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1.Breeding the restorer lines of *japonica* Hybrid Rice –bottleneck
 The difficult problems of the development of *japonica* hybrid rice are deficient in restorer genes and the stigma of sterile lines being small and not exerted.

To solve the problem of deficiency in restorer genes and low seed setting rate of F1 cross between indica and japonica rice via breeding different types of wide-compatibility varieties, restorer lines and intermediat material with the technique "building a bridge between indica and japonica"



1.Breeding of the restorer lines in *japonica* hybrid rice

- Fast oriented transfer of the restorer line in japonica hybrid rice
- $\square P \longrightarrow A(CMS line)$

 $\Box A/R \longrightarrow A//A/R \longrightarrow A///A//A/R \longrightarrow RP$

A fine variety is oriented transfer to be a restorer line successfully

Breeding of the restorer lines in *japonica* hybrid rice

C57, the first heterobeltiosis restorer line, served as a prelude to the japonica hybrid rice.

C418, the japonica restorer line with high combining ability, is the japonica elite restorer line in China up to now.











Breeding of the sterile lines with high PES

Collection of the japonica lines with high PES
 The gene of big stigma in *indica* rice was transformed into *japonica* rice to select a batch of japonica sterile lines with high glume-closed exerted stigma rate and cross seed setting rate.

Marker-assisted selection

Breeding of the sterile lines with high PES

- PES is a quantity trait controlled by nuclear genes. A population of F₂ (168 lines) derived from a cross between a high-PES variety DS and a low-PES variety C9083 was used to analysis QTL of PES.
- A major QTL was detected which LOD score was 19.69 with phenotypic variation explained by 41.91%. It provides valuable reference for fine mapping, gene cloning and marker -assisted selection (MAS) of the sterility gene and stigmas exserted gene.



3.Heterotic hybrid variety development

- **Breeding of japonica hybrid rice of large-panicle type:**
 - F_1 consist of primary branches in the base and high upper secondary branches rate.
- Properties of grain filling of super japonica hybrid rice of large-panicle type
 - the grain filling of low part of a panicle started early and the panicle displayed strong sustainable grain filling ability.
- Fine grain filling character is one of the biophysiological foundation for super-high yield.

Heterotic hybrid variety development -breeding parent with high combining ability to improve yield advantage

- **100 combinations GCA analysis that perform positive competitive advantage**
- "high GCA /high GCA" combinations type:63%
 - "high / low" or "low / high" combinations type:28%
- □ "low / low" combination type:8.6%

It is easy to obtain the combination with high specific combining ability (SCA) using parents with high GCA.

Heterotic hybrid variety development -heterotic group ----J/I, P/T

Heterotic japonica hybrid rice of northern area of China

cross between the low temperature resistant photoperiodthermo-sensitive genic male sterile line with early *japonica* background and the restorer line with *indica* background.

- Heterotic japonica hybrid rice of middle rice area of China cross between the sterile line sensitive to photoperiod and the restorer line sensitive to temperature
- Heterotic japonica hybrid rice of south area of China

cross between the high temperature resistant sterile line with *indica* background and *japonica* background R line.

In our practice, the most likely heterotic combinations are between the sterile line with *japonica* background and the restorer line with *indica* background or the sterile line sensitive to photoperiod and the restorer line sensitive to temperature.

Heterotic hybrid variety development

- 1. "Breeding high yield parents" combinates the overparent heterosis and competitive advantage
- 2. Breeding high combining ability parents to improve the yield advantage
- **3** Increasing seed setting rate and number of panicle is the major factor to improve hybrid rice yield.
- 4. Utilization the genetic and indica difference between both parents to improve yield advantage. Heterotic group of japonica hybrid rice.

4.Breeding of the two-line japonica hybrid rice

The obstacles of three-line

- Three-line system is more complication and need restore line as its male parents, it is very difficulty to found a elite restorer line among the local inbred japonica cultivar
- The sterility of BT type CMS is unstable ,affected by high temperature
- □ F1 seed setting unstable ,affected by lower temperature

Breeding of the two-line japonica hybrid rice

photoperiodsensitive genic male sterile line (PGMS)

------The main technique to solve the

above problem is breeding the japonica

two-line genic male sterile line

Current situation of two-line

- The breeding technique of two-line *indica* hybrid rice is mature and its application area
 developing steadily in southern China.
- But two-line japonica hybrid rice developing slowly. The immature breeding technique of japonica photoperiod--sensitive genic male sterile (PGMS simple call S line) line is the main cause.

Experimental progress

- The authors began to breeding the japonica PGMS line in Tianjin and Northeast China since 2008 Several good japonica PGMS lines that adapt to be applied in the Northern rice area were bred successfully after increasing 8 generations in Hainan Base.
- Now they are being used to testcross to identify the heterotic combinations
- In our opinion, the successes of breeding can be summarized as fellow

Parents genetic background influence the separation of infertility individual plant

The PTGMS gene expressed related to the parental genetic background. If the ratio of separated sterile individual plant showed remarkable difference when different combinations in the same place or the same combination in different place affected by the photoperiod and temperature condition.

DS is able to be took as a donor and the elites local varieties as receptor.

In Tianjin 天津 DS/LD3 25% DS/131 30% DS/LJ26 18% The sterile line with steady sterile is easy to select in Tianjin because the photoperiod and temperature condition of Tianjin is better for the expression of the PGMS gene.

✓ Day length

June- 20) July-20	Aug-10	Agu-20
15h.	14.55h.	14.00h.	13.52h

✓ Temperature (1954—2008)
 lowest average temperature
 July 10- August 18 ≥22 °C
 July 14- August 12 ≥23 °C

Breeding technique of two-line genic male sterile line in the North of Tianjin

Environment

Long day and low temperature

Breeding Progress

- Take DS as a donor and local cold tolerance elite line as receptor
- □ Using greenhouse to guarantee to found the sterile lines in F₂ and BCF₂;
- Identify the sterile individual plant through MAS and then select and backcross.

How to select the PGMS line with the lower critical temperature of sterile converting

- The sterile line selected in the long day and low temperature condition of Northeast China;
- ✓ The selected individual whose fertility converting late or not converting in Tianjin ;
- The individual plant of converting to sterile earliest during the winter breeding in Sanya.

The key characters for PGMS line select

- ✓ The characters of practical
 PGMS lines are glume-closed
 after flowered;
 ✓ Exerted stigma after glum closed;
 ✓ early flowering time ;
- ✓White and slender anther.



Varieties

✓ DS

✓ L62S

✓ L6S

✓ Longyou 619(L6S/R19)

DS D Phytology characters

Plant height 80 cm, Strong tillering

loose panicle type,

Per panicle about 150 spikelets

Panicle length 18-21 cm

1000-grain weight 25 g

Good grain quality

High resistance to rice blast

Average leaf number 14.5 leaves



The sterility stage of DS

DS reproduction in Sanya



DS application

- ✓ The critical temperature of DS is lower (\leq 23.0 °C);
- The stigma exposed rate is high, more than
 90% after glumes closed ;
- $\checkmark~$ DS as donor parent ,we have derived L62S
 - L6S, L39S,.

L62S

Phytology characters

Plant height 70 cm, Strong tillering Impact panicle type, Per panicle about 160 spikele Panicle length 20 cm 1000-grain weight 25 g Good grain quality High resistance to rice blast Average leaf number 14 leaves





L6S

Phytology characters

Plant height 118 cm, Strong tillering loose panicle type, Per panicle about 130 spikelets Panicle length 20 cm 1000-grain weight 26 g Good grain quality High resistance to rice blast Average leaf number 14 leaves



Sterility



Field identified 1000 plants above, sterility plant rate was 100%; Bagged more than 500 bags self-seed-setting 0%; Typical aborted pollens, July 20-August 20 with aborted pollen rate of 100%; Infertility critical temperature is lower, safety period long, adapt to the southern Tianjin.

Flowering behaves and outcrossing rate of L6S

Flowering time is earlier, summer sunny day in the beginning at 10:35, peak time at 11:35-12:40;L6S glum open angle is big, the stigma is large and exposed rate is high, after glume closed stigma exposed rate is 83% or so, on average seed setting rate is nearly 60% in seed production fields



Longyou 619(L6S/R19)



Conclusion

- DS can be took as a donor for PGMS and stigma expose genes;
- Sanya is a best place for seed reproduction;
- The best place for sterile expression is south of Tianjin.Tianjin is good place for hybrid seed production;
- Breeding, reproduction and seed production in different place is the strategy for tow line japonica hybrid rice breeding

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