Risk control of two line hybrid rice seed production

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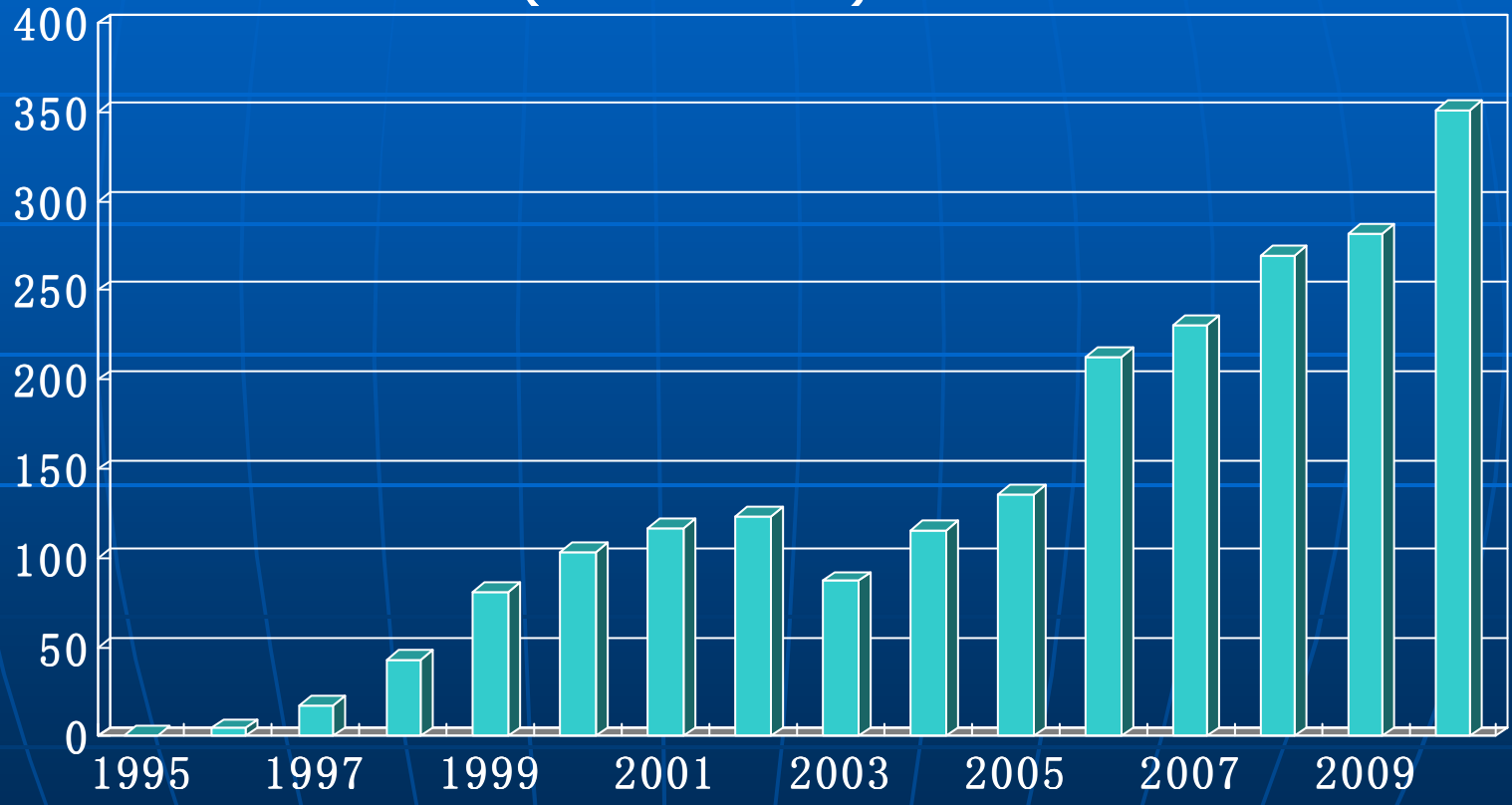
1、Development of two line hybrid rice and seed production in China

1.1 History of two line hybrid rice research and application

- The first PGMS line breeding successfully in 1986.
- Identified thermo sensitivity in 1989 after a unusual low temperature occurred in Summer
- PA64S was the first S line with CT of 23.5℃ released in 1991.
- Declared success of two line hybrid rice research in 1995.
- The first two line super hybrid rice variety Liangyoupeijiu released in 2000.

- Released super two line hybrid rice varieties Liangyou 0293, Y liangyou 1 and Zhun Liangyou 527 with yield potential of 12 T/ha in 2004.
- 《Technical Rules For Seed Producing System of Two Line Hybrid Rice》as a Hunan provincial technical norms released in 2006.
- 《Technical Rules For Seed Producing System of Two Line Hybrid Rice》 redacted in 2010, and will be released as a national technical norms.
- The super two line hybrids Y liangyou 2 reached 13.5 T/ha in 2011.

1.2 Planting area of two line hybrid rice in China (10000 ha)



1.3、**Two line hybrid rice development in China in 2010**

Planting area 3.51 million ha.

- According to planting acreage, among top 10, 5 of them were two line hybrids.
- The first 3 were two line hybrids.
- Seed production area about 25000 ha.
- Average seed yield about 2.0 T/ha, the highest was 4.22 T/ha in about 10 ha area in 2011.

2、The main risk of two line hybrid rice seed production

2.1、The thermo sensitivity of TGMS

The threshold temperature of fertility reversing also called critical temperature (CT).

The S lines used in China were belong to High temperature inducing male sterility type with CT around 23℃～24℃。

The S line with lower CT would be lesser risk when put in seed production.

Abnormal low temperature occurred when the sensitive stage of S line during seed production, is the main risk.

2.1、

CT of the main S line currently used in seed production

line	CT	line	CT
PA64S	23.5	Zhun S	24.0
GZ63S	24.0	Zhu 1S	23.0
Y58S	23.5	XL 628S	23.0
P88S	24.0	HD9802S	23.5
C815S	23.0	1892S	24.0

2.2、**Genetic drift of CT**

Within a S line population, CT of individual plant could be different. The one with higher CT would get more seeds during seed increasing and the number of those plants raised faster than others which inducing CT of population getting higher.

The CT of PA64S identified as 23.3℃ in 1991，after 3 generations seed increasing，it rise to 24.2℃ in 1993。

CT genetic drift increased the risk of two line seed production.

- 2.3、**Bio-mixture during seed increasing and the isomorphic fertile plant**

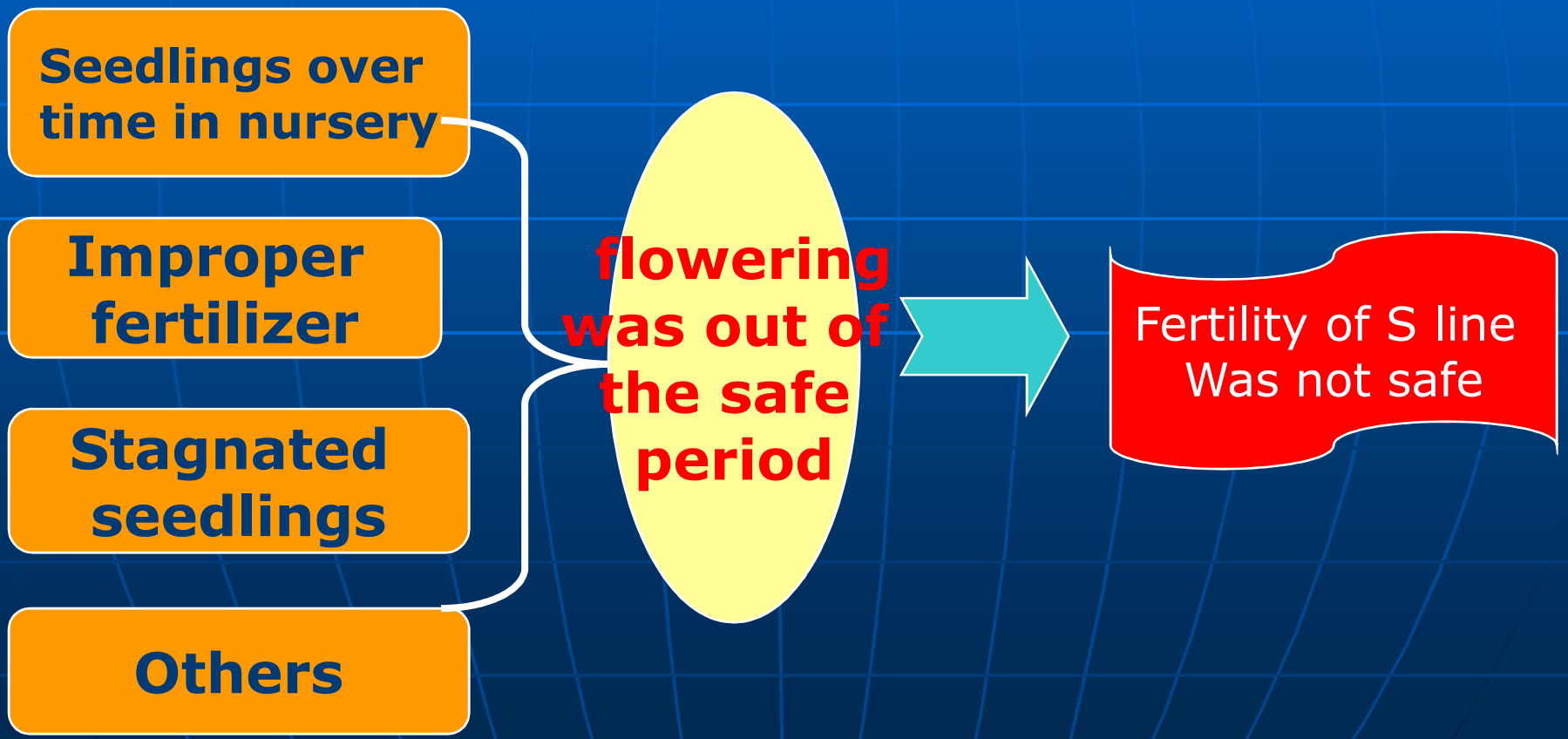
S line could be contaminated by external pollen due to its partial fertility.

When bio-mixture occurred and after 3-4 generations seed increasing, there were some isomorphic fertile plants mixed in the population which had the same morphological traits as the sterile plant but with fertile pollen.

Those fertile plants were difficult to be put out during seed production and the purity of produced seeds could be reduced.

- 2.4、isomorphic fertile plant

2.5 flowering time may out of the expected safe period because of inappropriate cultivation

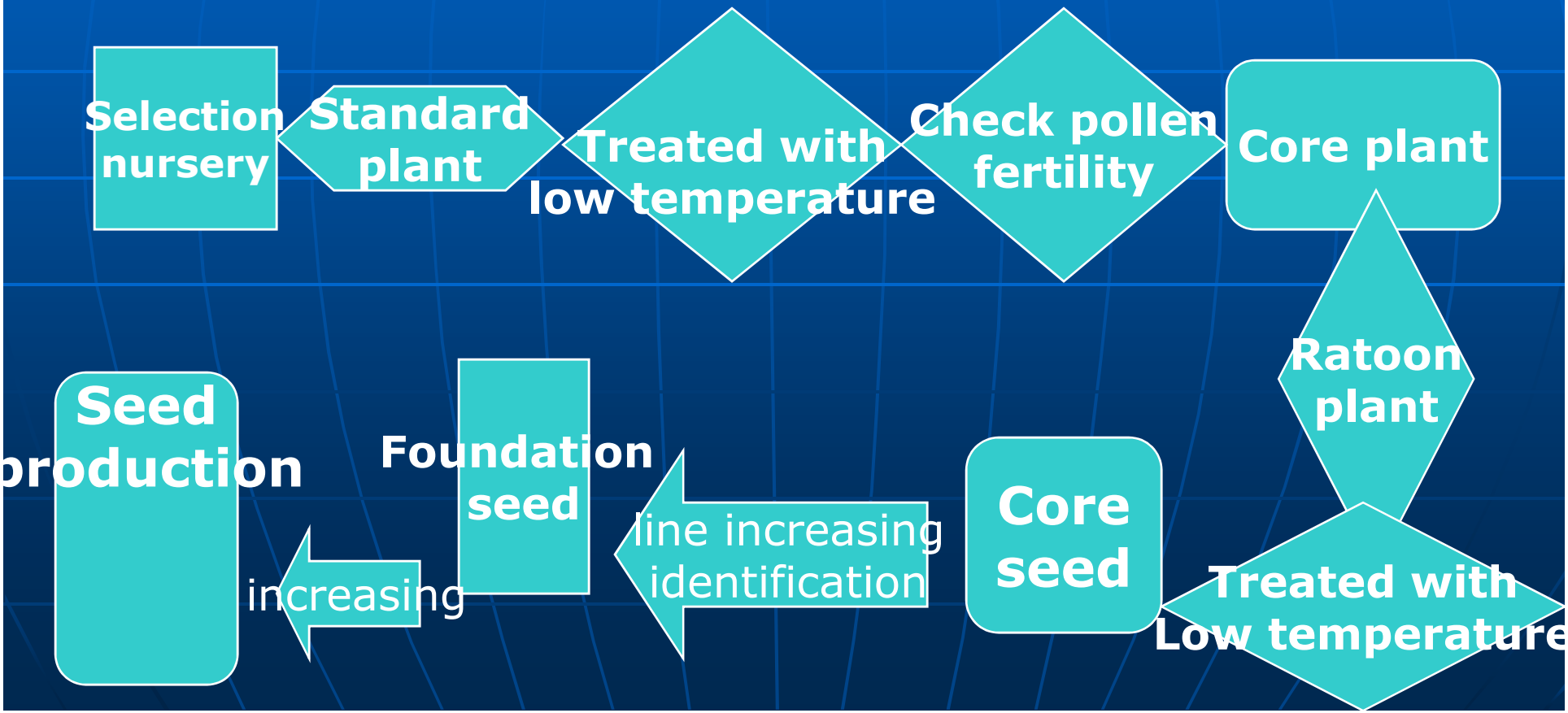


3.1、Use S line with lower CT for seed production

Use a S line with CT of 23.0℃, could select seeds base with larger area and longer time to arrange the sensitive stage.

Different site of seed base and different season had different environment temperature condition, then could use S lines with different CT for seed production.

3.2、S line core seed and foundation seed producing and limited the foundation seed increasing generation used for hybrid seed production.



3.3、S line seed high yielding and high quality increasing

Cold water treatment: Select field had sufficient cold water supply with temperature around 18-20℃. Irrigated with deep water and keep the young panicle submerged. The water temperature should be more than 2℃ lower than the CT of the S line.

Winter season: Increasing seed at south part of Hainan province(N 18°) during winter season, arrange the sensitive stage at early February when the temperature was lowest in a year.

Summer season at high altitude site: Selected land at altitude about 1600m at Yunnan province(N 23°)where was cool in Summer. Arranged the flowering time at early to middle of August.

S line seed increasing

Cole water irrigation

S line seed increasing

Y58S

Winter season at Hainan

S line seed increasing

High altitude at Yunnan

3.3、Techniques for improving S line seed quality during seed increasing

(1) Seed source came from the procedure of core seed to foundation seed and the foundation used no more than two generations.

(2) Strictly isolation. The seed fields should be more then 300m away from other pollen source or the flowering time must be more than 30 days earlier or later than other pollen source near by.

(3) Roguring. Specially at tillering stage, before and after initial heading and before harvesting.

3.4、Selected favorable eco-condition for two line hybrid seed production

Two line and three line seed production were with same techniques except need to insure the male sterility safe.

Index of safe male sterility

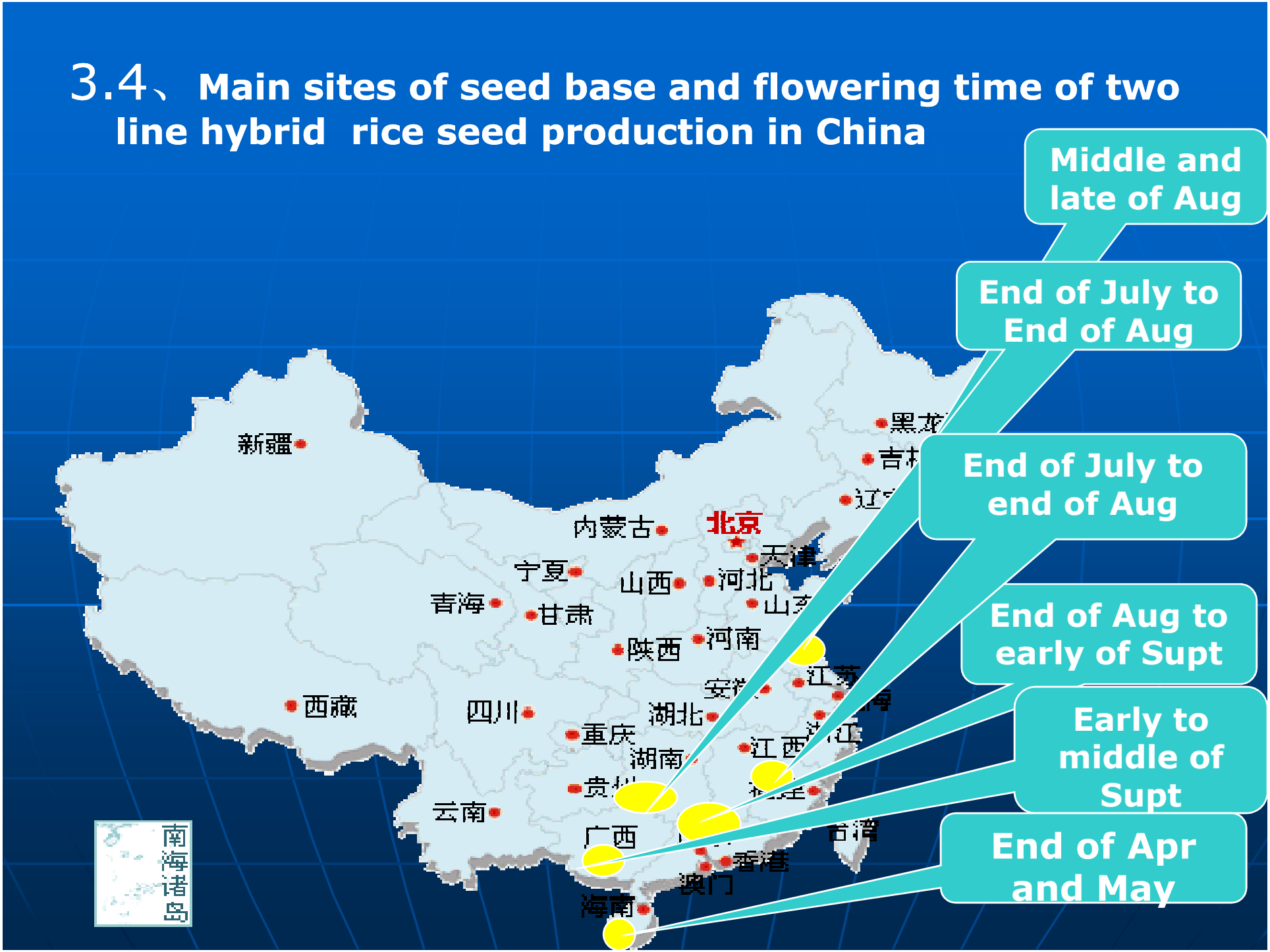
3.4、 **Formula of fertility safe：**

$$\text{Index of fertility safe} = 1 - \frac{\text{Sum of years had continuous 3 days with temperature lower than the CT of used S line}}{\text{All data collected years}}$$

- When the index was 1 means no risk for seed production
- When the index was over 0.95，there were some risk, seed production area should be limited.
- When the index was lesser than 0.95，the risk could be high，should not take commercial seed production.

Two line hybrid rice seed production had to select favorable site as the seed base and favorable season to insure the male sterility stable.

3.4、Main sites of seed base and flowering time of two line hybrid rice seed production in China



3.5、Normative cultivation of S line to make the flowering time within the expected favorable period

- **Techniques：**

  Using enough S line seed .

  Rare seeding in nursery for raising vigour seedling.

  transplanting seedlings on time.

  Early apply fertilizer and control N at middle and late growing stage

  Control late tillers

- **Targets:**

  Developing a uniformly female population with less late tiller and flowering under expected time.

3.6、**Monitoring fertility of S line and forecast seed purity**

- Monitoring fertility of S line：

（1）Temperature analysis

Observe and analysis environmental temperature during the fertility sensitive stage of S line.

（2）Check pollen and anther

check pollen fertility under microscope and observe appearance of anther.

（3）Isolated planting

Take sample plants from seed field and move to a strictly isolated place to check the self seed set rate.

3.6、forecast seed purity

- Formula of seed purity：

$$X(\%) = 100 - \left(a + \frac{n}{m} \times 100\right)$$

X: estimated seed purity（%）;

a: off type（%）, number from field inspection.

n: Self seed number from isolated planting（%）;

m: outcrossing seed set number from the seed production fields（%）。


Thanks
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