# Development and application of molecular markers in wheat quality improvement

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# Outlines

- Chinese wheat quality
- Molecular marker development
- KASP platform and breeding chips
- Cultivar development

# Chinese wheat quality

# Major cereal production in China, 2017

| Crop | Area<br>Mha | Yield<br>T/ha | Production<br>Mt | %<br>World |
|---|---|---|---|---|
| Rice | 30 | 6.9 | 209 | 28 |
| Maize | 35 | 6.1 | 216 | 21 |
| Wheat | 24 | 5.4 | 130 | 17 |

Data source: Chinese Ministry of Agriculture, 2018

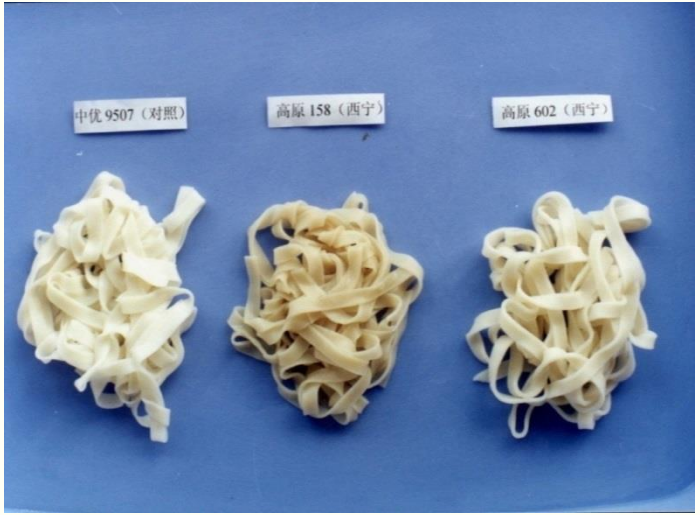
# Percentage of wheat based foods in China

| Food type | % |
|---|---|
| Steamed bread including flat bread | 46 |
| Noodles and dumplings | 39 |
| Cookies and biscuits | 6 |
| Western bread | 5 |
| Others | 4 |
| Total | 100 |

# Chinese wheat with weak dough quality

| Food type | Recommended stability (min) |
|---|---|
| Pan bread | 10-15 |
| Noodle | 7-9 |
| Steamed bread | 5-6 |
| Imported Canada wheat | 13 |
| Chinese wheat | 4 |

# Significant genetic variation for noodle color



# Quality priority

- **Priority I: improving dough quality for making pan bread and blending purpose**
- **Priority II: improving color related traits for traditional products**
- **Significant progress made in research and breeding during the last 20 years**

# Constrains for marker application

- **Gene-specific marker is ideal in breeding program, very limited number of markers is available**
- **Shortage of high-throughput platform, marker testing expensive and time consuming**
- **A lot of research, but limited application**

# Objectives

- **Gene-specific markers development and application**
- **KASP platform and breeding chips**
- **Quality cultivar development**

# Molecular marker development

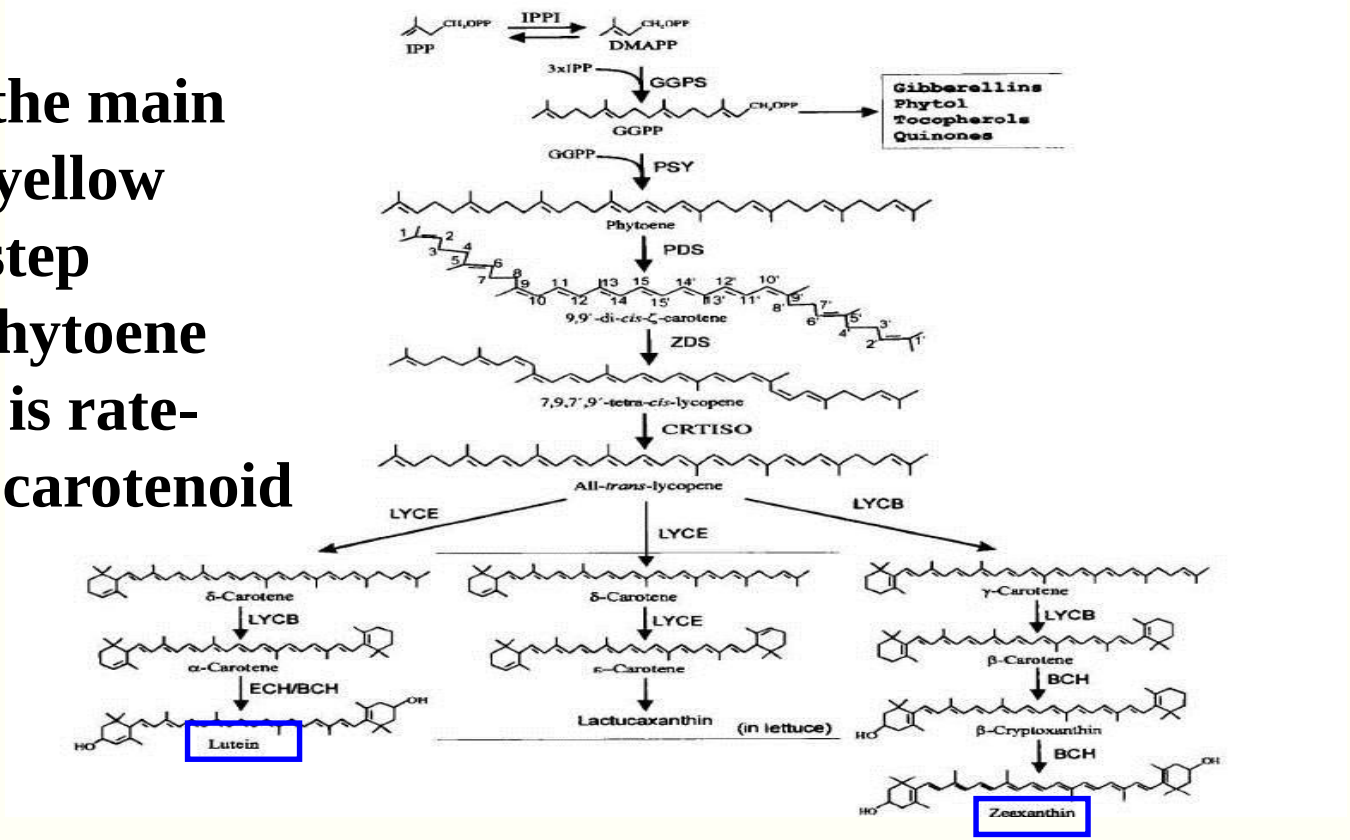
# Genomic approach

- **Rapid progress has been made in crop genomics, can we transfer it to quality breeding?**
- **Genomic information in rice, maize, and wheat can be used to clone wheat genes, and develop gene-specific markers**
- **Wheat is ahead of rice and maize in gene-specific marker development and application (Kage et al., 2015)**

# Background

- **A bright white to creamy color is preferred for Chinese noodles and steamed bread, and a bright yellow color is desirable for yellow alkaline noodles (YAN)**
- **Yellow pigment (YP) is highly associated with the color of these products, high YP is desirable for YAN and durum wheat pasta, but undesirable for Chinese noodles and steamed bread**
- **Genetic manipulation of YP is an important objective for bread and durum wheat breeding programs**

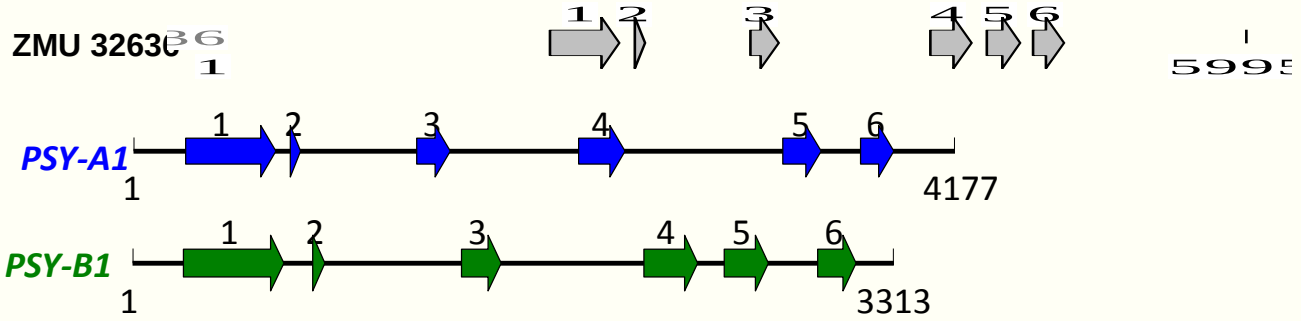
**Carotenoid is the main component of yellow pigment. The step catalyzed by phytoene synthase (Psy) is rate-limiting in the carotenoid biosynthesis**




**Biosynthetic pathway for carotenoid, Zhu et al., 2003**

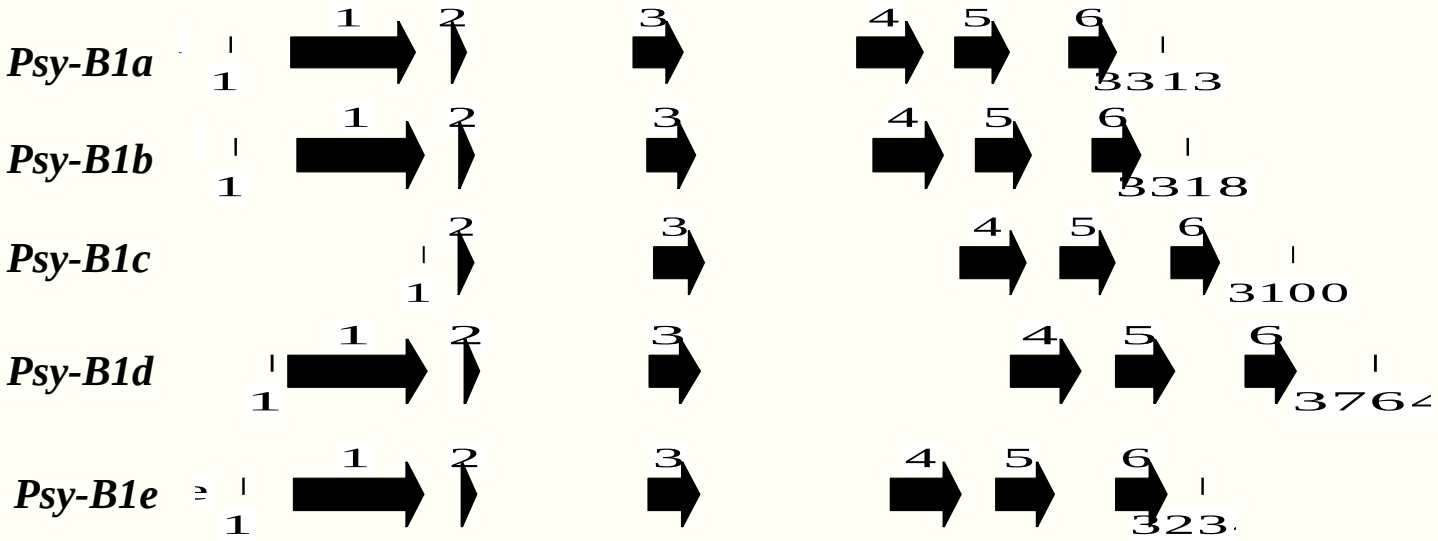
# Cloned *Psy* genes on wheat chr 7A and 7B

| Allele | Coding seq (bp) | Intron | cDNA (bp) | | | Deduced amino acids | |
|---|---|---|---|---|---|---|---|
| | | | 5’ UTR | ORF | 3’ UTR | Residues | Mass (kD) |
| ***PSY-A1*** | 4177 bp | 5 | 221 | 1284 | 303 | 428 | 47.8 |
| ***PSY-B1*** | 3313 bp | 5 | 222 | 1263 | 156 | 421 | 47.0 |



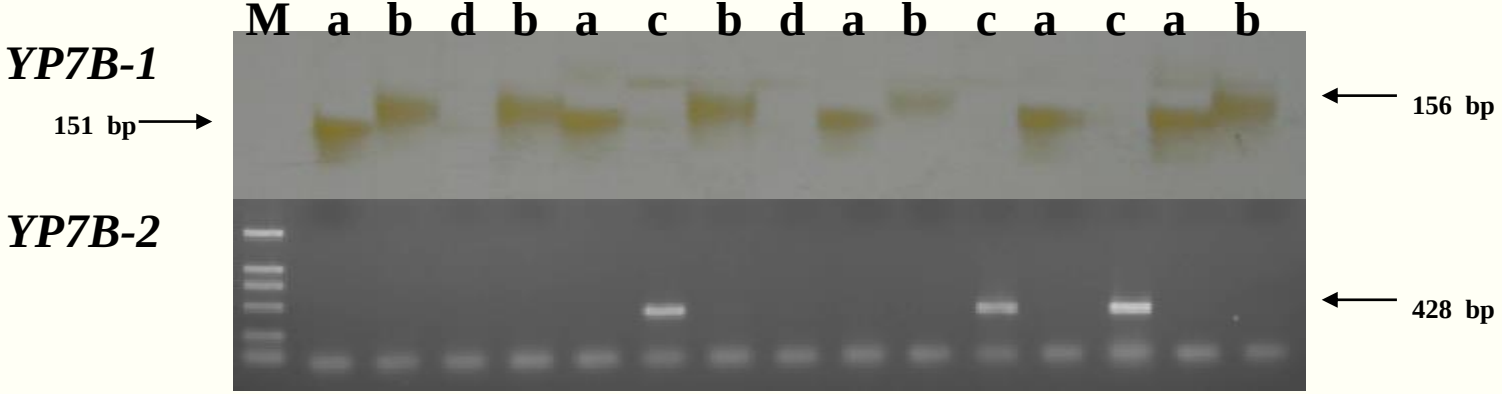

**He et al., 2008, TAG, 116: 213-221**

# Allelic variants for *Psy-B1* gene on chr 7B




He et al., 2009, Mol Breeding, 23: 553-563

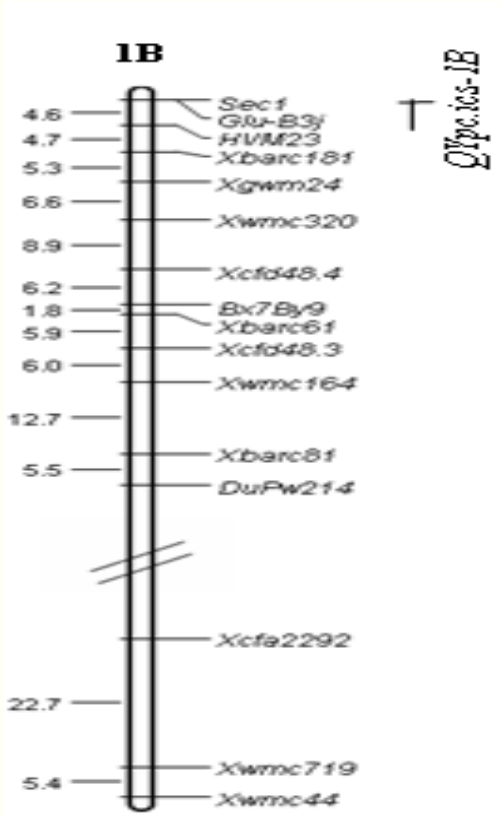
# PCR amplification with *YP7B-1* and *YP7B-2* in Chinese cultivars




**The 156-bp fragment amplified by *YP7B-1* (*Psy-B1b*) is associated with lower YP content, whereas the 151-bp (*Psy-B1a*) and the 428-bp (*Psy-B1c*) fragments amplified by *YP7B-1* and *YP7B-2*, respectively, are associated with higher YP content**

**He et al., 2009, Mol Breeding, 23: 553-563**

# 1BL·1RS translocation increases yellow pigment




- **Accounts for 32% of the phenotypic variation**
- **Widely present in China and other countries**

**Zhang et al., 2009, Euphytica,165:435-444**

# Association between *Psy-A1*/*Psy-B1*/1BL·1RS and YP content

| Genotype | Accession number | Mean (mg/kg) | Range |
|---|---|---|---|
| *Psy-A1a*/*Psy-B1c*/1BL·1RS | 18 | 2.42a | 1.04-3.42 |
| *Psy-A1a*/*Psy-B1a*/1BL·1RS | 35 | 2.08b | 1.22-3.36 |
| *Psy-A1a*/*Psy-B1b*/1BL·1RS | 26 | 1.80c | 0.93-2.69 |
| *Psy-A1a*/*Psy-B1a*/non-1BL·1RS | 23 | 1.60c | 1.08-2.91 |
| *Psy-A1a*/*Psy-B1b*/non-1BL·1RS | 27 | 1.30d | 0.62-2.27 |
| *Psy-A1b*/*Psy-B1a*/non-1BL·1RS | 19 | 1.16d | 0.57-1.84 |
| *Psy-A1b*/*Psy-B1b*/non-1BL·1RS | 30 | 1.15d | 0.48-1.78 |

**Different letters indicate significant differences at $P<0.05$**

**He et al., 2009, Mol Breeding, 23:553-563**

# Gene characterization and gene-specific marker development in flour color related traits

| Gene | Chr. | Marker | Allele | Fragment size (bp) | Phenotype |
|---|---|---|---|---|---|
| Phytoene synthase (*Psy*) | 7A | YP7A | *Psy-A1a/Psy-A1c* | 194 | High |
| | | | *Psy-A1b* | 213 | Low |
| | 7B | YP7B | *Psy-B1a* | 151 | Medium |
| | | | *Psy-B1b* | 156 | Low |
| Phytoene desaturase (*Pds*) | 4B | YP4B-1 | *TaPds-B1b* | 562 | High |
| | | YP4B-2 | *TaPds-B1a* | 382 | Low |
| Lycopeneε-cyclase (*Lcye*) | 3A | e-LCY3A-3 | *e-LCY3Aa* | 537 | - |
| | | | *e-LCY3Ab* | 309 & 230 | |
| | 3B | YP3B-1 | *TaLcye-B1a* | 635 | - |
| | | | *TaLcye-B1b* | No | |
| 1BL.1RS | 1B | H20F/R | Y | 1598 | High |
| | | | N | No | Low |

# Gene characterization and gene-specific marker development in flour color related traits, continued

| Gene | Chr. | Marker | Allele | Fragment size (bp) | Phenotype |
|---|---|---|---|---|---|
| Polyphenol oxidase (*Ppo*) | 2A | PPO18 | *Ppo-A1a* | 685 | High |
| | | | *Ppo-A1b* | 876 | Low |
| | 2B | F-8 | *Ppo-B1a* | 400 & 600 | Low |
| | | | *Ppo-B1b* | 400 | High |
| | 2D | PPO16 | *Ppo-D1a* | 713 | Low |
| | | PPO29 | *Ppo-D1b* | 490 | High |
| Lipoxygenase (*Lox*) | 4B | LOX16 | *Lox-B1a* | 489 | High |
| | | LOX18 | *Lox-B1b* | 791 | Low |
| Peroxidase (*Pod*) | 3A | Pod-3A1 | *Pod-3Aa* | 291 | Low |
| | | Pod-3A2 | *Pod-3Ab* | 766 | High |

# Sixty-three markers in quality traits

- **HMW-GS: *Ax2**, *Bx7*, *Bx7$^{OE}$*, *Bx17+By18*, *Bx14+By15*, *By8*, *By9*, *By16*, *Dx5*, and *Dy10***
- **LMW-GS: 20 markers for *Glu-A3* and *Glu-B3***
- **Polyphenol oxidase: *PPO16*, *PPO18*, *PPO29*, and *PPO33***
- **Yellow pigment: *Psy-A*, *Psy-B*, and *Psy-D***
- **Grain hardness: *Pina-D1b*, *Pinb-D1b*, and *Pinb-D1p***
- **Sprouting tolerance: *Vp1B3*, and *TaSdr*…**
- **Starch: *Wx-A1*, *Wx-B1*, and *Wx-D1***

# SNP array in wheat

- **QTL mapping based on SSR markers is disappearing**
- **90K and 660K SNP arrays, a dramatic improvement over 9K SNP and SSR, in the numbers of gene-based markers for constructing high-density linkage maps**
- **QTL mapping and genome-wide association study (GWAS) based on SNP array are very powerful tools in identifying and locating candidate genes, and for marker-assisted selection**

# Flour color associated traits

- L* is the brightness, from 0 (dark) to 100 (white), a* is a function of the green to red difference, and b* is a function of the blue to the yellow difference
- Yellow pigment
- Polyphenol oxidase (PPO) associated with dough darkening
- Peroxidase (POD) associated with…
- Black point

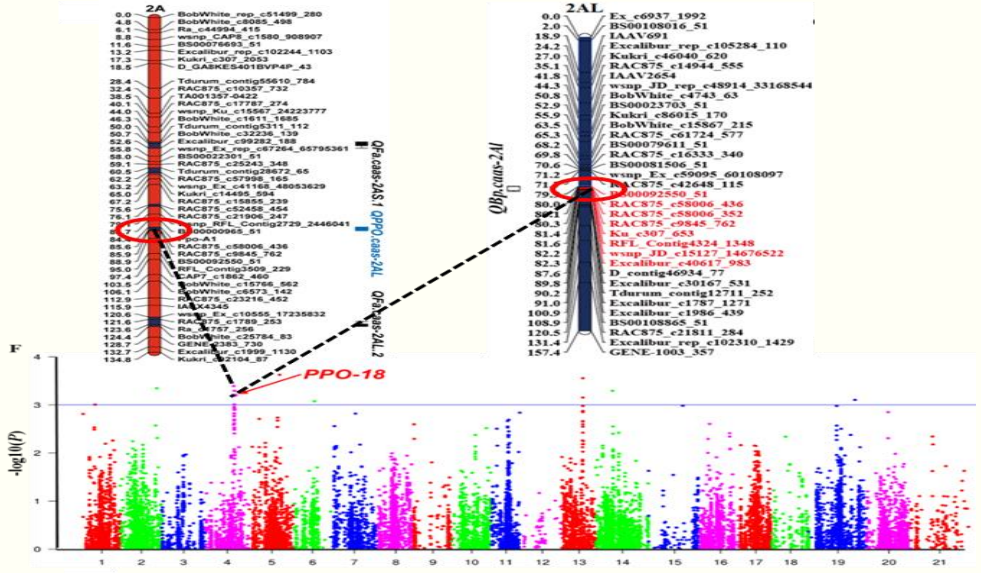
# Black point

- Characterized by dark discoloration at the embryo end of the kernel
- Marketing limitation on the incidence of black point, less than 10% in China
- Use of core parents susceptible to black point

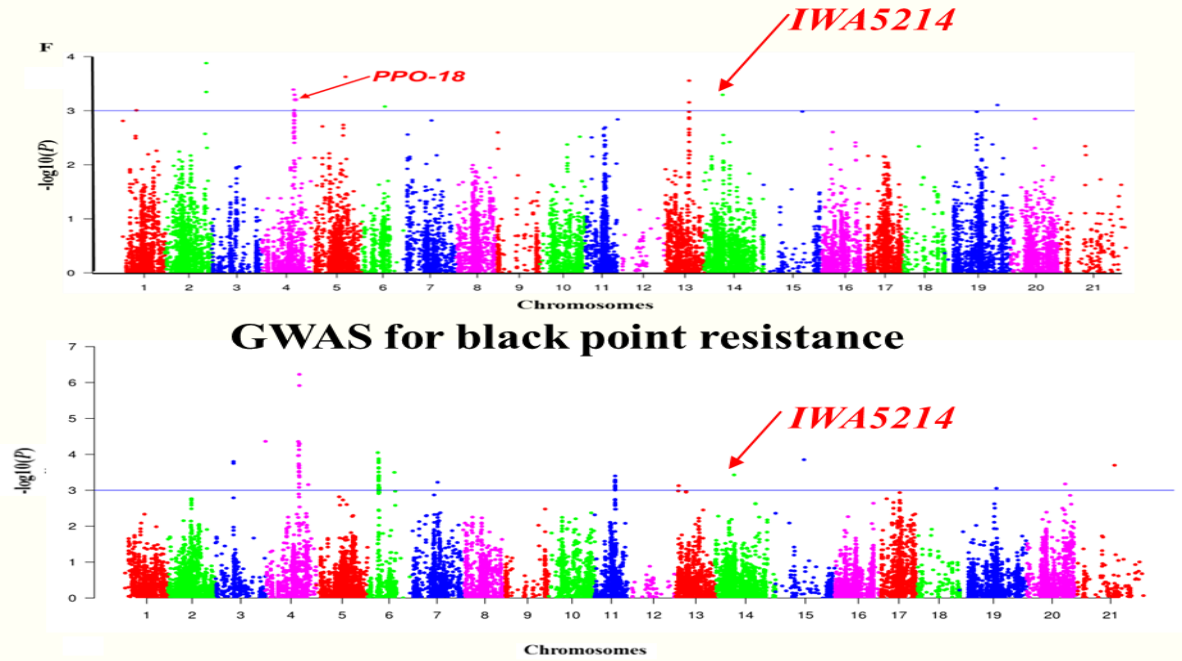
# Factors associated with black point

- **Fungus such as *Alternaria alternate*, *Bipolaris sorokiniana* and *Fusarium proliferatum***
- **Enzymatic browning such as POD (peroxidases), PPO (polyphenol oxidase) and LOX (lipoxygenase), also associated with product color**
- **Environmental factors such as high temperature and humidity, largely dependent upon season**

# Relationship between *PPO-A1* and black point resistance QTL on chromosome 2AL

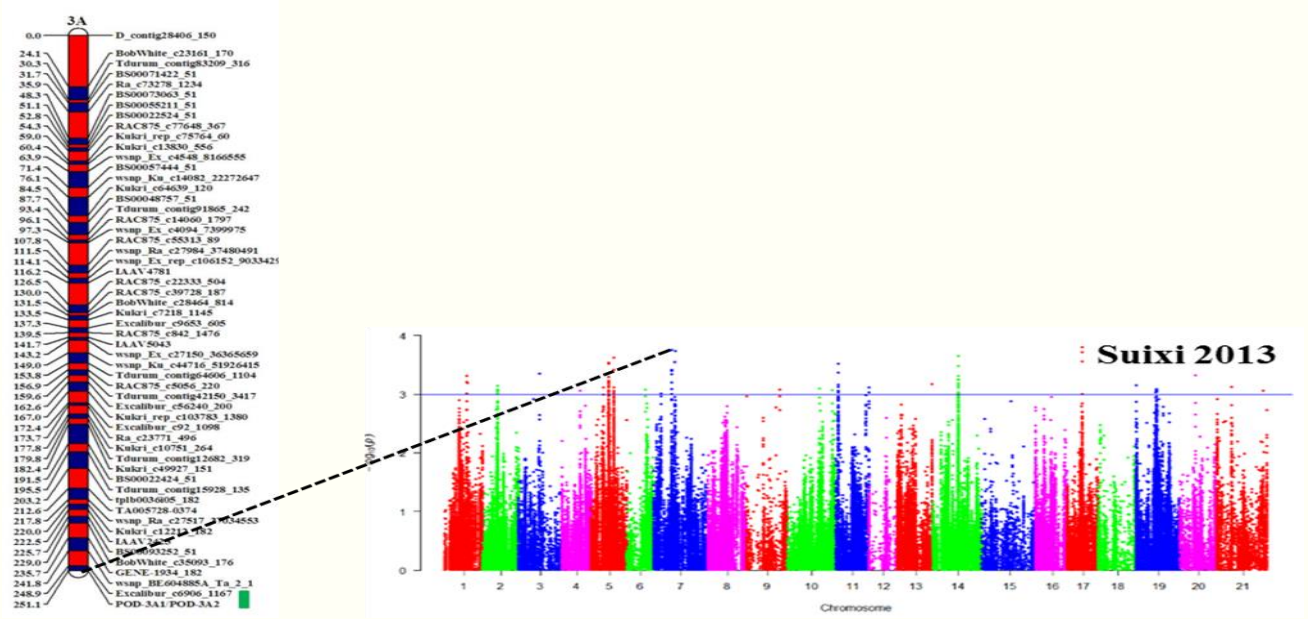



- ***QPpo.caas-2AL* (*Ppo-A1*) and *QBp.caas-2AL* shared the same locus**
- ***PPO18*, the gene specific marker of *Ppo-A1*, significantly associated with black point resistance (Zhai et al. 2016)**




***IWA5214*** **located on 5B, significantly associated with black point resistance and PPO activity (Zhai et al., 2018, Frontiers in Plant Science, under revision)**

# Relationship between *QPod.caas-3AL* and black point resistance QTL on chromosome 3AL




**The resistance loci on chromosome 3AL identified by GWAS is overlapped with the *QPod.caas-3AL*(Wei et al., 2015)**

# Summary for flour color traits

- **Wheat genomics is making rapid progress, gene cloning, discovering , and molecular marker development have entered a new era**
- **SNP array is very powerful tool in identifying new genes, but markers are not evenly distributed among genome and chromosome, a lot of improvements are needed**
- **Resistance for black point can be selected by the markers of PPO genes on 2AL and 5B, and POD genes on 3AL**

# KASP development and application

# Advantages of gene-specific markers

- **Enables novel breeding strategies**
  - ✓ **The ideal marker for breeding program, can be applied to all genotypes after validation**
  - ✓ **Targeted genes can be identified and introduced into cultivars**
  - ✓ **Gene/QTL pyramiding to combine essential genes**
- **Around 150 gene-specific markers available**

# Disadvantages of gel based markers

- **All gene-specific markers in wheat are PCR-gel based markers, limitations in breeding application**
  - ✓ **Higher cost in labor and chemistries**
  - ✓ **Longer time**
  - ✓ **Flexibility and accuracy need improvement, good skills and high quality chemistries**
- **KASP is the most desirable technology for SNP genotyping**
  - ✓ **Desirable flexibility**
  - ✓ **High throughput**
  - ✓ **Low cost**

# KASP platform (Kompetitive Allele Specific PCR)

- **Development**
  - ✓ 60 KASP markers, 32 public available, 28 from CIMMYT Mexico
  - ✓ 95 KASP markers developed by CAAS-CIMMYT
  - ✓ All gene-specific markers for qualities, agronomic traits…
- **Validation by SNPLINE from LGC**
  - ✓ 3000 cultivars from China and other countries
  - ✓ Four mapping populations
- **Application**
  - ✓ Development of central facility for genotyping
  - ✓ Development of breeding chip by adding more SNPs associated with phenotype from 90K/660K

# Tested data on KASP from China

- **Time: 1584 cultivars can be genotyped with 142 available markers in 2-3 days**
- **Cost: 9 cents per data point including DNA extraction**
- **Highly consistent with results from PCR markers**

# Summary of KASP markers in wheat

| Trait | Locus number | Marker number | Allele number |
|---|---|---|---|
| Quality trait | 35 | 63 | 91 |
| Agronomic trait | 38 | 61 | 109 |
| Disease resistance | 17 | 18 | 18 |
| Total | 90 | 142 | 218 |

**Data source: CAAS-CIMMYT, 2018**

# Wheat SNP Array

- 90K Infinium array, 100 USD per genotype
- 35K Breeder's array, unknown
- 660K Affymetrix array, 200 USD per genotype
- 820K Affymetrix array, unknown
- Above chips very costly or have huge number of SNPs without any application in genetics and breeding
- Can we develop chip for research and breeding application with reduced cost?

# 50K Wheat SNP Array

- **Less cost: 20% lower than current SNP array**
- **High quality markers and uniform genome coverage: polymorphic across genetic backgrounds, each chromosome has over 2000 SNP markers with uniform distribution**
- **Functional genes: over 100 SNPs from functional genes for agronomic, quality and disease resistance traits**
- **QTLs from published markers: 700 SNPs from published QTLs and GWAS**

# Next step

- **15K Wheat SNP Array, much cheaper (around 35 USD)**
- **Triticum-Genesizer technology, allele discovery and identification by sequencing, cost reduction by 50%**
- **Collaboration with Chinese companies**

# Summary-KASP and breeding chips

- **KASP assay is a high-throughput genotyping technology, with low cost, much shorter time, and great flexibility**
- **KASP has great application in breeding program**
- **50K SNP array has a lot of advantages**

# Cultivar development

# Marker application

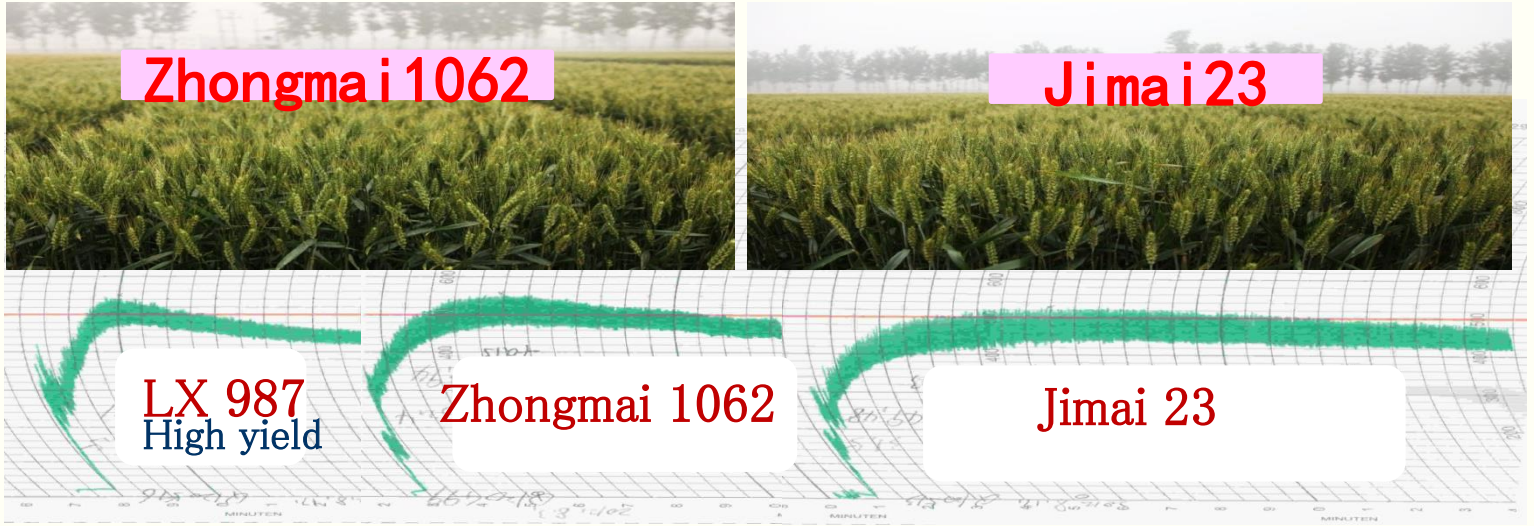
- About 100 markers are routinely used in parental characterization and advanced lines confirmation
- Around 10 crosses per year for MAS program, focused on quality improvement
- Six cultivars have been released in 2016 and 2017

# Key points for MAS

- **Excellent knowledge on breeding parents, both phenotype and genotype information**
- **Target dough quality and color traits**
- **Single cross or limited backcross**
- **Large population size, 500-600 plants for backcross**
- **Fully integrated with conventional breeding program, and combination of field selection, marker testing and quality evaluation**

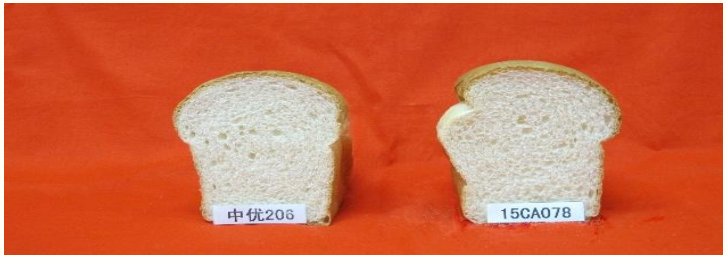
# Cultivars released from MAS

- **Jimai 23 with high yield and quality released in Shandong, in collaboration with Shandong AAS**
- **Zhongmai 1062 with high yield and quality released in Northern**



# Zhongmai 578

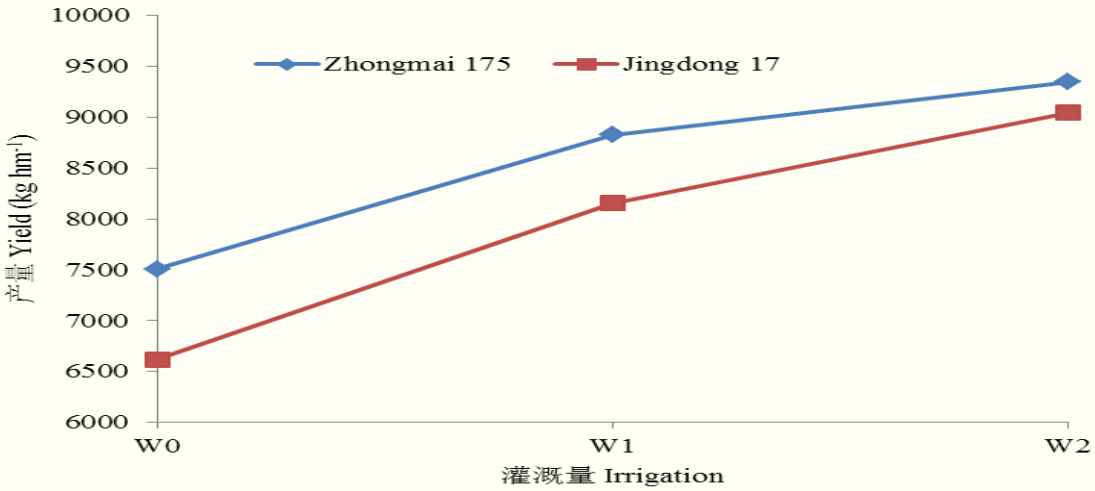
- **Excellent bread-making quality**
- **Three days earlier than check cultivar, 3.2% better yield**
- **Resistance to rusts and powdery mildew**
- **Expect to be released in Yellow and Huai Valleys in 2018**



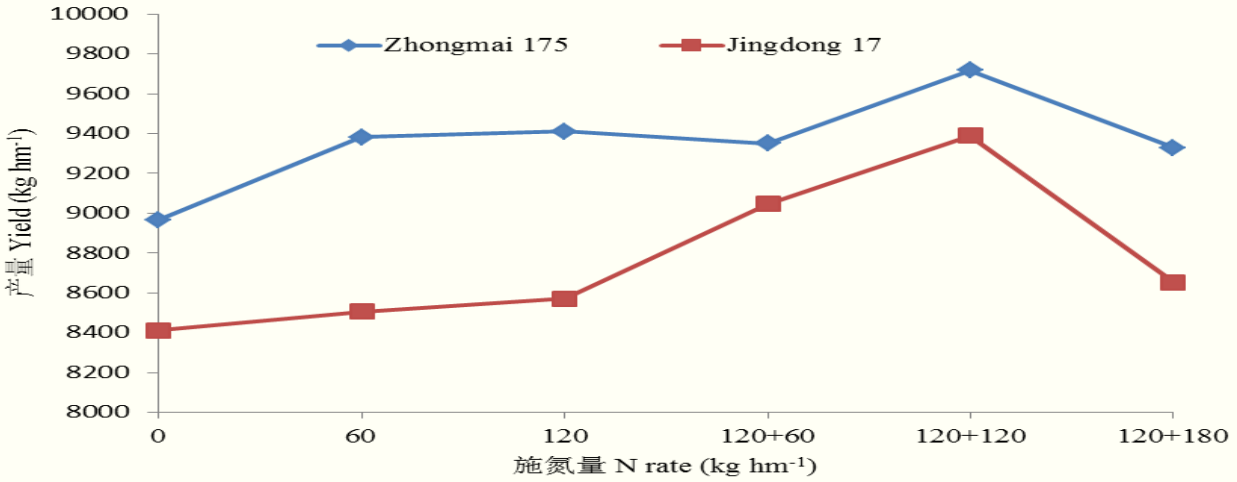

# Zhongmai 175

- High yield potential, excellent noodle quality, high use efficiency in water/N/P, and broad adaptation
- Released in eight provinces, check cultivar for the Northern China Plain Winter Wheat Region
- Leading cultivar with 400, 000 ha in 2017, 30-40% area


# Zhongmai 175 outyielded check cultivar under various irrigations

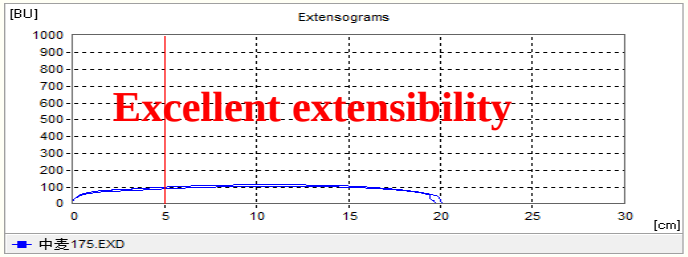


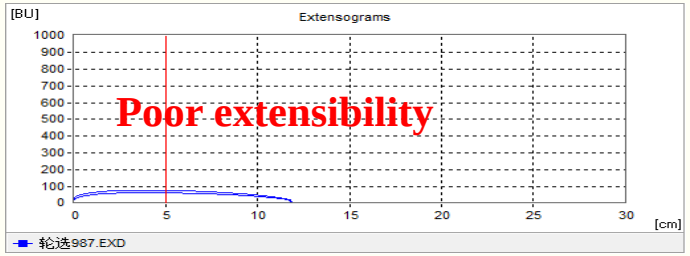
# Zhongmai 175 outyielded check cultivar at six different fertilizer levels



# What we learned

- Molecular markers can be effectively used in improving dough strength and color traits
- Dough extensibility is very important for pan bread and noodle qualities, at least one parent must confer excellent extensibility
- Markers will play more significant role in the future



# Quality improvement in the future

- **Consumers concern health and nutritional qualities as well as processing quality**
- **Much more work is needed to reduce DON level from head scab under wheat/maize rotation system**
- **Farmers need a balance between yield and quality**
- **Molecular marker technology must be fully integrated with conventional breeding and quality testing program**

# Take home messages

- **Eight genes associated with flour color have been well characterized and molecular markers developed and validated**
- **The Second and Third-generation Sequencing Technologies, SNP arrays and wheat genome sequences provide great opportunities for gene discovering and cloning, and marker development**
- **KASP assay has great application in breeding program**

# Acknowledgement


- **CIMMYT: Ravi Singh, Matthew Reynolds, Carlos Guzman, Pawan Singh, Susanne Dreisigacker, Caixia Lan, Xinyao He, and Tom Payne**
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