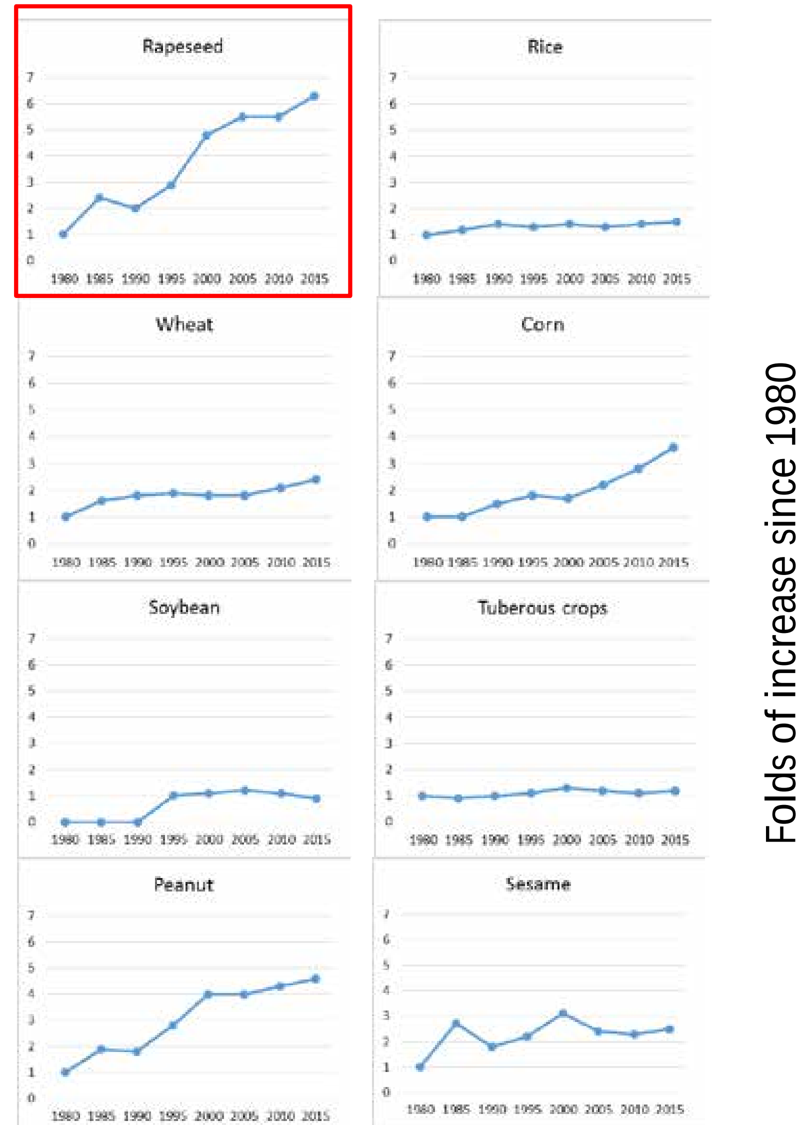


Data of the year 2016

Rapeseed is the most important source of plant oil in China. Rapeseed planting area takes up 54% of the total planting area of oil crops including peanuts , sunflowers, 'Sesame and Flax . Taken together, oil crops accounts for 8.4% of the total planting area among the major field crops, ranked before legumes, tubes, fodders, cotton, medical plants, sugars, and tobacco, but behind cereals, vegetables and fruits.

The increase of rapeseed production since 1980



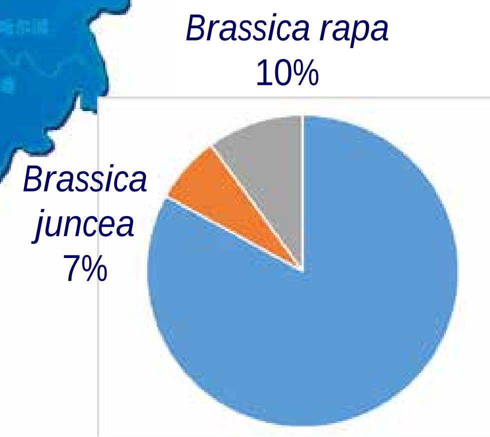


Among the major field crops in China, the total production of rapeseed is relatively small in comparison with those of corn, rice, wheat, sugarcane, and even soybean and peanuts. However, in the past 37 years, rapeseed is the crop which has the largest growth rate in terms of total production.

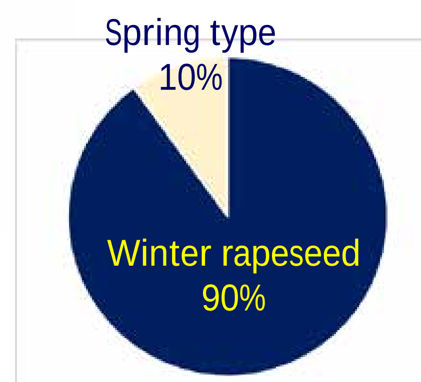
The distribution of rapeseed production areas in China

Data source: http://www.Chinabgao.gov.cn

Rapeseed is mainly grown in the Yangtze-River-Valley as a winter crop. So winter rapeseed takes up 90% of total production, and spring rapeseed which is grown in Qing-Hai High Plateau, in Xin-Jiang, Mongolia and northeast China, accounts for 10%.

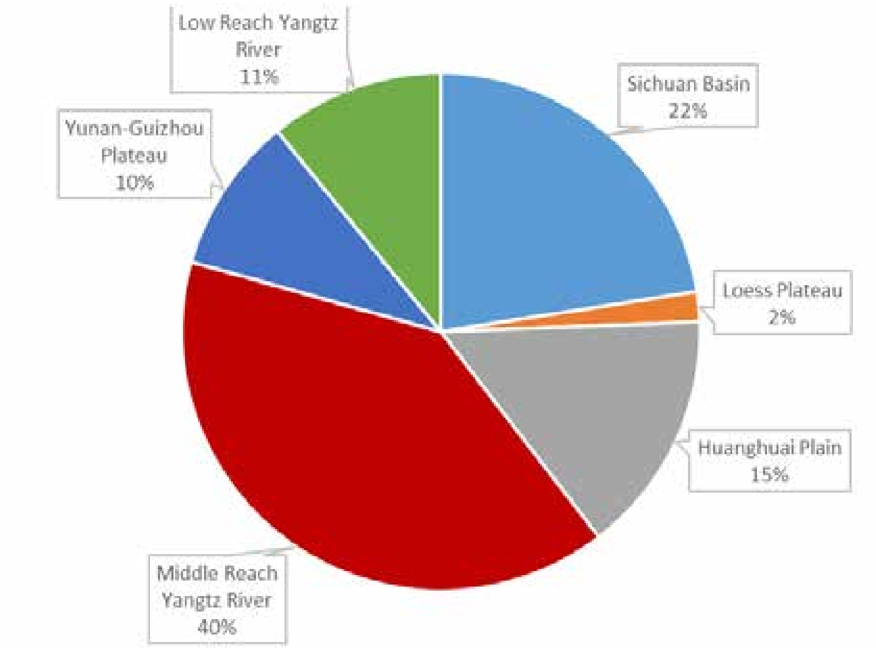


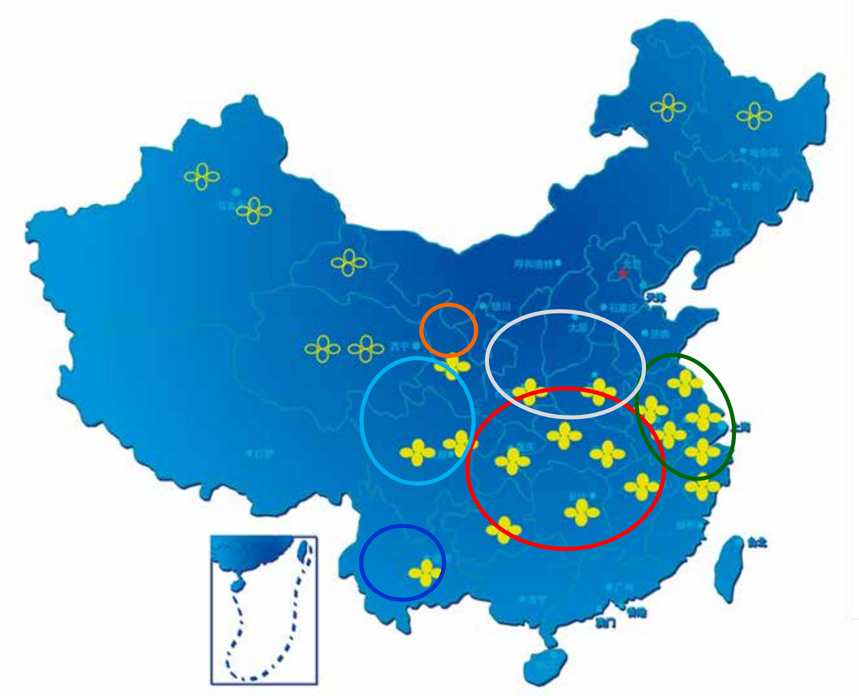
83% of rapeseed in China is *Brassica napu*, 10% is *Brassica rapa*, 7% is *Brassica juncea*.



The major rapeseed production areas in China

Data source: National Bureau of statistics (http://www.stats.gov.cn/)

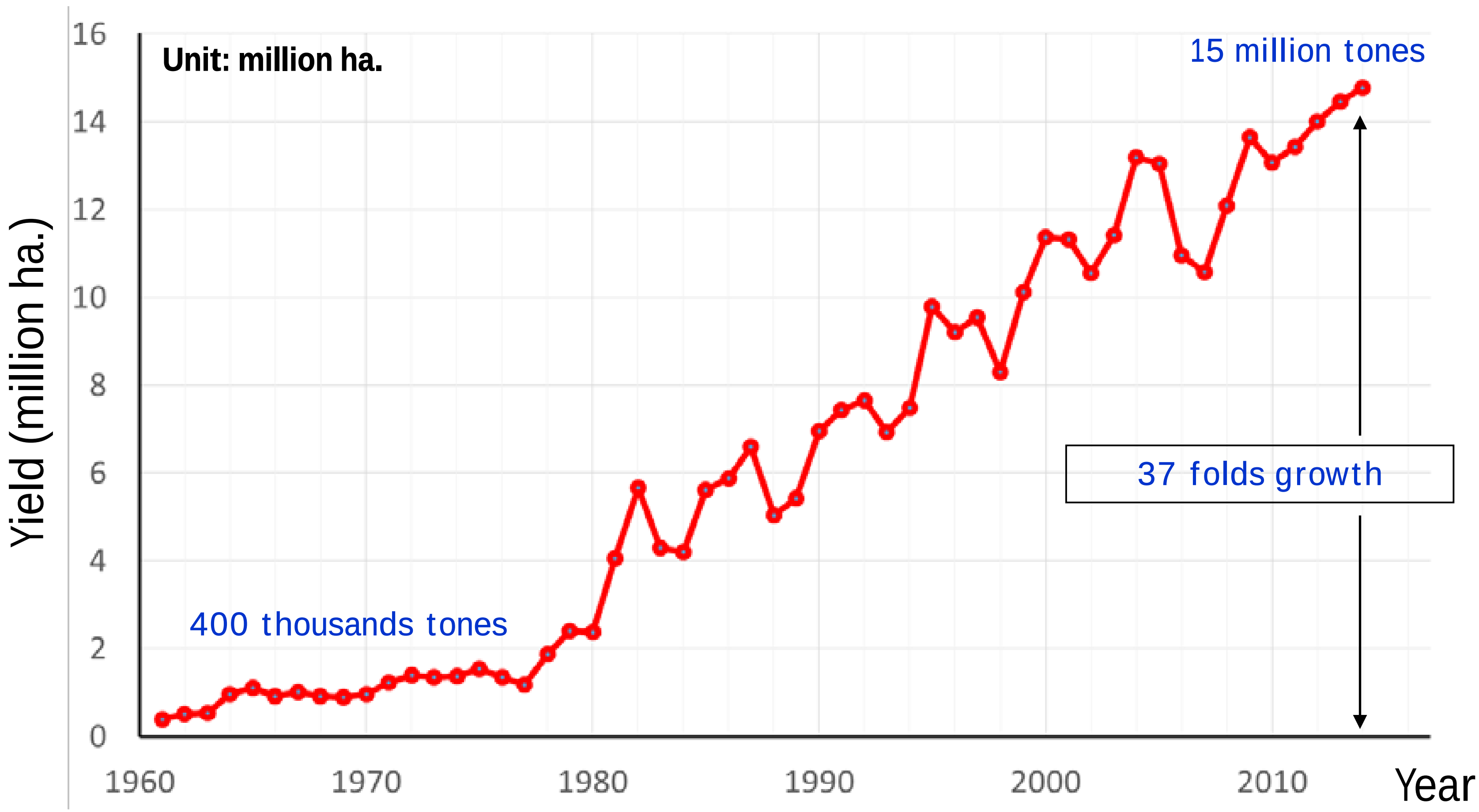




Geographically, we can divide the main Chinese rapeseed production area in six parts. The biggest one is middle reach of Yangtze-Valley, followed by Sichuan Basin, low reach of Yangtze-Valley, Huang-Huai River Plain, Yunan-Guizhou High Plateau, and Loess Plateau.

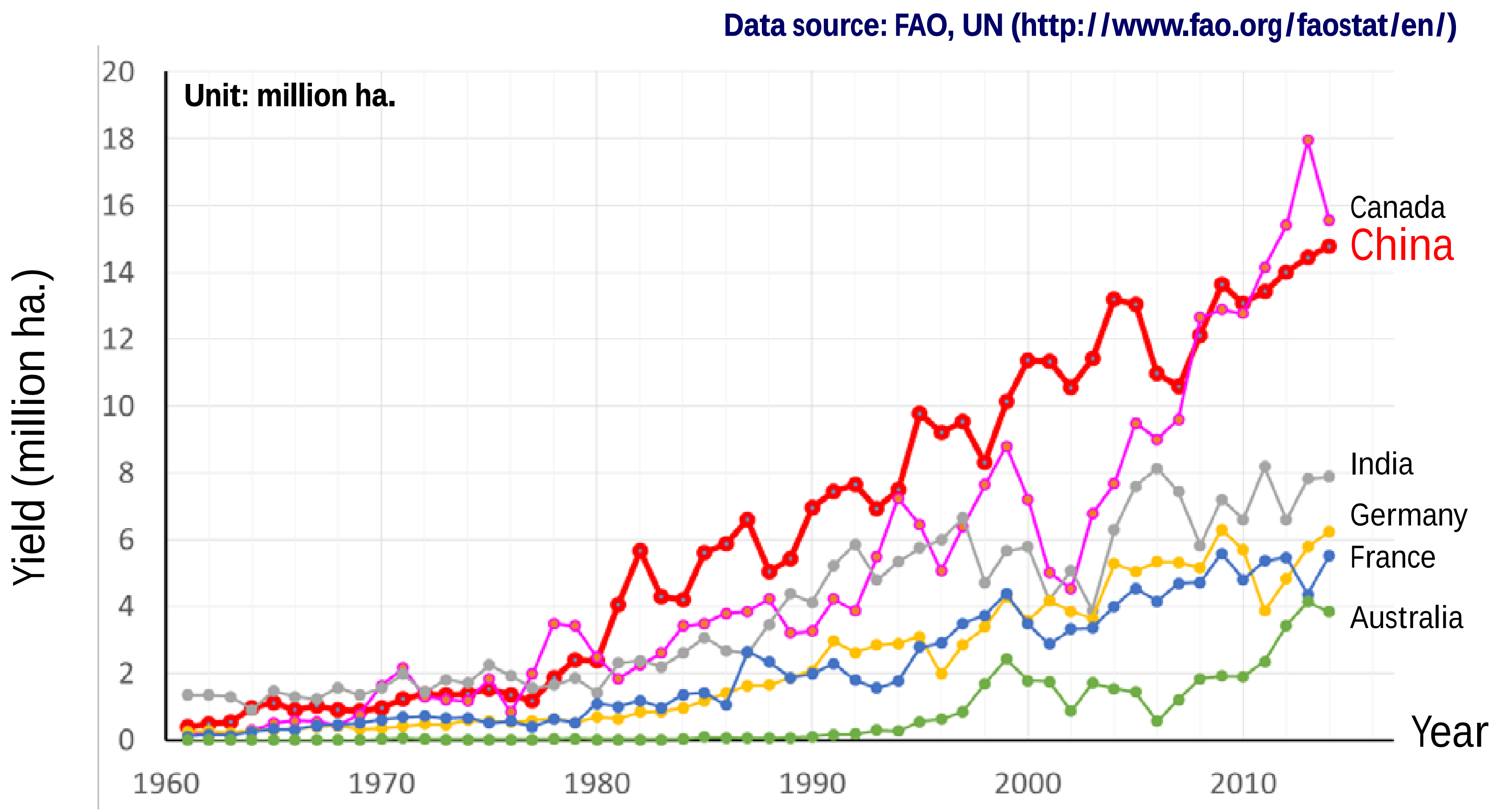
The increase of rapeseed total production in China since 1961

Data source: FAO, UN (http://www.fao.org/faostat/en/)



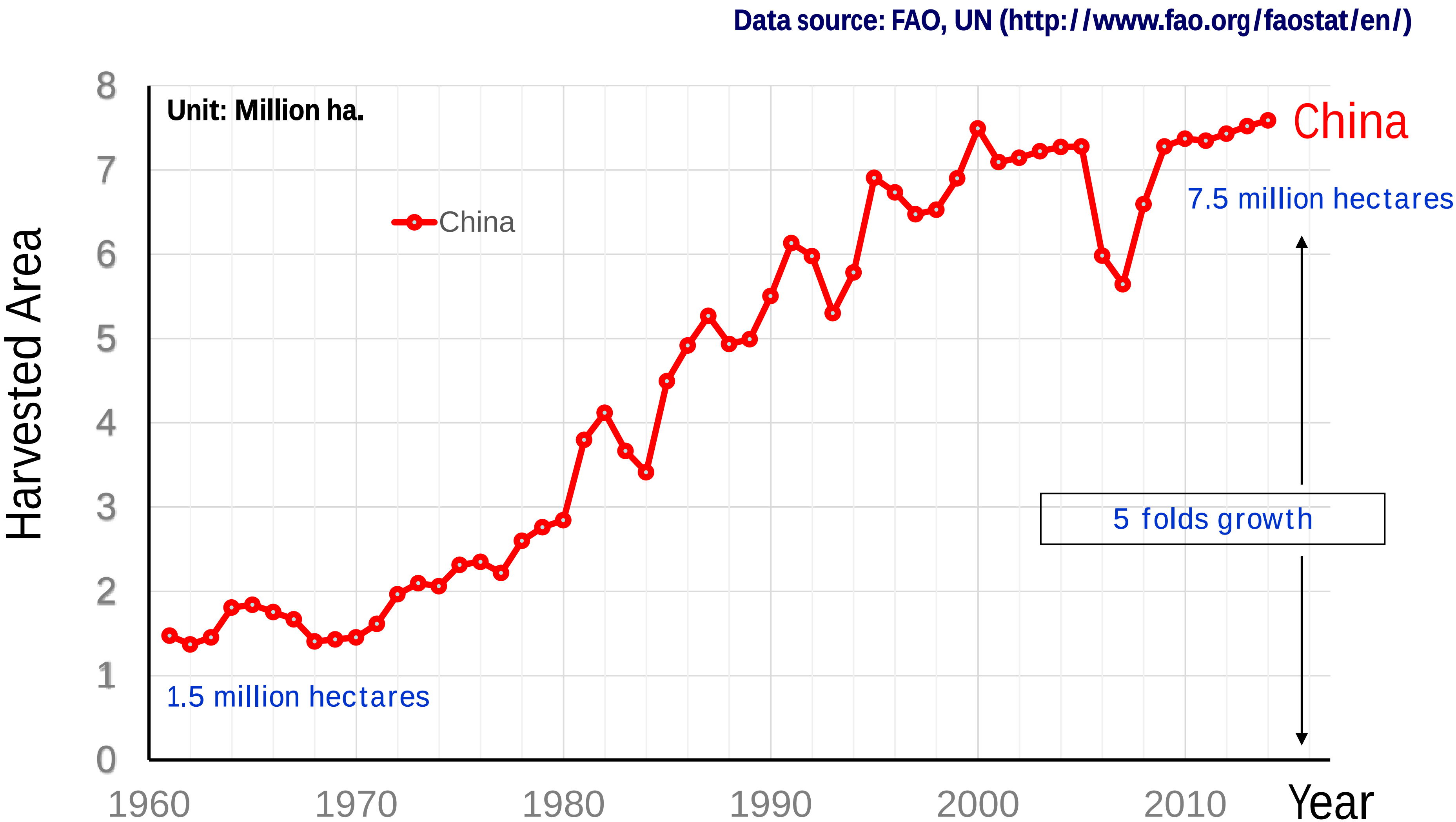
China went through a drastic increase of rapeseed production in the past half a century, from nearly 400 thousand tones in 1960 to 15 million tons in 2015, which is a 37-folds increase.

The increase of rapeseed production in China and some other countries



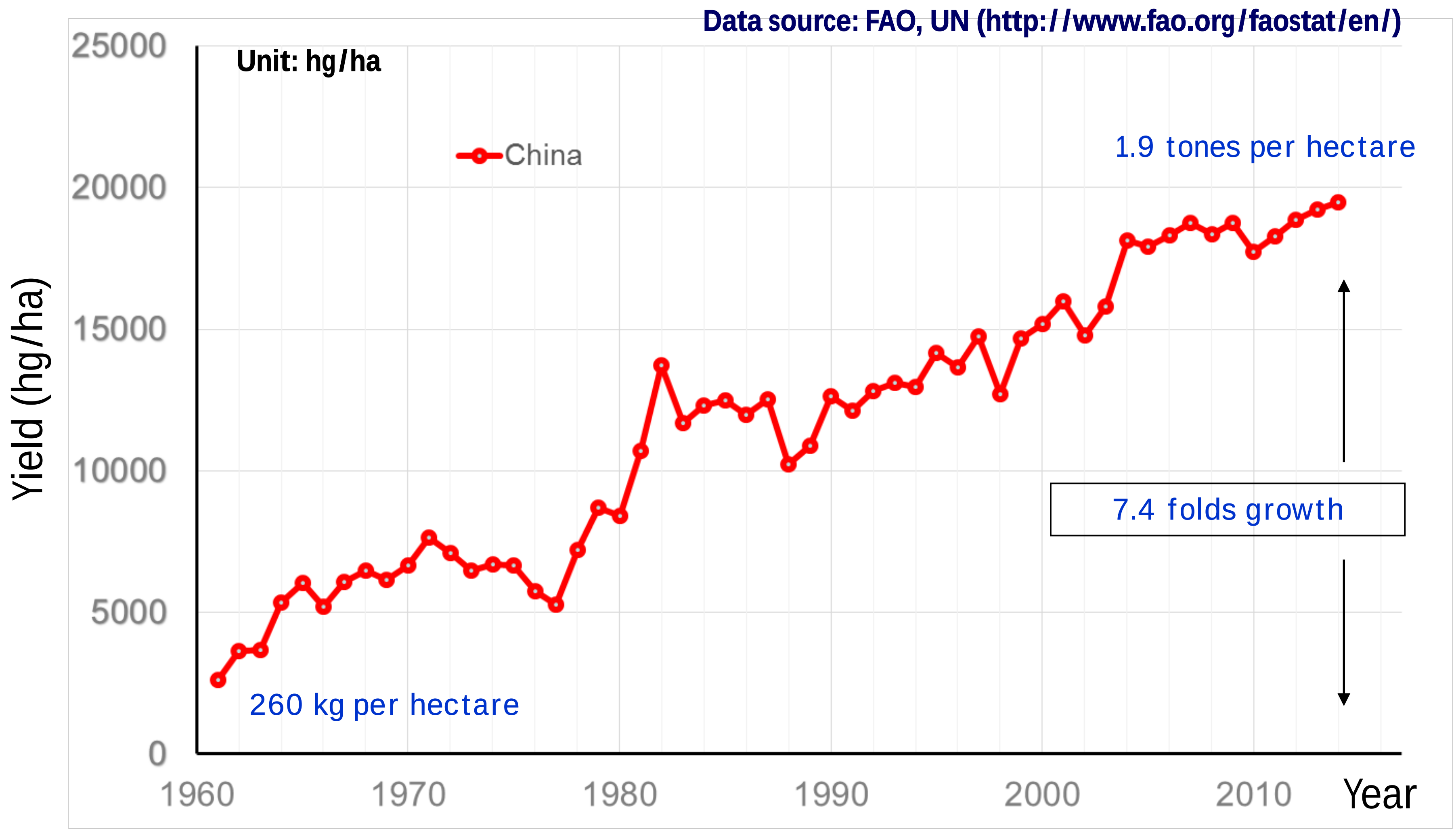
However, if we look at the world outside China, the total rapeseed production in Canada increased with even a more remarkable growth rate in the past half a century, especially in the recent 10 years.

The expansion rapeseed planting area in China since 1961



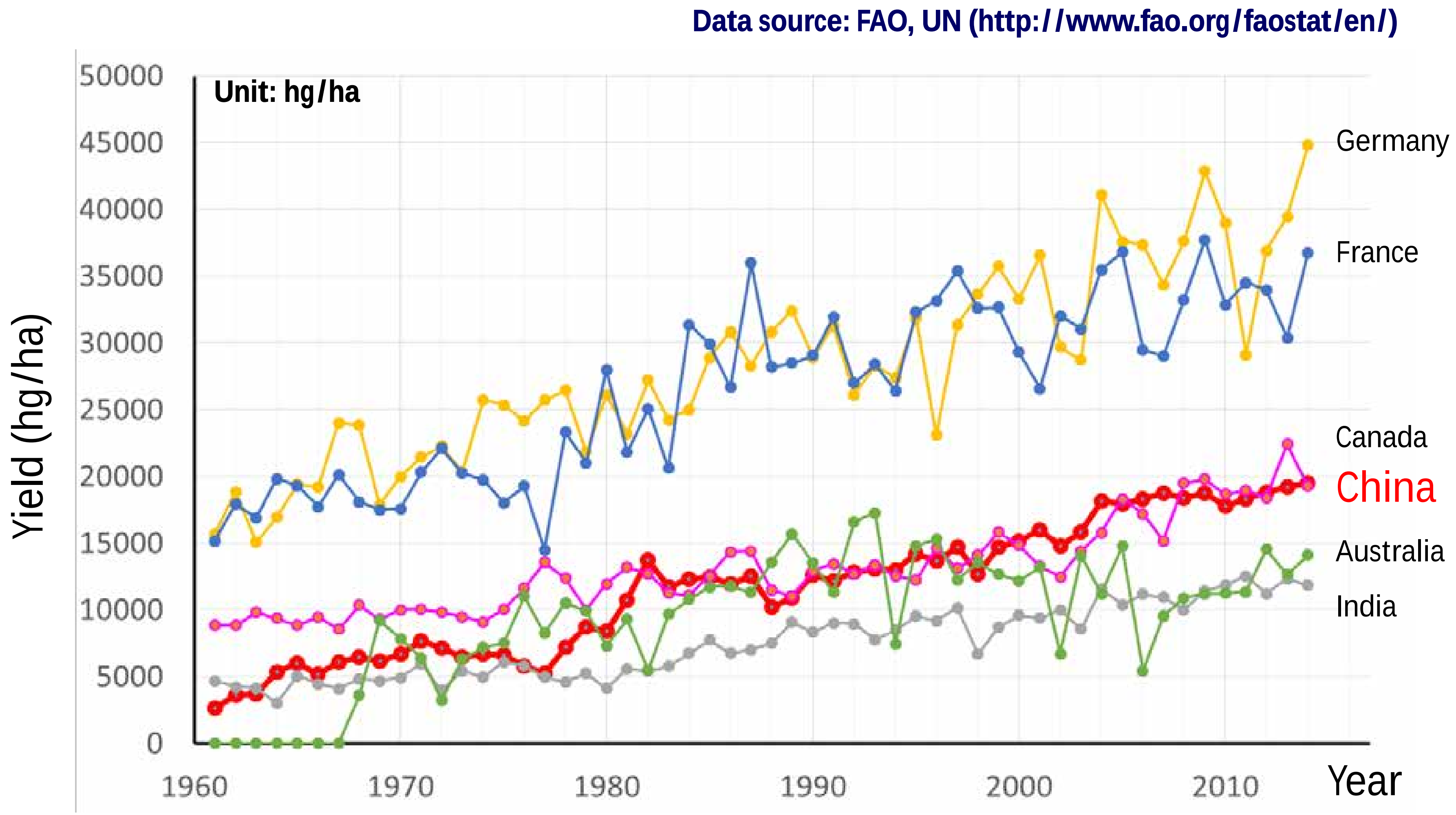
The increase of rapeseed production in China was partly due to the expansion of planting area, from 1.5 million hectares in the year 1961 to 7.5 million hectares in 2015, which was a 5-folds growth.

The increases of rapeseed yield in China since 1961



But also, thanks to the improvement of yield, which was from only 260 kg per hectare in 1961 to the current level of about 1.9 tons per hectare.

The increase of rapeseed yield in China and some other countries



However, the yield level of Chinese winter rapeseed is only 42% that of German winter rapeseed, and 54% that of French winter rapeseed, but better than the yield levels in Australia and India.

Technical reasons attributed to the increase of rapeseed production in China

- The replacement of *Brassica rapa* with *Brassica napus* in the 1950s
- The breeding of *Brassica napu* cultivars with early maturity and semi-dwarfism type in 60-70s
- The successful development of F1 hybrids in70-80s, and
- The improvement of seed quality starting from the beginning of 1980s.

The drastic increase of rapeseed production in China attributed to many reasons including political reasons, climate reason and technical reasons. **Politically,** the economic reform especially the change from commune system to family-contract system in China starting from late 70s, greatly inspired the motivation of the farmers. **Meteoro'logically**, the global warming starting in the 1980s, in my opinion, was favorite to crop growth. And the technical reasons include….

The three cropping system in Yangtze-River Region of China

Above all, early maturity and short life cycle is essential for the cropping system in Yangtze-Valley rapeseed production area.

45 D

Rice Nursery
Mar 10 – April 25

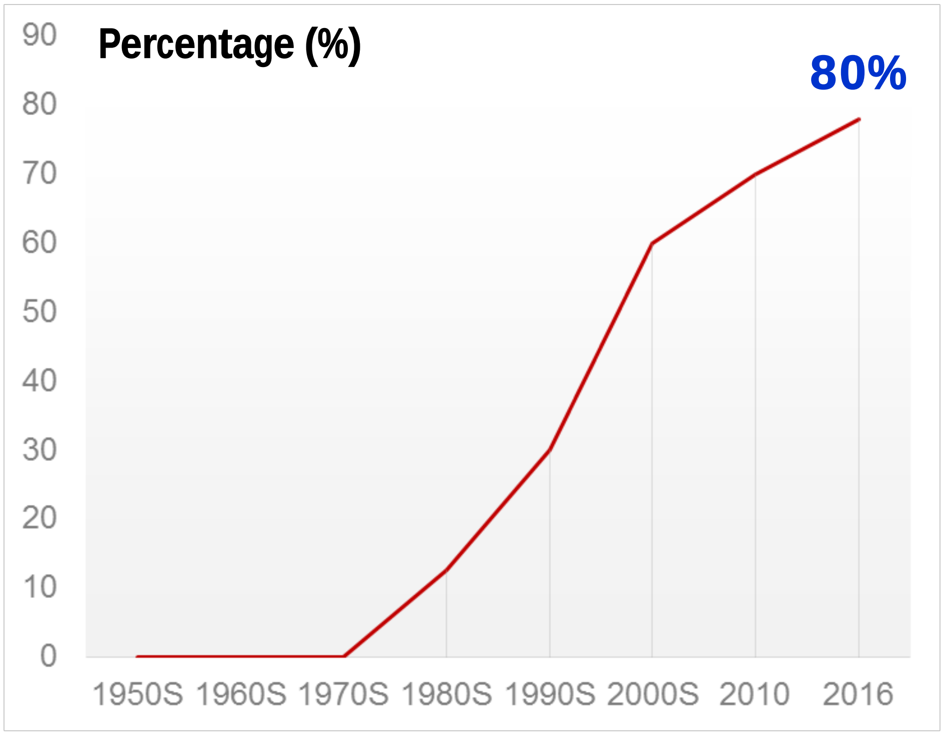
Rapeseed	1st Rice	2nd Rice
Nov 15 – May 30	April 20 – July 25	August 1 – Nov 15
165 D	95 D	105 D

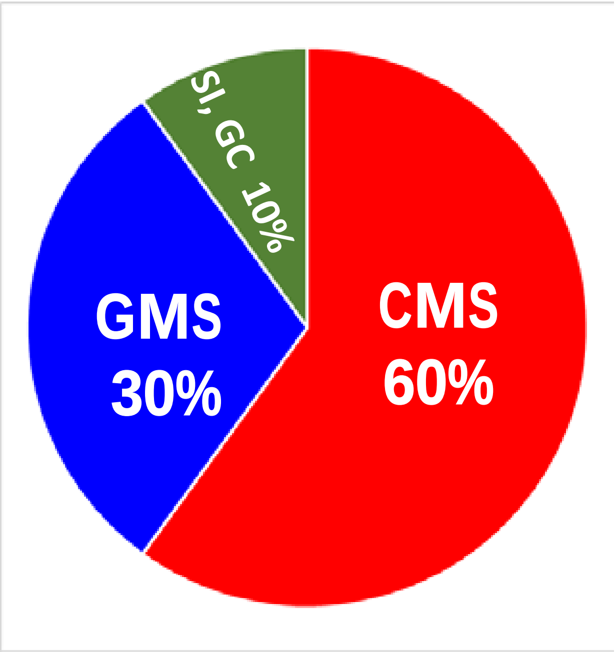
Rapeseed Nursery
Oct 1 – Nov 15

45 D

The utilization of heterosis of rapeseed in China

Data source: http://www.Chinabgao.gov.cn



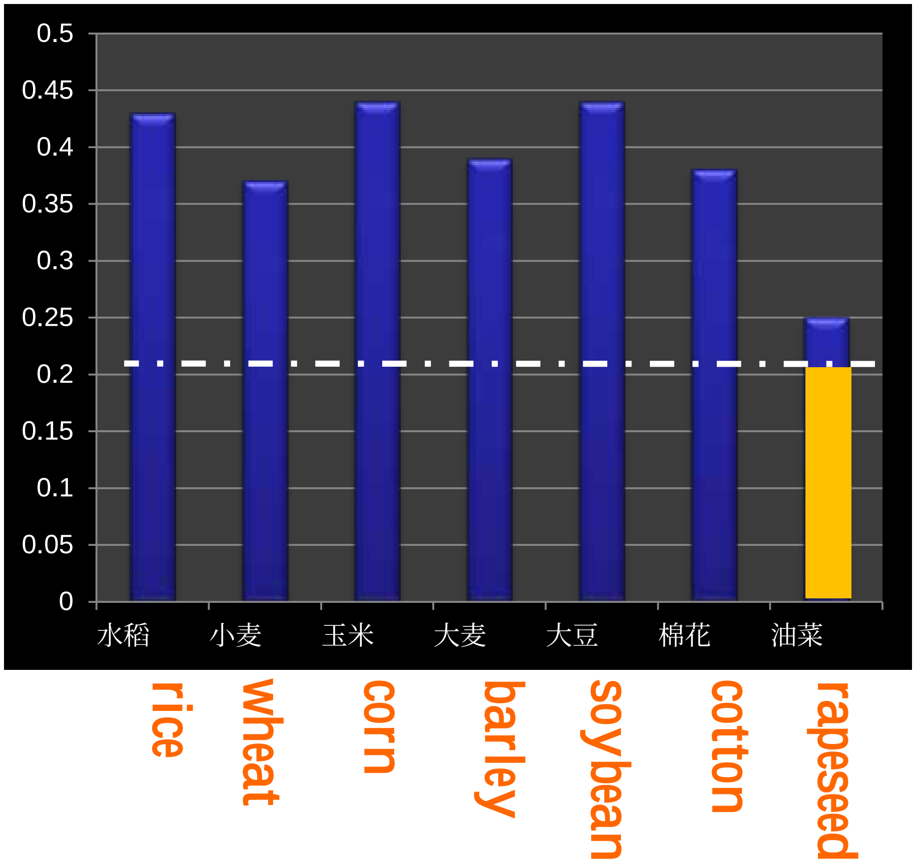


China is the first country that successfully utilized the rapeseed heterosis. The first commercial rapeseed hybrid, Qin-You 2, was released in 1985. Today, more than 80% rapeseed planting area is covered by hybrids. CMS, GMS and SI or other systems accounts for 90%, 30% and 10% of total hybrids, respectively.

To increase harvest index is essential for hybrid development

Yan et al., 2017, data unpublished

The harvest index of major field crops



Average heterosis of 50 F1 hybrid lines

- Heterosis for biomass: ~ 32%
- Heterosis for seed yield: ~ 6.5%
  - Pods per plant: ~ 75.4%
  - Seeds per pod: ~ 16.6%
  - TSW: ~ 1.5%
- Heterosis for seed oil content: ~2.7%
- Heterosis for protein content: ~5.8%

The potential of hybrid development is to increase the harvest index. Among the major field crops, rapeseed featured with the lowest HI, which is only about 0.25. Rapeseed hybrids have even a smaller HI.

We examined the heterosis of 50 hybrid lines last summer, and found that .....

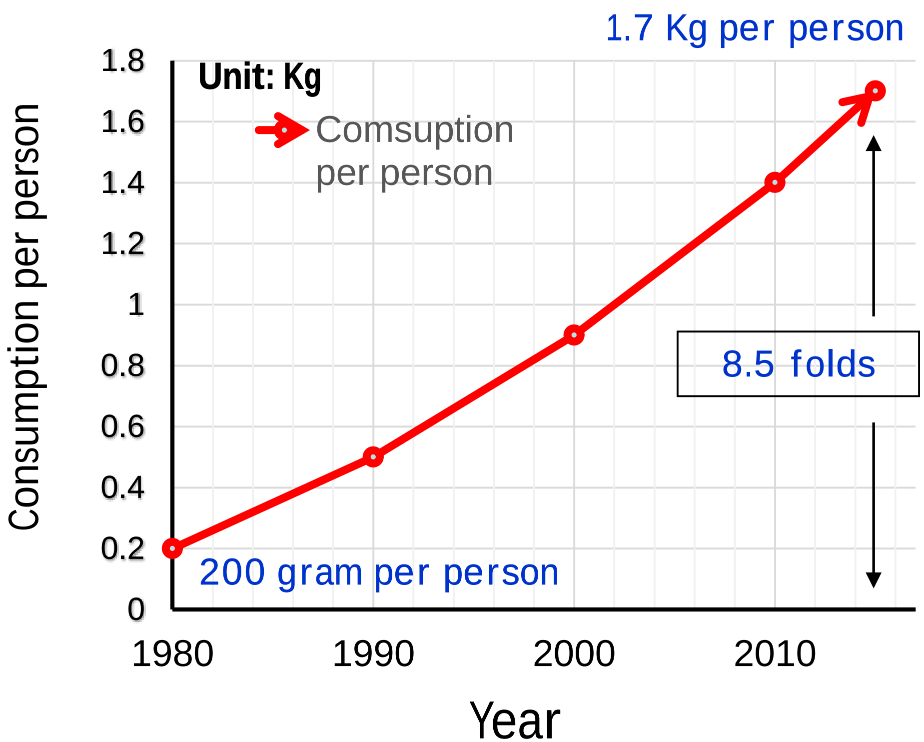
The technical reasons attributed to the increase of rapeseed production in China

- The replacement of *Brassica rapa* with *Brassica napus* in the 1950s
- The breeding of *Brassica napu* cultivars with early maturity and semi-dwarfism type in 60-70s
- The successful development of F1 hybrids in70-80s, and
- The introduction of Canola type from Canada and Europe in 1980s.

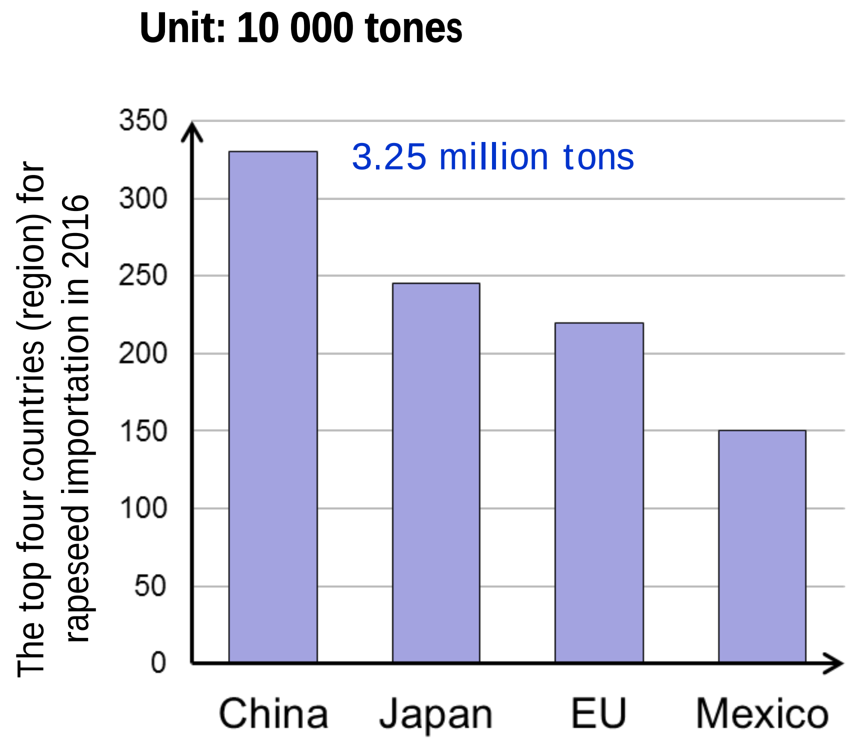
The breeding for double-low seed quality started in China at the beginning of 1980s. Canada helped us a lot in this process. At least five provincial institutions received aid-funds for double-low quality breeding from Canada in the 1980s. At the turn-of-the-century, most of Chinese rapeseed cultivars became double-low double-low seed quality.

The increase of personal consumption of rapeseed oil in China since 1980

Data source: FAO, UN (http://www.fao.org/faostat/en/)



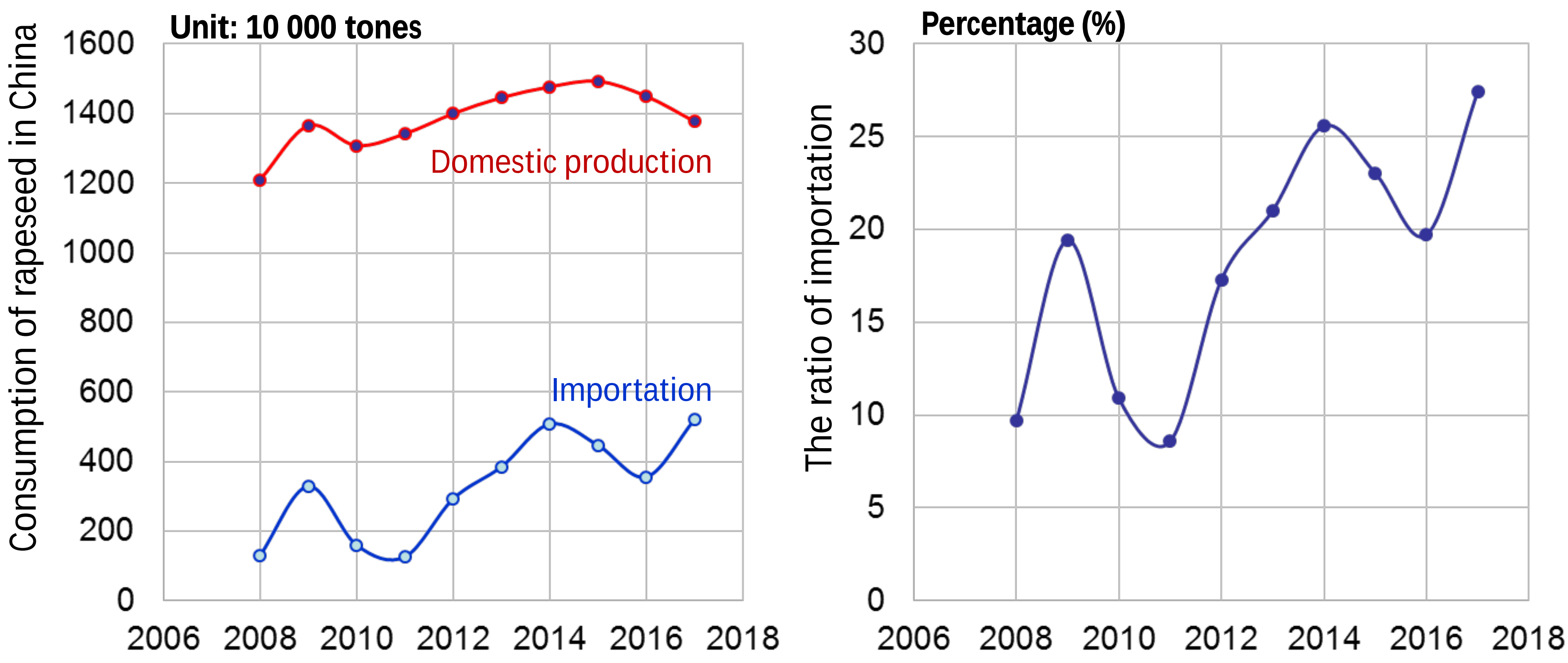
The improvement of people's life China led to a significant increase of edible oil consumption from 200 grams per person at 1980 to 1.7 KG per person at present, which is 8.5 folds increase.



The increase of consumption makes China the No. 1 country for rapeseed importation, with an annual importation of about 3.5 million tons. Most importation is from Canada.

The ratio of imported rapeseed in China

Data source: National Bureau of statistics (http://www.stats.gov.cn/)



The proportion of importation increased significantly in the last 10 years, with a big fluctuation between different years though, by contrast, the domestic rapeseed production in China declined in the recent four years, especially in the year 2017.

Reasons for the low competitiveness of domestic rapeseed

- Lower yield and high manpower cost arise from:
  - The sensitiveness of early mature cultivars to diseases and frost damage;
  - The difficulty of ‘mechanized production of rapeseed in paddy field.

- Bad seed quality arises from:
  - The rapid rising temperature in April sometimes late March that results in lower seed oil content.
  - Short life cycle adapting to the three- or two- cropping system in the Yantze-River rapeseed production area
  - The dilemma of heterosis and high seed quality (*erucic acid : two recessive genes; glucosinolate: three recessive genes*)

Paddy fields

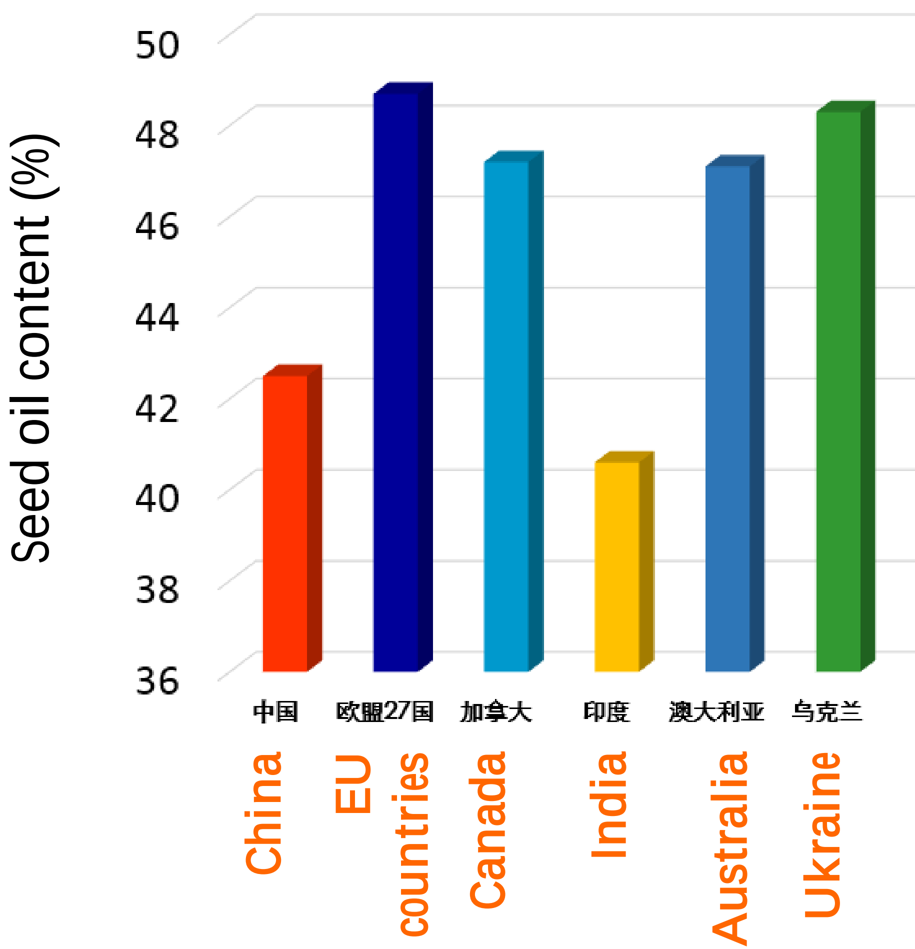
Rapeseed fields

Reasons for the enlarging gap between domestic production and demanding

- **Higher price from lower yield**:
  - In particular, early mature cultivars are more sensitive to diseases and frost damage, and yield less.
  - The difficulty of mechanized production of rapeseed in paddy field suitable to rice production.

- **Bad seed quality arises from**:
  - Short life cycle adapting to the three- or two- cropping system
  - The rapid rising temperature in spring that results in low SW and SOC.
  - The conflicts of heterosis and high seed quality. Quatitaive traits are normally controlled by recessive genes, but heterosis comes from the complementary combination of dominant alleles.

The biggest seed quality problem for Chinese rapeseed is low SOC



Seed oil content (%, dry weight):

- Chinese varieties of 10-15 years ago: ~40%
- Current Chinese varieties applied for production: 42-45%
- The potential breeding materials: 55-60%

The biggest seed quality problem for Chinese rapeseed is low SOC. In comparison with the SOC of Canadian and European rapeseed, the SOC of Chinese rapeseed is very low, only 42-45% in average.

The two China 973 projects for understanding molecular mechanism of SOC

China Key Projects for Basic Research (abbreviated as 973), financed by MOST, Chinese central government

Dr. Hanzhong WANG
China Oil Crops Research Institute, Wuhan → Maternal effect of SOC

Dr. Lixi JIANG
Zhejiang University, Hangzhou → Identification of inhibitors and reducers of seed lipid

Dr. Yongming ZHOU, Dr. GS Yang
Huazhong Agricultural University, Wuhan → Potential of embryonic lipid biosynthesis

Prof. Chunyun GUAN
Hunan Agricultural University, Changsha → Stabilize SOC in adverse environmental conditions

Dr. Jiana LI
Southwest University, Chongqing → Seeking for high efficient flow of photosynthates from source to sink

1st 973 project → **2006-2010**

2nd 973 project → **2015-2019**

Therefore, we are interested in the improvement of SOC and we have made many efforts to understand how oil accumulates in seeds in the framework of China 973-project. My group at Zhejiang University is working on the identification of inhibitors and reducers of seed lipid biosynthesis. My colleagues in other institutions are working to understand the mechanism from other approaches.

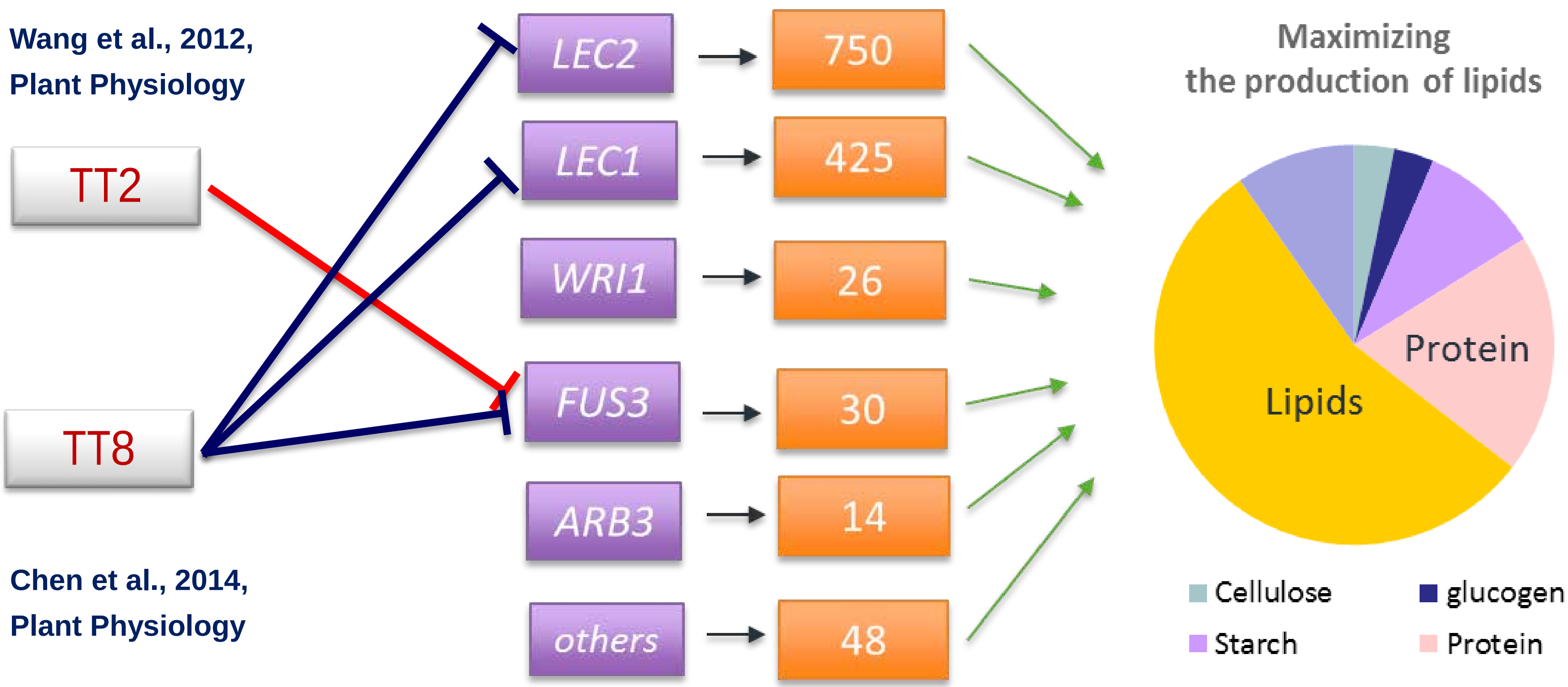
(1)
Identification of inhibitors for embryonic lipid synthesis

Identification of inhibitors for embryonic development

Chen et al., 2012, Plant Physiology

Wang et al., 2012, Plant Physiology

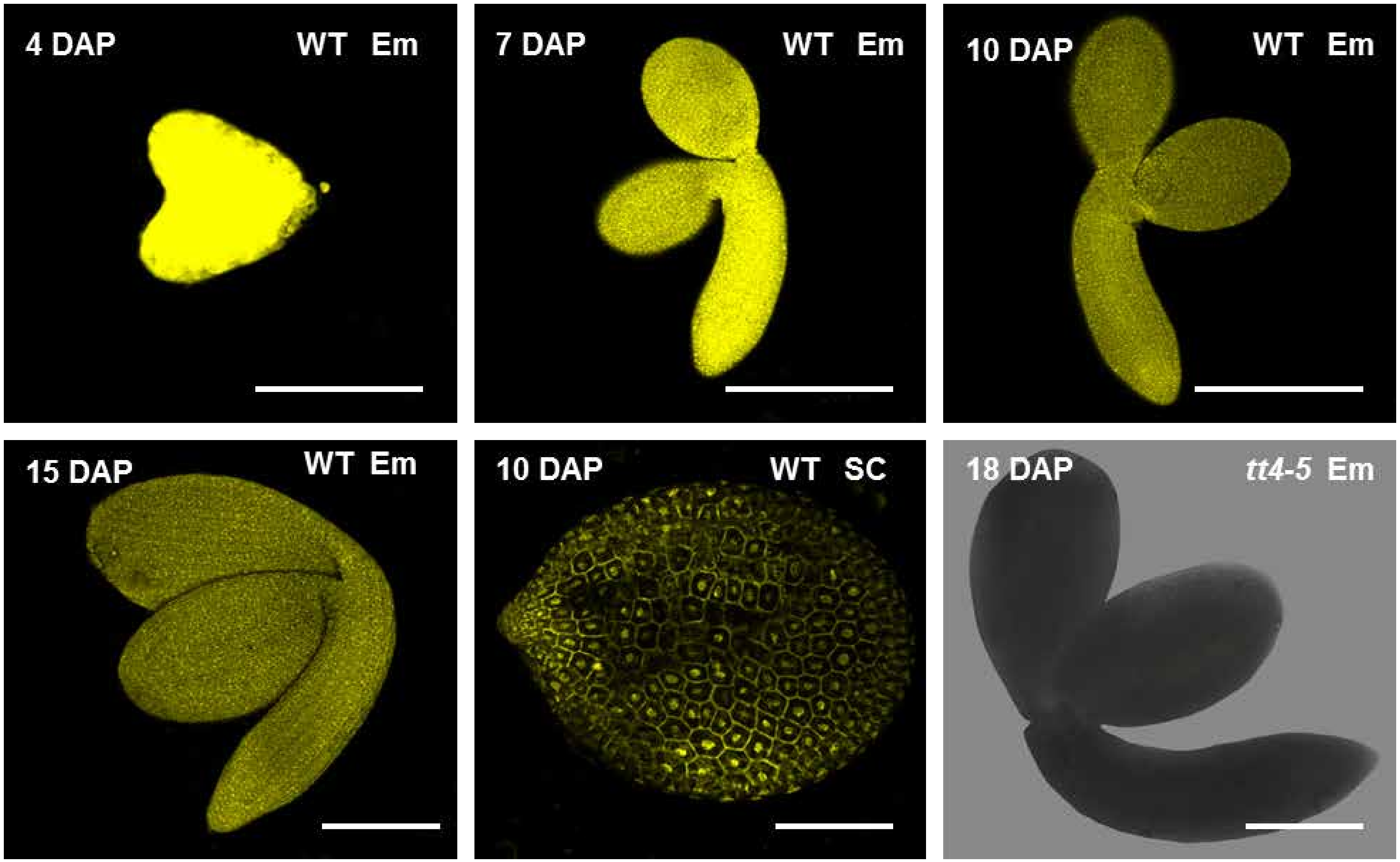
Chen et al., 2014, Plant Physiology



We know that these five transcriptional factors, which have the most number of downstream targets, are very important for the accumulation of seed storage compounds such as oil. We found that *TT2* combines with the 5-end regulatory region of FUSCA3, and negatively affects its function. *TT8* combines with the promoters of *LEC1, LEC2* and negative influences their function. The removal of these inhibitors gives rise SOC increase.

Flavonoids determining seed color are produced not only in seed coat but also in embryo, and diminish along with embryo development in WT plants

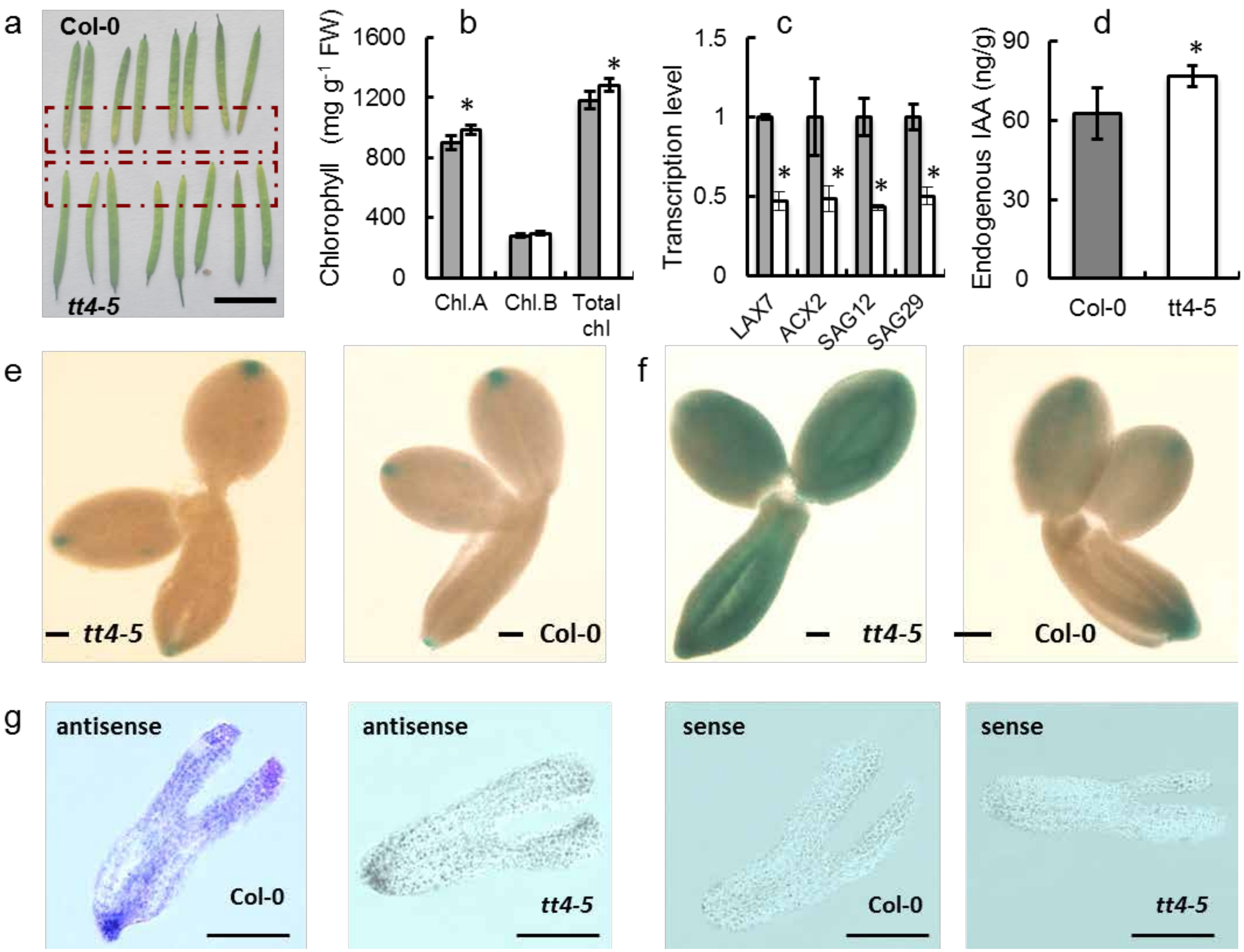
Xuan et al., 2017, Data unpublished



We found that flavonoids are produced not only in seed coat but also in embryos, and they diminish along with the process of embryo development

TT genes are responsible for the biosynthesis of flavonoids in seed coats. The yellow-seeded rapeseed has been interested by breeders for many years. We try to understand why yellow seeds yield more oil.

The flavonoid-repression on FA synthesis associates with the alteration of embryonic auxin concentration



In the Arabidopsis mutant embryo where flavonoids were absent, the auxin content was significantly higher than that of in WT.

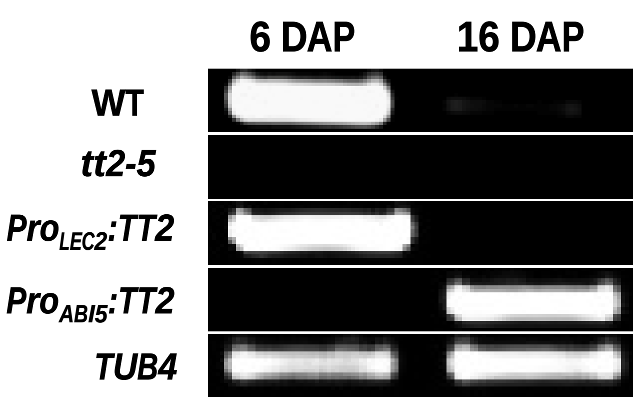
Under the condition with excessive auxin in culturing medium, normal embryo maintains a polar distribution of auxin in the two cotyledons, whereas, mutant embryos lost the function.

The auxin transporter PIN4 was abundant in WT but somehow absent in the mutant embryo without flavonoids.

Xuan et al., 2017, Data unpublished

Silence of TT2 at early stage of embryonic development results in reduction of SOC

	C16:0	C18:0	C18:1	C18:2	C18:3	VLCFAs	Total FAs
WT (Ws)	9.61±0.27	3.52±0.21	17.74±0.24	31.74±0.17	21.48±0.52	15.15±0.29	259.19±8.21
tt2-3	9.14±0.34	2.71±0.13	14.41±0.38	31.59±0.75	24.63±0.89	16.92±0.22	467.35±2.96
tt2-3fae1-2	9.29±0.19	2.87±0.85	19.01±0.33	31.74±0.55	26.61±0.68	9.98±0.13	398.77±8.03
WT (Col-0)	6.68±0.23	2.99±0.14	16.45±0.16	34.62±0.82	23.51±0.97	15.45±0.39	283.21±5.49
tt2-5	6.34±0.28	3.04±0.41	18.29±0.21	29.07±1.15	24.68±1.01	18.58±0.33	506.88±3.58
fae1-2	10.46±0.15	2.81±0.94	17.2±0.88	31.93±0.68	28.12±1.76	9.22±0.19	288.20±3.94
tt2-5fae1-2	10.55±0.48	3.44±0.71	16.49±0.75	32.67±1.21	27.24±0.87	9.40±0.29	393.54±1.92
Pro_{LEC2}:TT2	7.92±0.25	3.43±0.31	16.77±0.67	29.43±1.06	22.98±0.95	19.46±0.34	229.98±6.37
Pro_{ABI5}:TT2	12.42±0.27	5.37±0.59	19.19±0.59	18.757±1.17	27.41±0.73	16.85±0.26	491.05±7.88



Wang et al., 2014, Plant Journal

TT2 is expressed in early stage of developing embryos.
Silencing *TT2* at early stage results in the reduction of SOC

When we drove *TT2* with *LEC2* promoter in seeds, the transgenic lines had very low SOC. By contrast, when we drove *TT2* with ABI5 promoter, the transgenic lines had high SOC

Allelic variation of *BnaC.TT2.a* associated with rapeseed seed color and total FAs

Zhou et al., 2016.

PLOS ONE

RESEARCH ARTICLE

Allelic Variation of *BnaC.TT2.a* and Its Association with Seed Coat Color and Fatty Acids in Rapeseed (*Brassica napus* L.)

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Table 6. DNA Polymorphism (SNPs and/or *Indels*) at *BnaC.TT2.a* gene locus and their association with seed coat color and FA content and composition among 83 *B. napus* genotypes. "#" represents the position need to be checked.

Gene	Position	Polymorphisms	Location	Trait	2010–2011		
					F	*P*	R^2
BnaC.TT2.a	166	T/A	Intron	C18:1	14.43	2.80E-04	0.1512
				C18:2	4.51	3.68E-02	0.0527
				C22:0	13.38	4.50E-04	0.1417
				C22:1	6.18	1.50E-02	0.0709
				TotalFA	4.72	3.27E-02	0.0551
	188	G/A	Intron	C18:1	25.31	0.00E+00	0.2381
				C18:2	14.98	2.20E-04	0.1561
				C22:0	5.15	2.59E-02	0.0598
				C22:1	14.52	2.70E-04	0.152
				TotalFA	7.54	7.40E-03	0.0852
	222	C/A	Intron	C18:1	25.31	0.00E+00	0.2381
				C18:2	14.98	2.20E-04	0.1561
				C22:0	5.15	2.59E-02	0.0598
				C22:1	14.52	2.70E-04	0.152
				TotalFA	7.54	7.40E-03	0.0852
	226	T/C	Intron	C18:1	25.31	0.00E+00	0.2381
				C18:2	14.98	2.20E-04	0.1561
				C22:0	5.15	2.59E-02	0.0598
				C22:1	14.52	2.70E-04	0.152
				TotalFA	7.54	7.40E-03	0.0852
	738	Indel	Exon	a*	3.93	2.35E-02	0.0895
				C18:2	3.33	4.07E-02	0.0769
				Total_FA	4.18	1.88E-02	0.0946
	790#	T/G	Exon	b*	6.41	2.60E-03	0.138
				L*	6.35	2.80E-03	0.137
				H°	5.75	4.60E-03	0.1257
				CIRG	5.21	7.50E-03	0.1153
				C18:2	3.26	4.35E-02	0.0754

doi:10.1371/journal.pone.0146661.t006

In light of the knowledge from Arabidopsis, we investigated allelic variations of *TT2* loci in rapeseed, we found that the changes of some SNPs and indels on C sub-genome significantly associated with seed coat color and total seed oil content in seeds.

Germination of yellow and black seeds in stressful environment

Flavonoids inhibit seed oil accumulation. But on the other hand, flavonoids and pigments actually are helpful for young seedling establishment in a stressful environment.

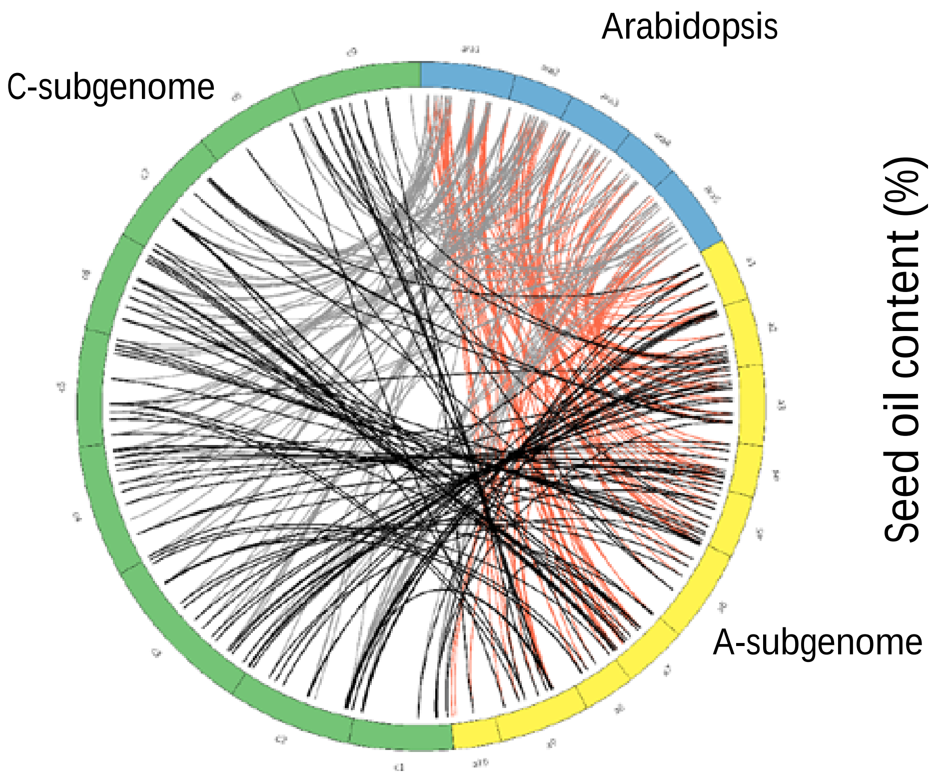
We reported that black seeds survived much better than the yellow seeds in salt solution with the concentration of 100 mM two days after sowing.

100 mM NaCL

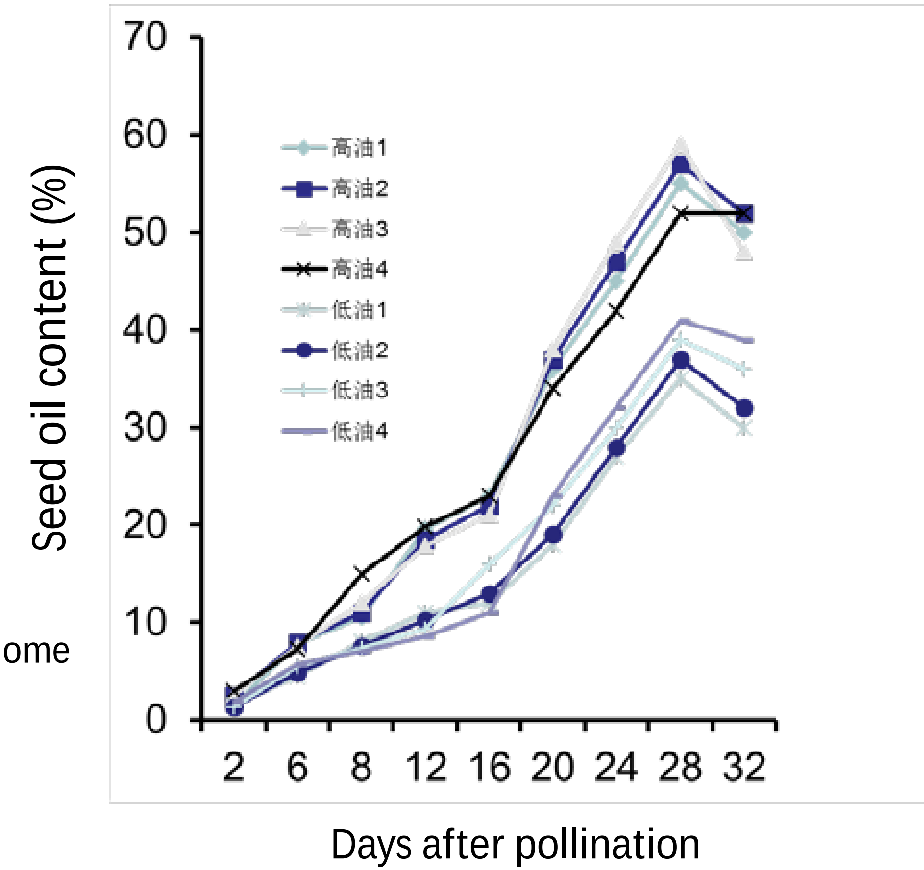
Xuan et al., 2015. Plant Growth Regulation.

(2)
Identification of *Seed Fatty Acid Reducers*

The definition of *SFARs*

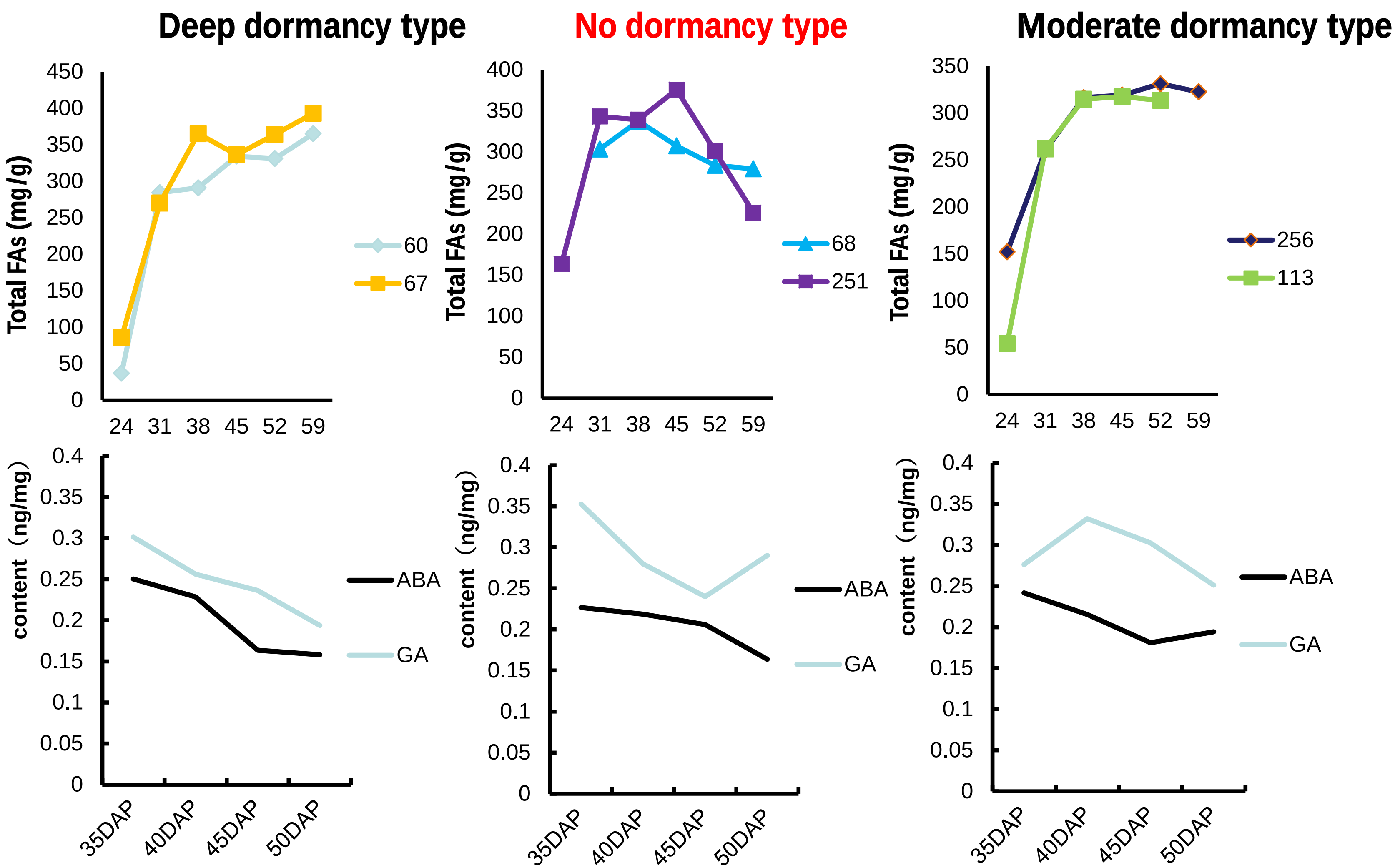


The GDSL genes in Arabidopsis and two rapeseed sub-genomes



The Seed Fatty Acid Reducers, abbreviated as *SFARs*, are defined as the GDSL type lipase that decompose lipids in seeds. There are about 108 GDSL genes in Arabidopsis and more than 300 hundreds GDSLs in rapeseed. The GDSLs in Arabidopsis and the two subgenomes of rapeseed have clear collinear relations. In some varieties of rapeseed, SOC declines significantly from a peak at late maturing stage before harvest.

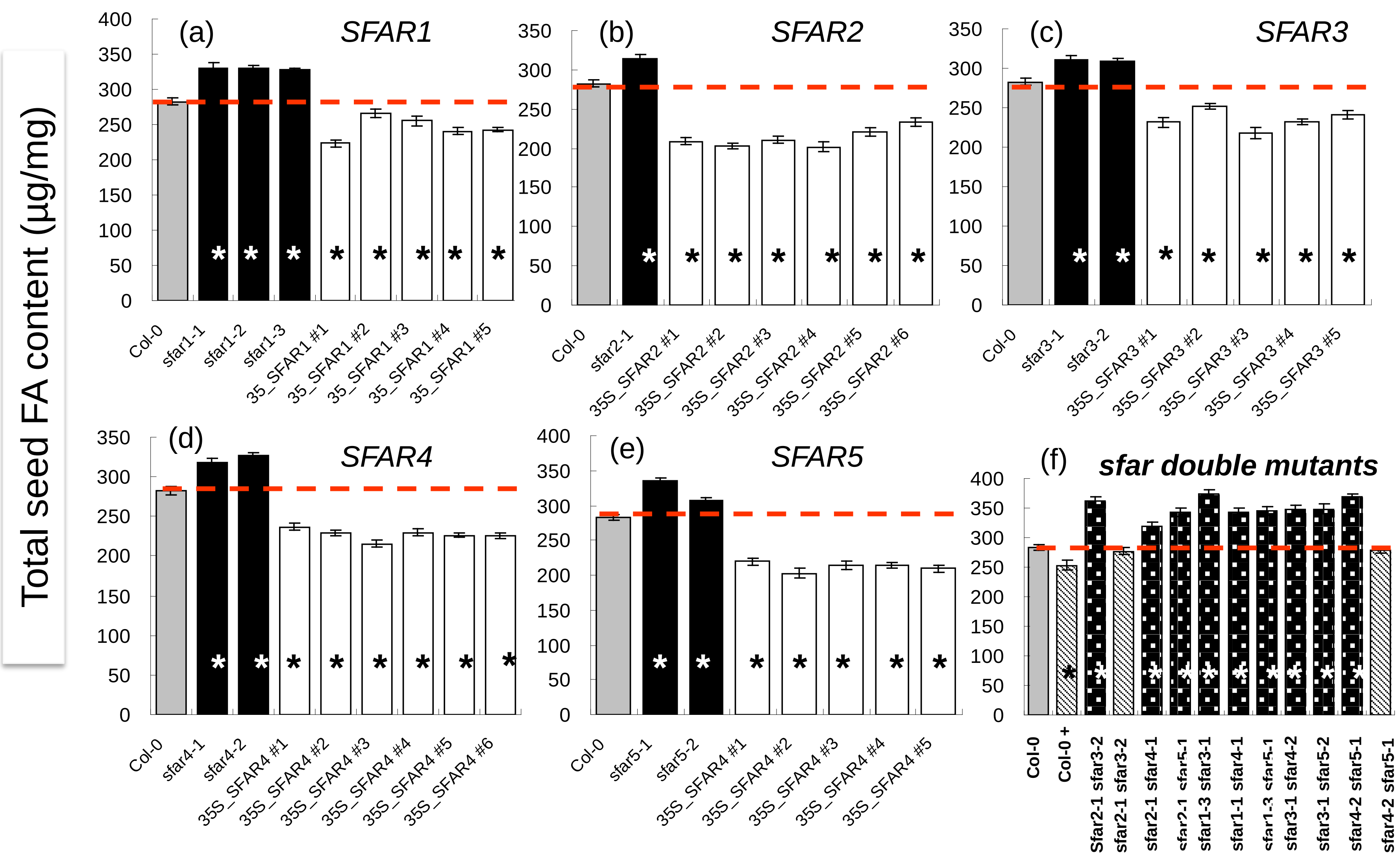
The association of dormancy degree and SOC reduction in rapeseed



The amount of SOC reduction associates with the dormancy degree which is different among genotypes. There are deep dormancy, moderate dormancy and little dormancy genotypes. The degree of dormancy relates to the ratio of endogenous ABA and GA concentrations.

Zhu et al., 2017 data unpublished

The effect of *SFAR1 – SFAR5* on seed total FAs

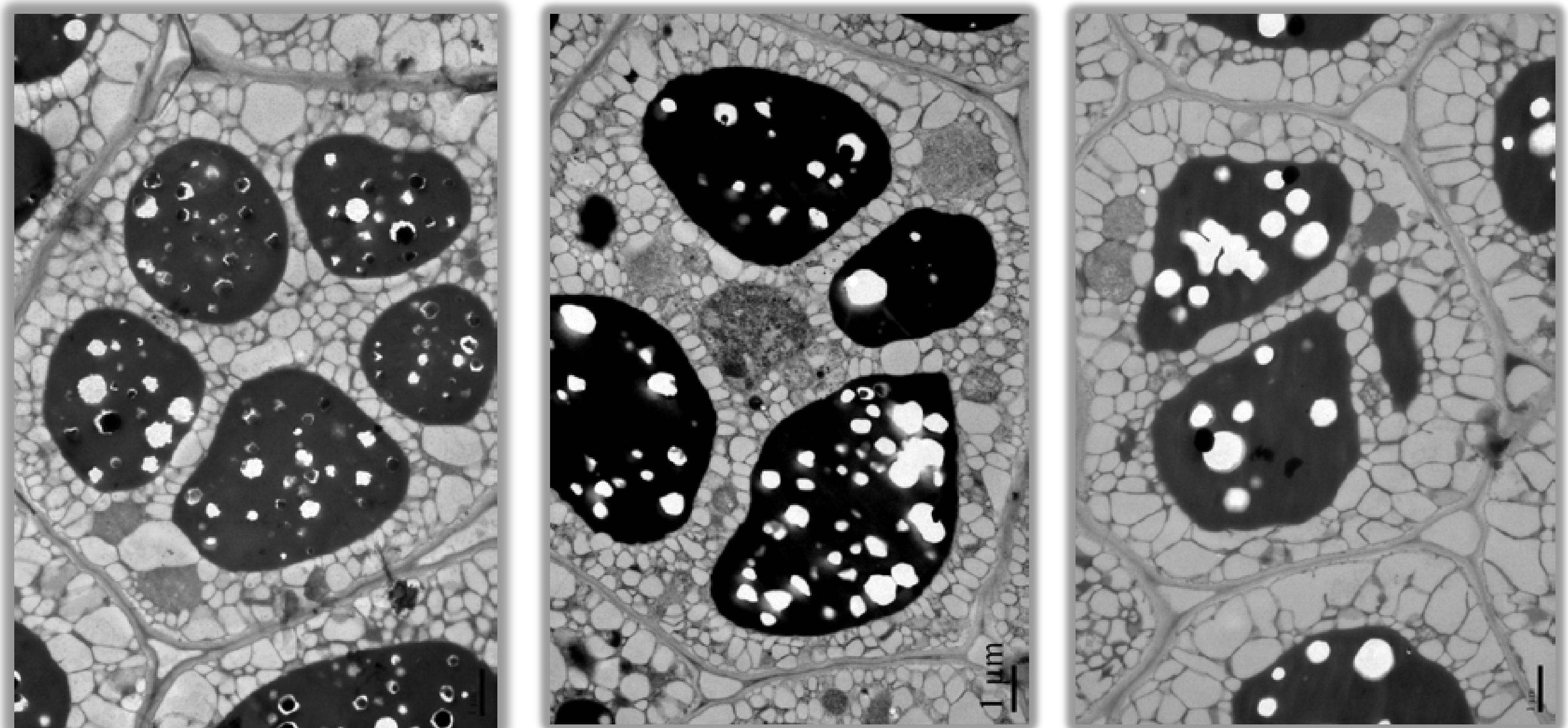


Chen et al., 2012. Plant Cell & Environment

We at first identified *SFARs (SFAR1 to SFAR5)* in Arabidopsis. We reported that the disruption of a *SFAR* resulted in significant increase of SOC. Moreover, the *sfar* double mutants even had higher SOC. On the other hand, overexpressing a *SFAR* gave rise to significant reduction of SOC.

The effect of *SFAR* on seed oilbody size

Chen et al., 2012. Plant Cell & Environment



WT **35_*SFAR1*** ***sfar2-1sfar3-2***

When we look at the seed oil bodies, we see that overexpression of a *SFAR* resulted in smaller oil bodies, by contrast, the disruption of *SAFRs* resulted in bigger oil bodies.

The GA regulated *SFAR* footprint

Chen et al., 2012. Plant Cell & Environment

	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0
Col-0+GA3☆	-0.79	-0.18	**+2.51**	**-2.99**	**-0.18**	-0.11	**+1.87**	-0.03	+0.04	-0.03
Ler + GA3 ★	+0.28	+0.66	**+6.84**	**-2.87**	**-7.44**	-0.08	**+2.42**	+0.06	-0.20	-0.01
Q/ga1–3 ★	+0.52	+0.61	**+7.14**	**-3.02**	**-7.17**	-0.08	**+2.33**	+0.16	-0.22	0.01
Q/WT ★	+0.20	+0.62	**+8.58**	**-3.49**	**-7.52**	-0.11	**+1.34**	-0.09	-0.32	-0.02
35S::SFAR1☆	+0.14	-0.12	**+0.54**	**-1.41**	**-0.22**	-0.10	**+1.32**	-0.12	-0.17	-0.09
35S::SFAR2☆	-0.16	-0.17	**+1.39**	**-1.38**	**-0.64**	-0.14	**+1.23**	-0.11	-0.11	-0.07
35S::SFAR3☆	-0.18	-0.09	**+0.47**	**-1.18**	**-0.31**	-0.09	**+1.48**	-0.10	-0.19	-0.02
35S::SFAR4☆	-0.64	-0.11	**+1.59**	**-0.68**	**-1.27**	-0.18	**+1.13**	-0.11	-0.18	-0.08
35S::SFAR5☆	+0.41	-0.17	**+0.29**	**-2.31**	**-2.89**	-0.11	**+1.03**	-0.10	-0.16	-0.01

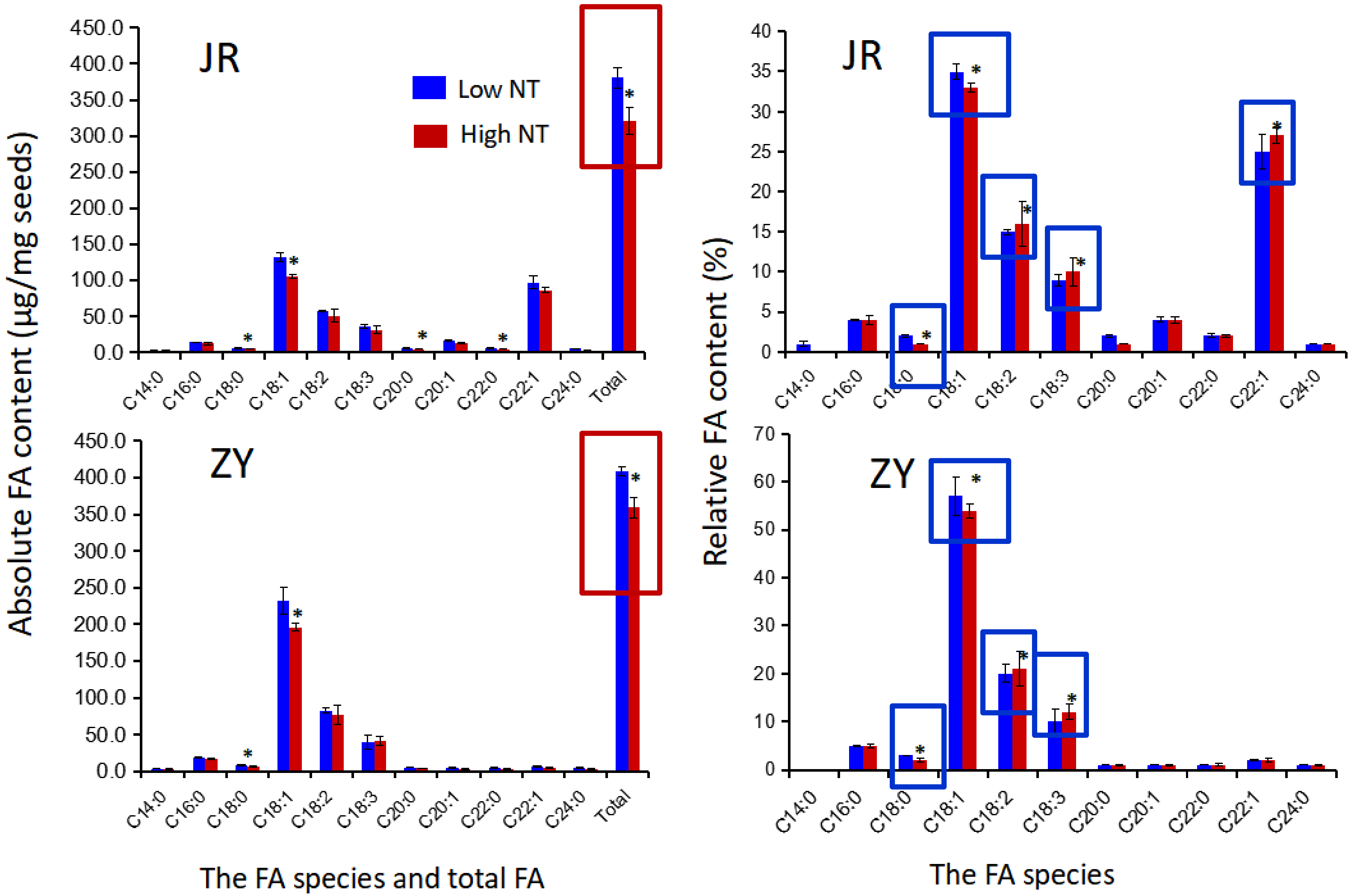
GA → SFAR → FA

The SFAR Footprint

We reported that a group of *SFARs* (namely *SFAR 1* to *SFAR5*) were up-regulated by GA, and the GA-regulation of *SFARs* resulted in a particular pattern of FA composition in seeds. We named it as the SFAR foot print.

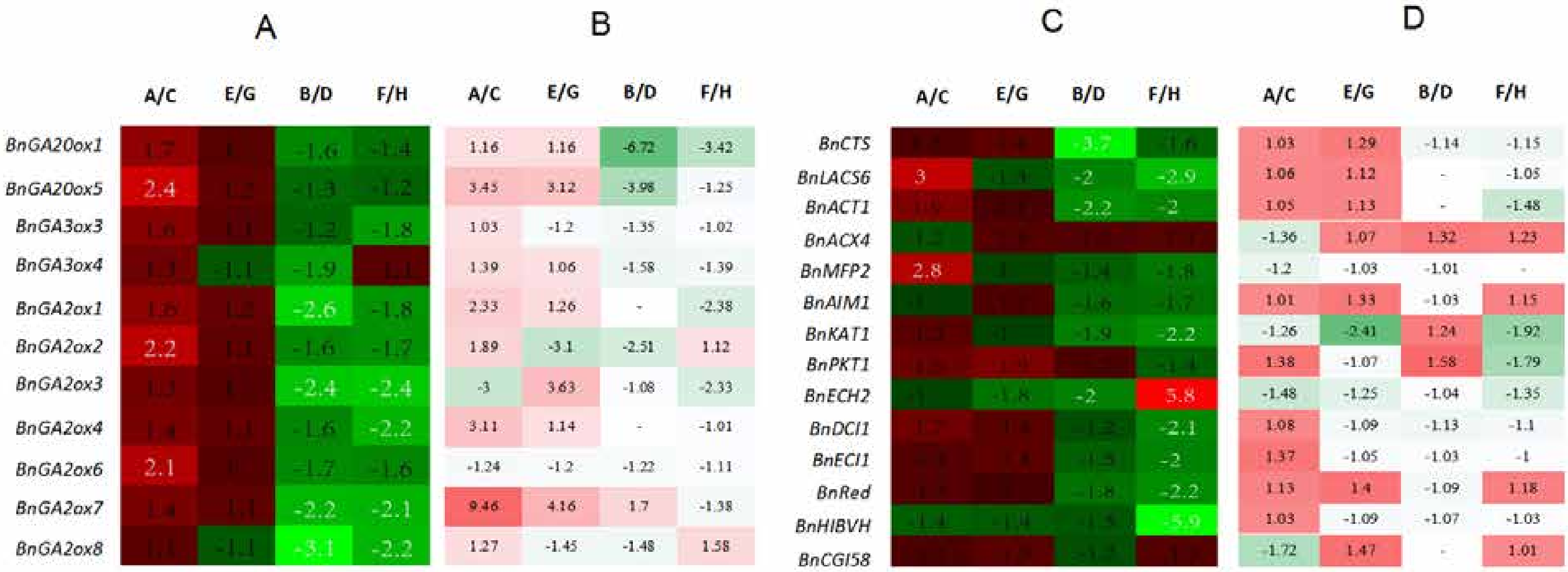
Higher night temperature gave rise to lower SOC in rapeseed

Zhou et al., 2017. JXB, under review



In Yangtze-Valley rapeseed production area, we have warm spring, especially warm spring nights in some years.
Higher NT resulted in lower SOC and the change of FA composition in both high and low SOC cultivars.

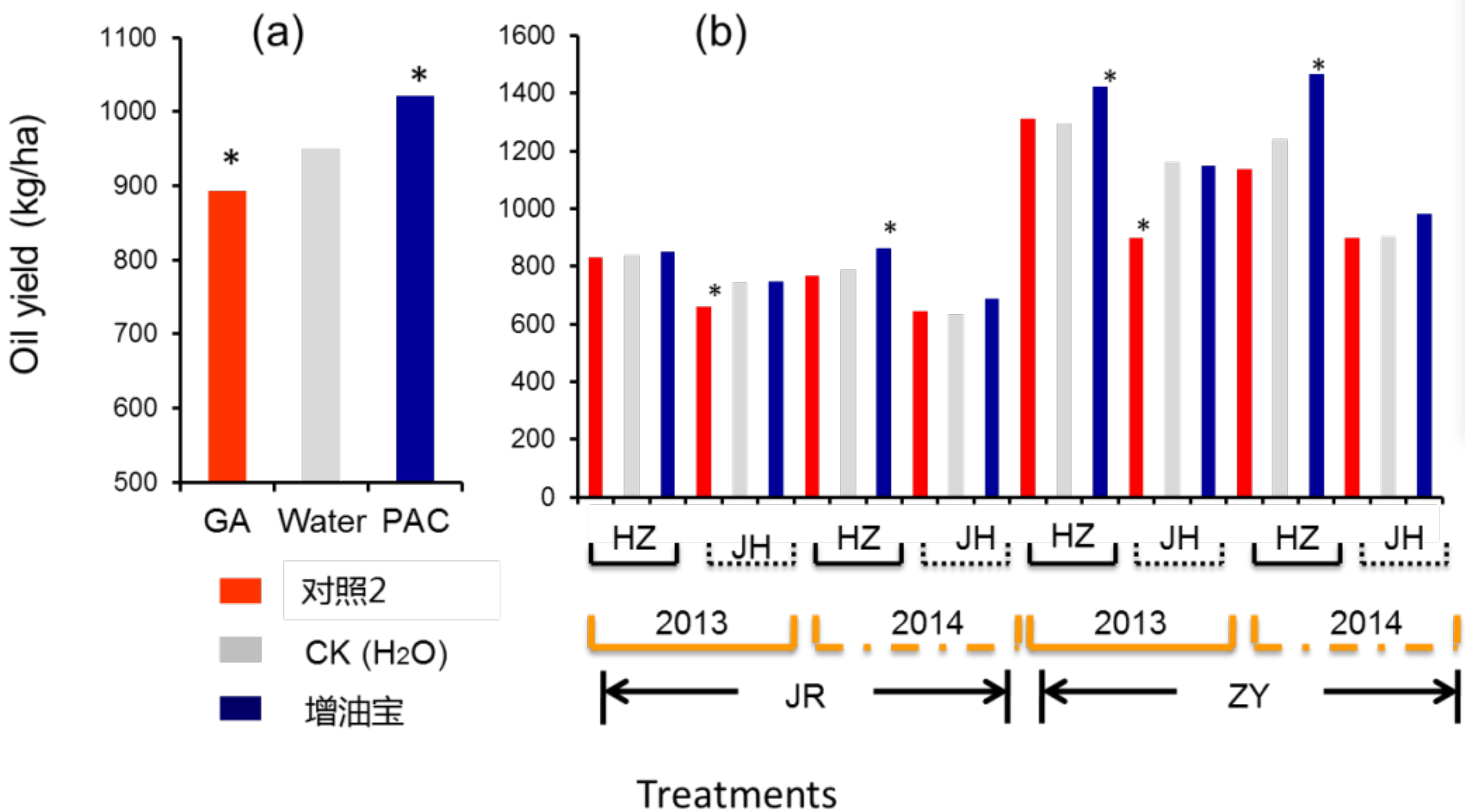
On-going Sino-German project to silence *SFARs* in rapeseed



When we compared the transcriptomes of seeds between different NT treatments, we found that high NT caused up-regulation of GA signaling, and consequently, higher expression of *SFAR* genes and higher expression of the enzymes on beta-oxidation and glyoxylate cycle pathways.

Zhou et al., 2017. under review

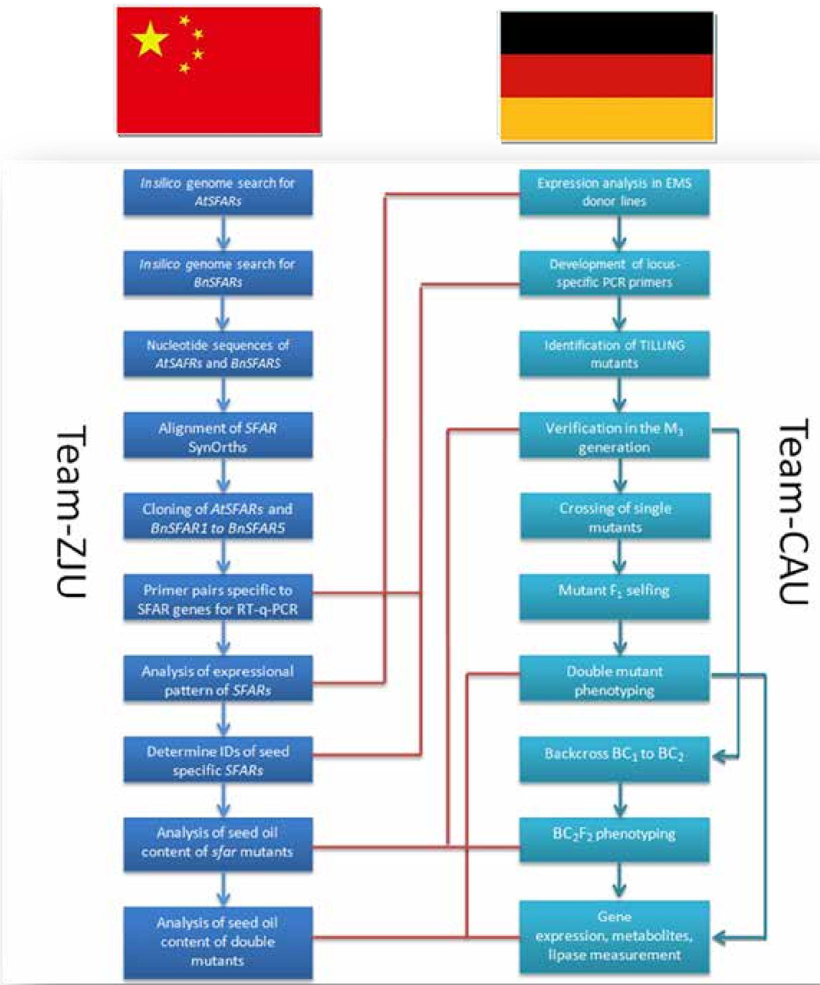
The application of PAC at pod filling stage resulted in an increase of SOC at a rate of 9%-18% in Zhejiang province



To prevent the up-regulation of *SFARs* by GA, we applied PAC to rapeseed at pod filling stage in a two-year two-location field experiment. We demonstrated that the application resulted in an increase of SOC at a rate of 9%-18% in our province.

Zhou et al., 2017. under review

On-going Sino-German project to silence *SFARs* in rapeseed



CN+DE 

CRISPR/Cas9

DE 

TILLING

CN 

Eco-TILLING

For the purpose to the genetic changes of *SFARs* in rapeseed, we are currently collaborating with German scientists (Prof. Jung's lab at University Kiel) in the range of a Sino-German project. We are doing CRISPA in editing *SFARs* and performing TILLING and ECO-TILLING in identify favorable *SFAR* alleles for breeding.

Summary

1. China ranks the 2nd country in term of rapeseed planting area and total production. The rapeseed production in China has increased more than 37-folds since 1961.
2. This great achievement is due mainly to (1) the replacement of *Brassica rapa* with *Brassica napus* in the 1950s, (2) the breeding of *Brassica napu* cultivars with early maturity and semi-dwarfism type in 60-70s, and (3) the successful utilization of heterosis in 80s.
3. There is an enlarging gap between the domestic supply and demand of rapeseed in China, which gives rise to an increasing importation. High manpower cost and low seed quality are the main reasons causing the low competitiveness of our rapeseed.
4. The strategies for the development of rapeseed production in China is to breed F1 hybrids and the cultivars with high SOC.
5. *TT* genes which encode flavonoids are repressor of seed oil biosynthesis; and the silencing of *SFARs* can prevent the reduction of SOC by decomposing lipids at maturing stage of rapeseed.

Thank you for your attention!

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