Construction and genetic evaluation of chromosome segment substitution lines (CSSLs)in tobacco (*Nicotiana tabacum* L.)

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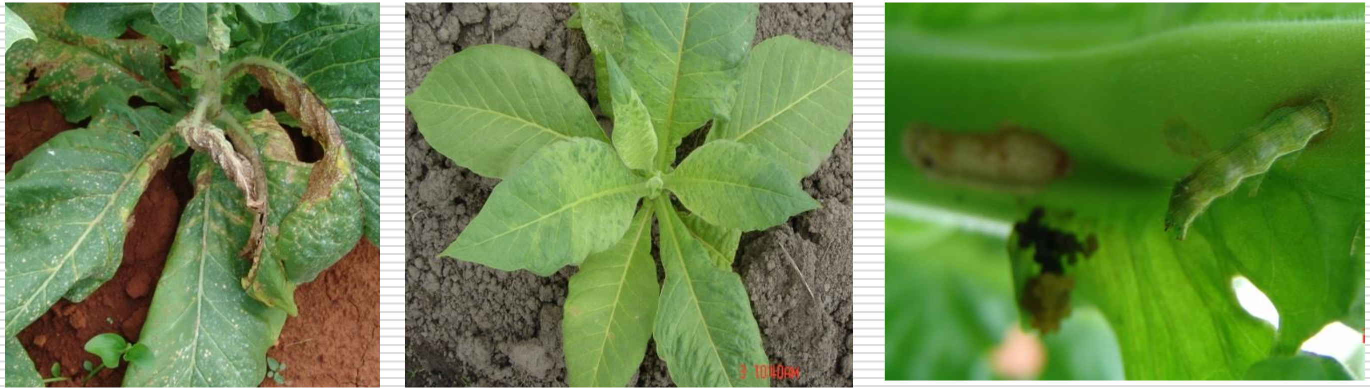
Outline

- **Background**
- **Methodology**
- **Results**
  - **Selection of polymorphic microsatellite markers of tobacco genome**
  - **Construction of tobacco CSSLs**
  - **Genetic Evaluation of CSSLs: Distribution, number and length**
- **Upcoming experiments**

Background

- **Leaf production is affected by:**
  - **Biotic stresses**



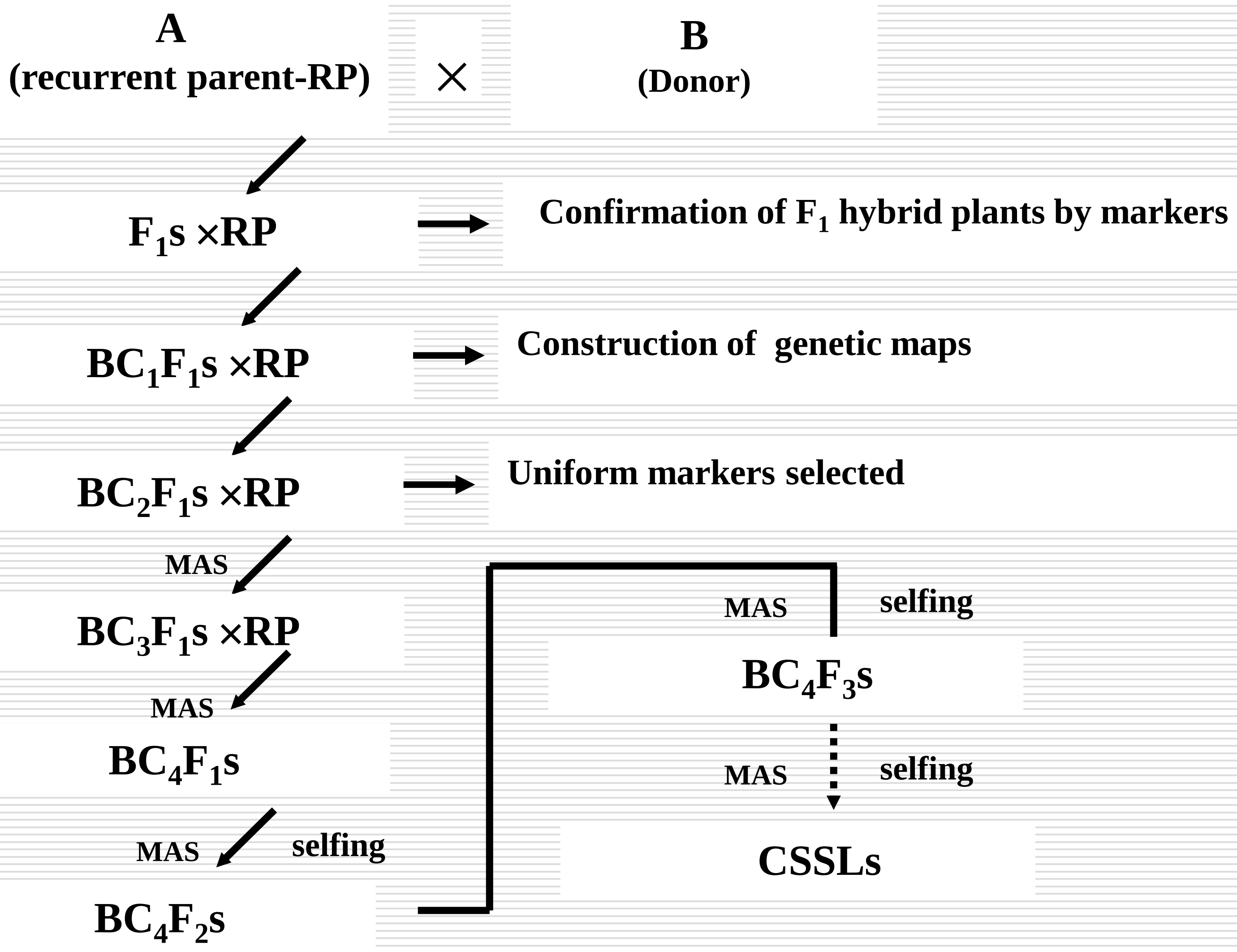
  - **Abiotic stresses**

Background

- It is Known that many important traits of tobacco are controlled by quantitative trait loci (QTL) . However,
  - The developed mapping populations of F_2 or BC_1 and even doubled-haploid and recombinant inbred lines, cannot be used to estimate single QTLs precisely.
  - These populations are not ideal for characterization of target QTLs and further intensive analysis.
  - Moreover, phenotype of QTLs are always influenced by genetic background and environmental factors
  - Great attention of CSSLs have been conducted in corn, rice and cotton.

Background

- **CSSLs ?**
  - **SSR markers were used**
  - **Target plant carries a single or a few chromosomal segments from the donor parent in the genetic background of RP were selected**
  - **Ideal CSSLs population: has ONLY one different chromosome segment, while the whole population cover the entire genome of the donor**
- **To our knowledge , this is the FIRST report on CSSLs research in tobacco**



A
(recurrent parent-RP) × **B**
(Donor)

F_1s ×RP → **Confirmation of F_1 hybrid plants by markers**

BC_1F_1s ×RP → **Construction of genetic maps**

BC_2F_1s ×RP → **Uniform markers selected**

MAS

BC_3F_1s ×RP

MAS

BC_4F_1s

MAS **selfing**

BC_4F_2s

MAS **selfing**

BC_4F_3s

MAS **selfing**

CSSLs

Methodology

- ☐ **Construction of CSSLs**
  - **BC1 populations were constructed in 2013.(300 plants)**
  - **A total of 100 SSR markers were produced in 2014**
  - **Stable and homozygous tobacco CSSLs were established in Feb.2016.**
- ☐ **Genomic DNA extraction and genotypic analysis of the substitution lines**
- ☐ **Evaluation of the substitution lines**

Selection of Polymorphism SSR markers in tobacco

- **Two preliminary analyses were conducted for tobacco CSSL construction.**
  - **13,645 pairs of tobacco SSR markers were used**
  - **Two SSR genetic maps (unpublished data) with 626 and 562 marker loci, respectively, were constructed based on the two BC_1F_1 plants derived from crosses between the donor parents (K326 and Beinhart1000-1) and the recipient parent (Y3) , in which 416 SSR markers were shared by the two maps and evenly distributed in the 24 linkage groups**

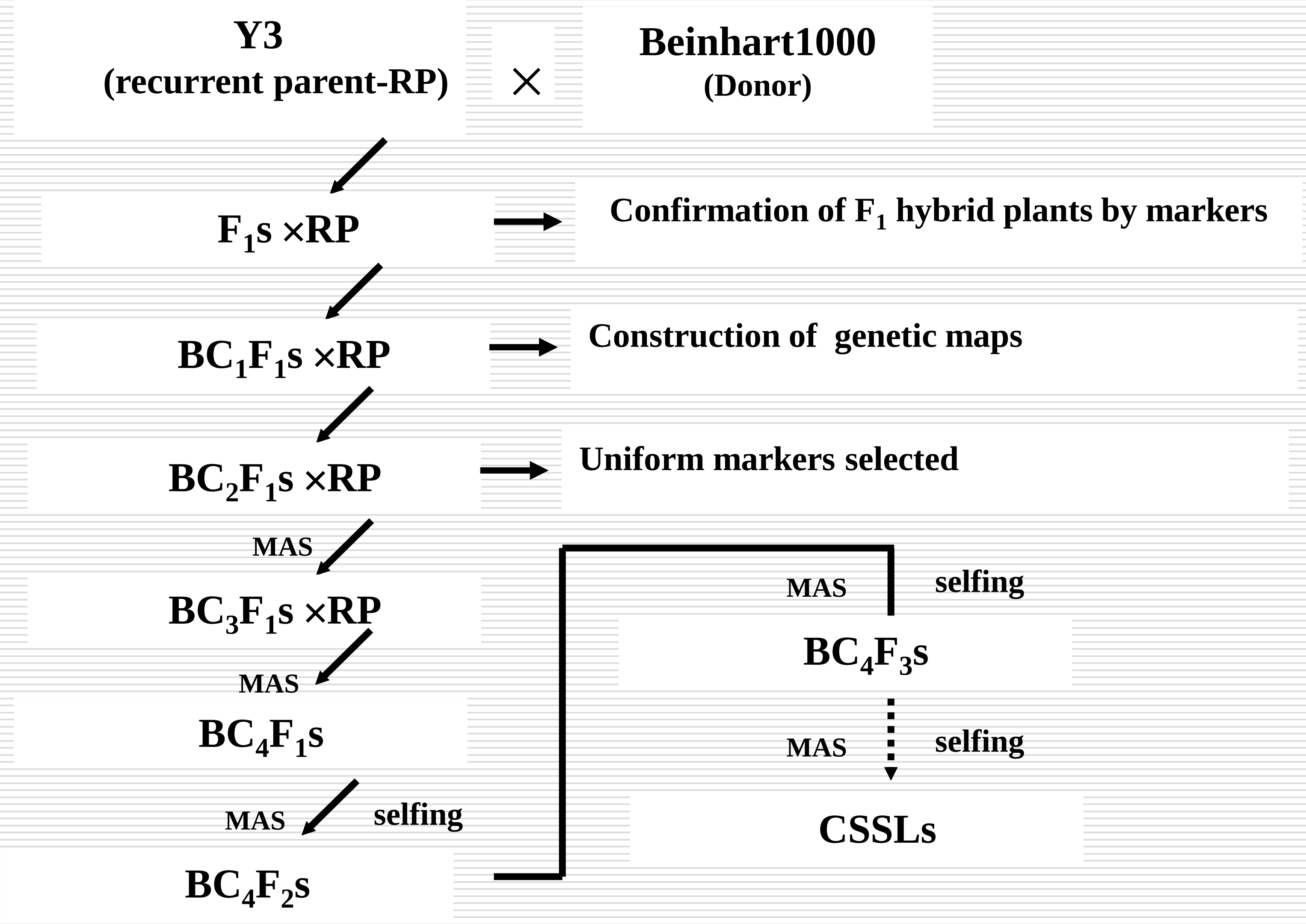
Polymorphism between the recipient and donors detected by tobacco SSR markers

Donor	No. of marker	No. of polymorphic marker	Ratio of polymorphism (%)	Average distance between polymorphic marker$^{2)}$(cM)	No. of shared polymorphic marker
K326	18764	626	3.34	1.79 (1120.45cM/626)	416
Beinhart1000-1	18764	562	3.00	2.39 (1341.18cM/562)	

Construction of CSSLs

☐ The procedure used for CSSL development is shown

- choose 10-20 cM for 1 SSR
- 100 SSR mark for tracking and detecting
- Distance range 0.05-36.88cM


Y3
(recurrent parent-RP)
×
Beinhart1000
(Donor)

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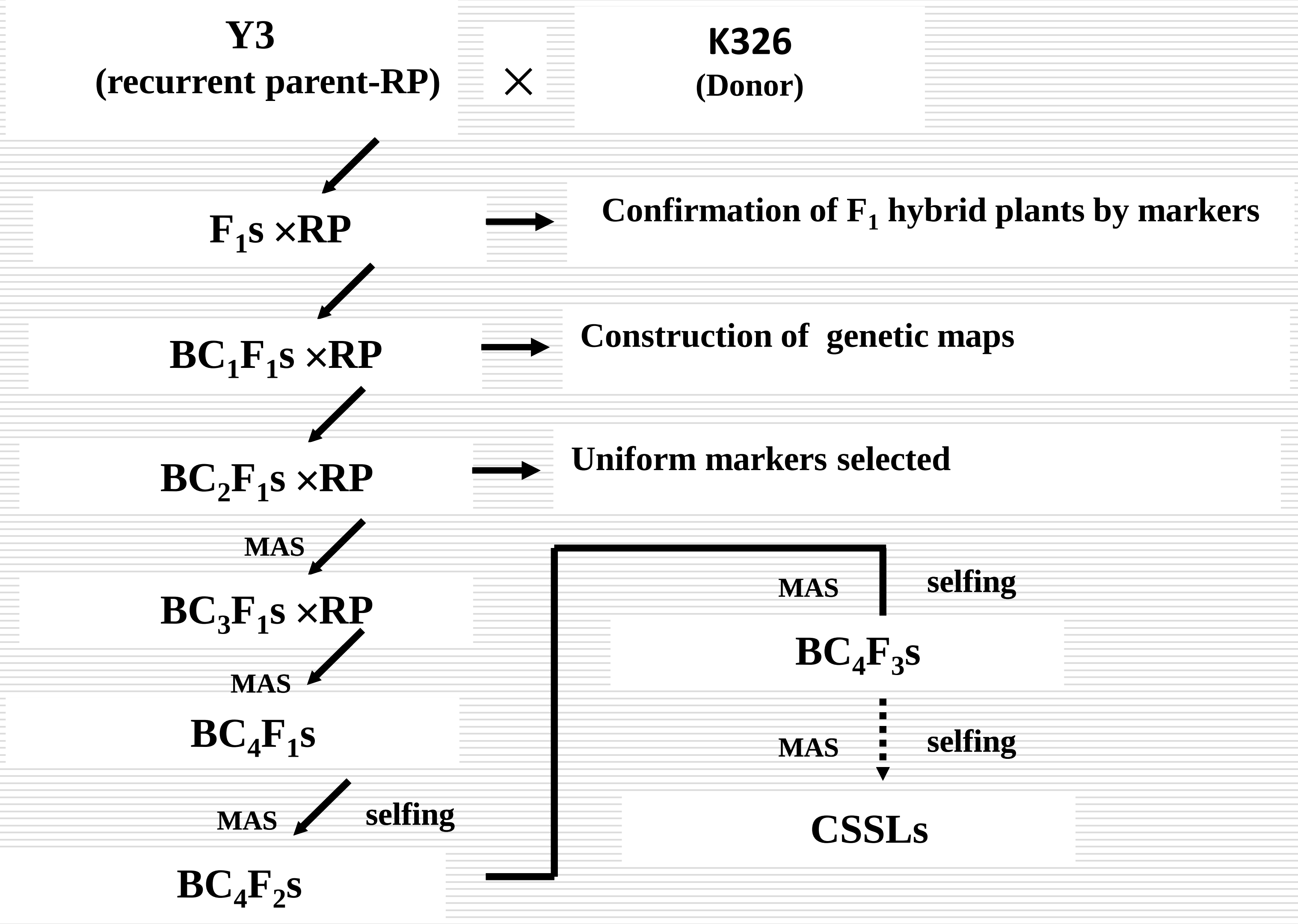

Y3
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F_1s ×RP → **Confirmation of F_1 hybrid plants by markers**

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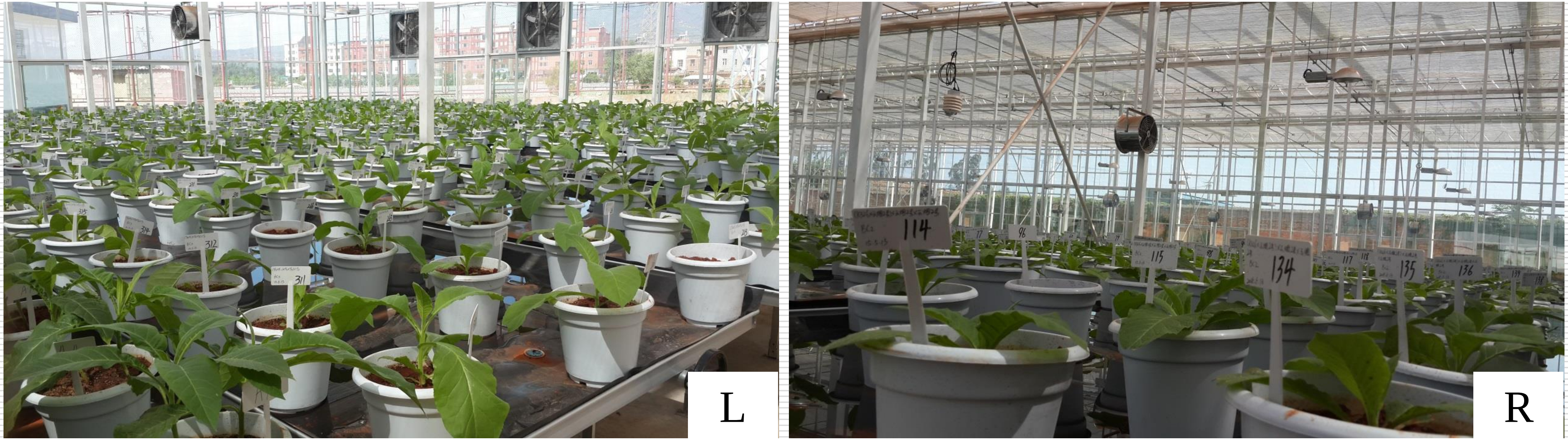
CSSLs



Selected BC_3F_1 lines（L:Y3/K326 .R:Y3/Beinhart1000-1) by tracking and detecting

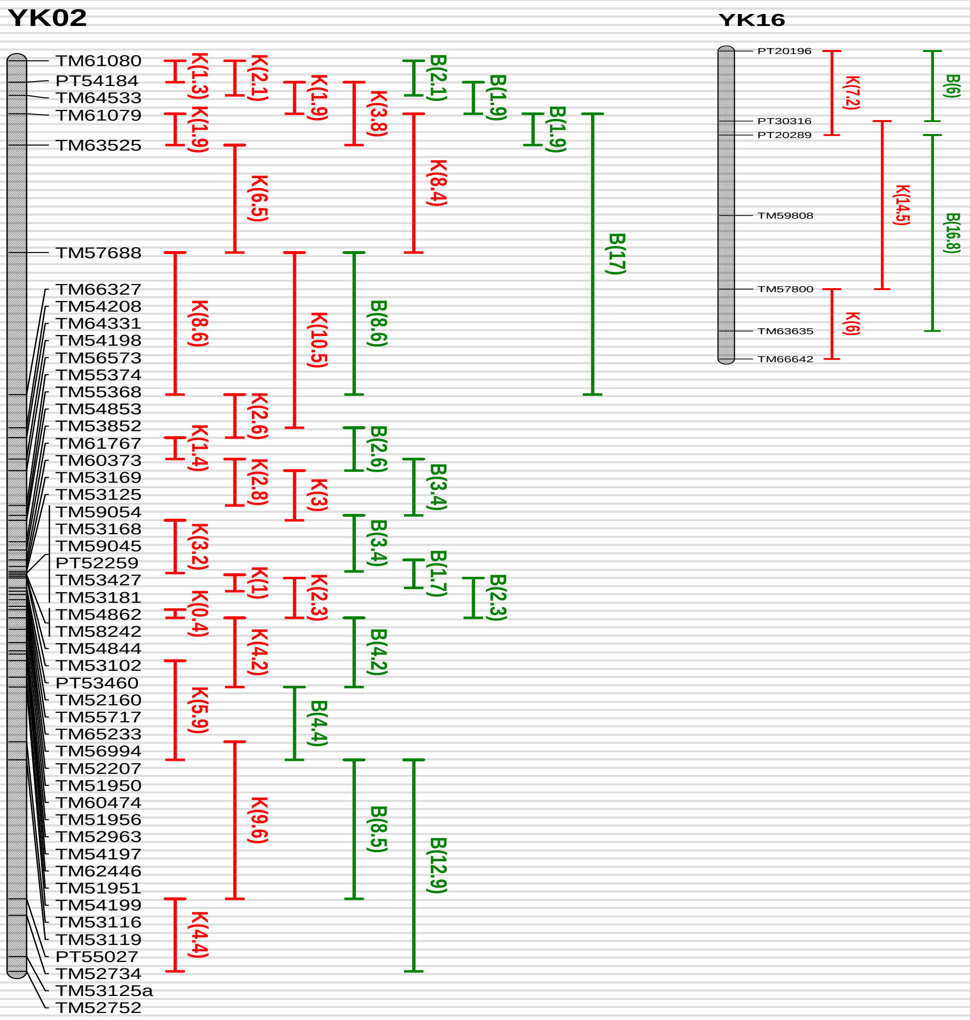
Genetic analysis of substituted chromosome segments in CSSLs

- **Totally, a set of CSSLs containing substitution chromosome segments from K326 and Beinhart1000-1 were generated**

Donor	Number CSSLs	Number segments	Segments/ CSSLs	Size of Segments (cM)
K326	131	215	1.64	0.05-36.88
Beihart1000-1	125	162	1.30	
Total	256	377	1.47(Ave.)	7.75(Ave.)

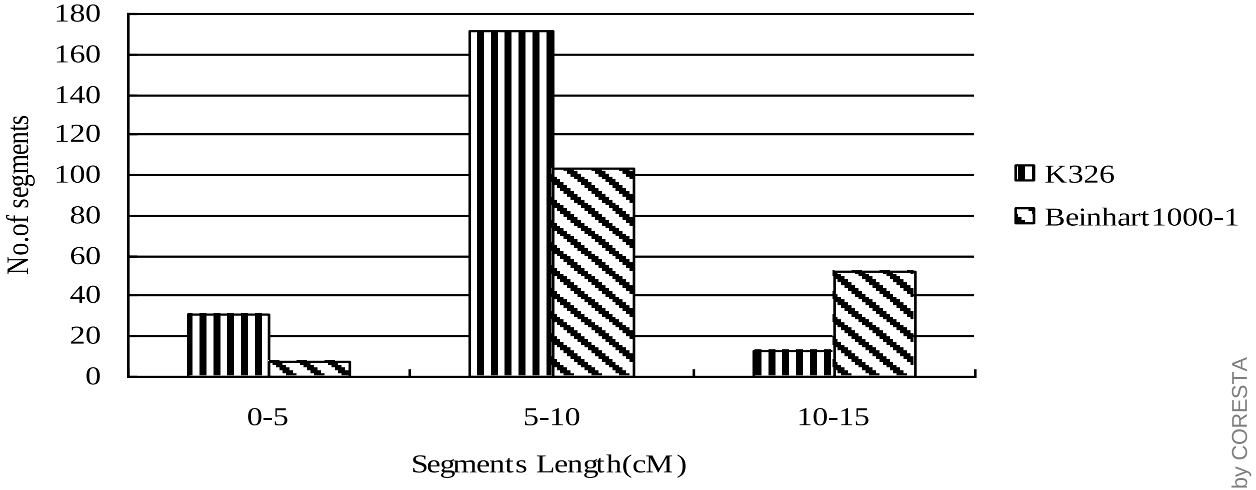
Genetic analysis of substituted chromosome segments in CSSLs

- Uneven distribution happened for 377 segment among the 24 linkage groups (LG).
  - The most introgressed segments found in LG2(35)
  - LG 16 having the least(5)
  - For 215 segments from K326,LG 2 contained the most segments(21), LG16 having the least(3)
  - For 162 segments from Beinhart1000-1,LG 2 also contained the most segments(14), LG16 having the least(2)



Genetic analysis of substituted chromosome segments in CSSLs

- **Segments from K326 and Beinhart1000-1 were smaller than 5 cM**
- **Major segments from K326 and from Beinhart1000-1 were from 5-10 cM**
- **While 13 segments from K326 and 52 segments from Beinhart1000-1 ranged from 10-15 cM.**



Conclusions

- The first set of CSSLs in tobacco has been constructed(ONLY carried 1-5 segments).
- The CSSL population provided a useful tool for gene identification, these CSSLs will also provide a foundation for tobacco improvement on disease resistance and leaf quality.

Upcoming Experiments

- **Continue to find new CSSLs.**
- **Elucidating a major gene for brown spot resistance .**

Thank you for your attention