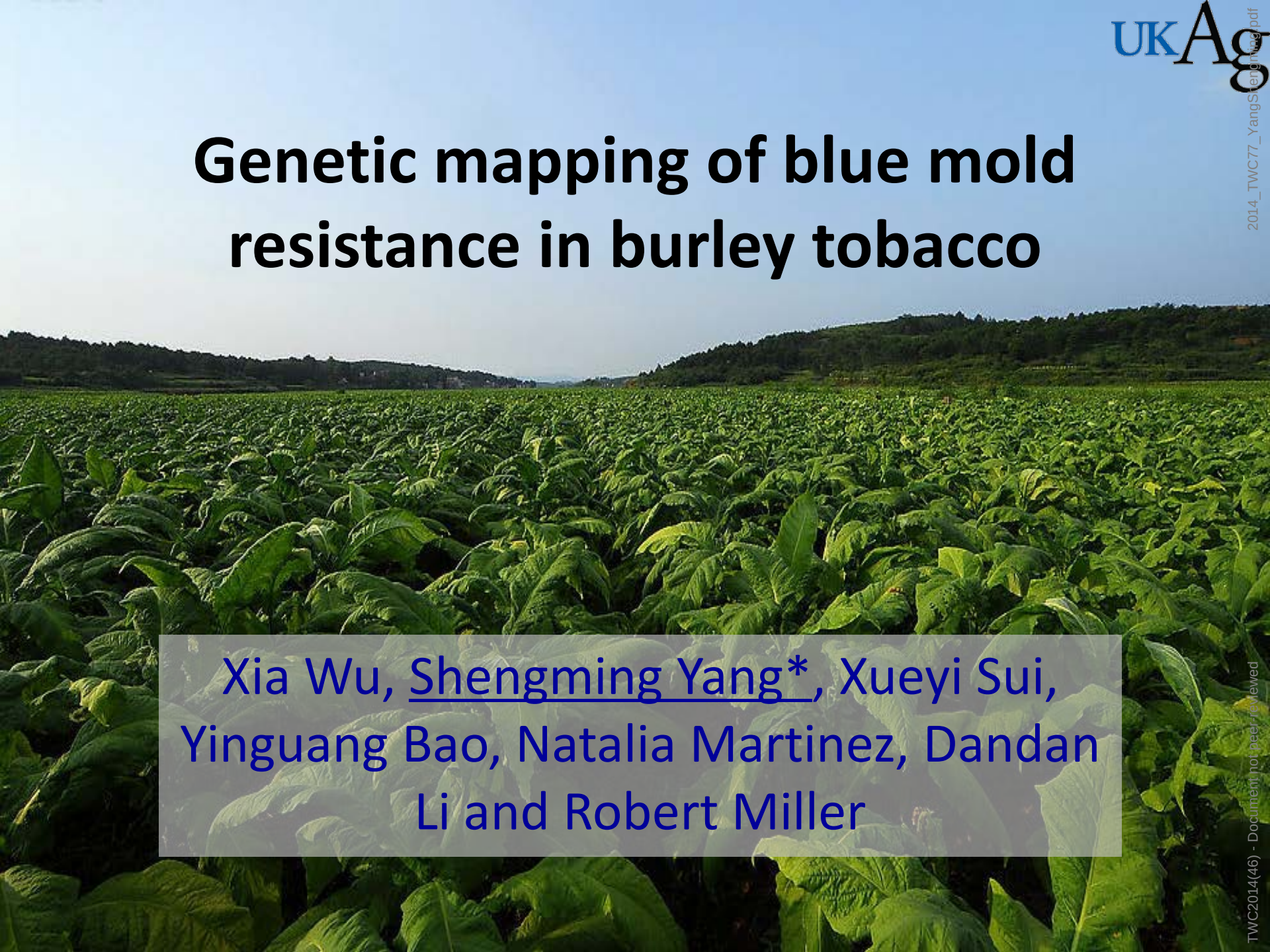
Genetic mapping of blue mold resistance in burley tobacco



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Background

- The Kentucky-Tennessee Tobacco Improvement Initiative (KTTII) has been very productive in releasing burley and dark tobacco varieties since its inception in 1999.
- About 80-85% of the burley tobacco crop and 20-25% of the dark tobacco crop in the United States is produced from varieties developed and released from KTTII
- The primary focus of the KTTII breeding program was the development of new disease resistant tobacco varieties through traditional back-cross and pedigree breeding schemes.
  - black shank, blue mold, black root rot, PVY and TMV
  - Molecular markers for resistance have been routinely used

Background

- In January 2014, a Tobacco Genomics Laboratory was founded supported by KTTII, KTRDC and the Department of Plant and Soil Sciences at the University of Kentucky.
  - To provide service for tobacco breeding program by means of development of user-friendly molecular markers
    - Disease resistance to black shank, blue mold and PVY
  - To understand molecular mechanisms underlying tobacco-specific traits, such as nicotine biosynthesis
    - With the impending FDA regulation, the levels of alkaloids in tobacco are assuming increasing importance.
    - Gene isolation for *Nic1*
    - Design co-dominant gene-specific markers for *Nic1*
  - To provide a service platform for the whole tobacco community
    - To generate a tobacco Tnt1-retrotransposon insertion mutant library

Blue Mold

- Blue mold is caused by the downy mildew pathogen *Peronospora tabacina* D. B. Adam (syn. *P. hyoscyami* de Bary).
- It was first reported in tobacco-growing areas of Australia during the 1800s.
- Blue mold epidemics have resulted in annual losses exceeding $200 million in North America (Heist et al., 2002) .

Blue Mold

- The pathogen can infect plants at any growth stage.
- It can spread quickly if weather conditions are favorable.
- The loss from disease could be only slight or completely destructive, depending on environmental conditions.

Symptoms of Blue Mold

- Single or groups of yellow circular spots occurs on the leaves.
- The grey to bluish downy mold grows on the lower surface of the tobacco leaves.

Symptoms of Blue Mold

- The affected vein becomes reddish-brown, and the area around the vein is yellowed and distorted.
- At last, the diseased leaves become twisted.
- The whole leaf could fall apart from the plant.

Resistance to blue mold

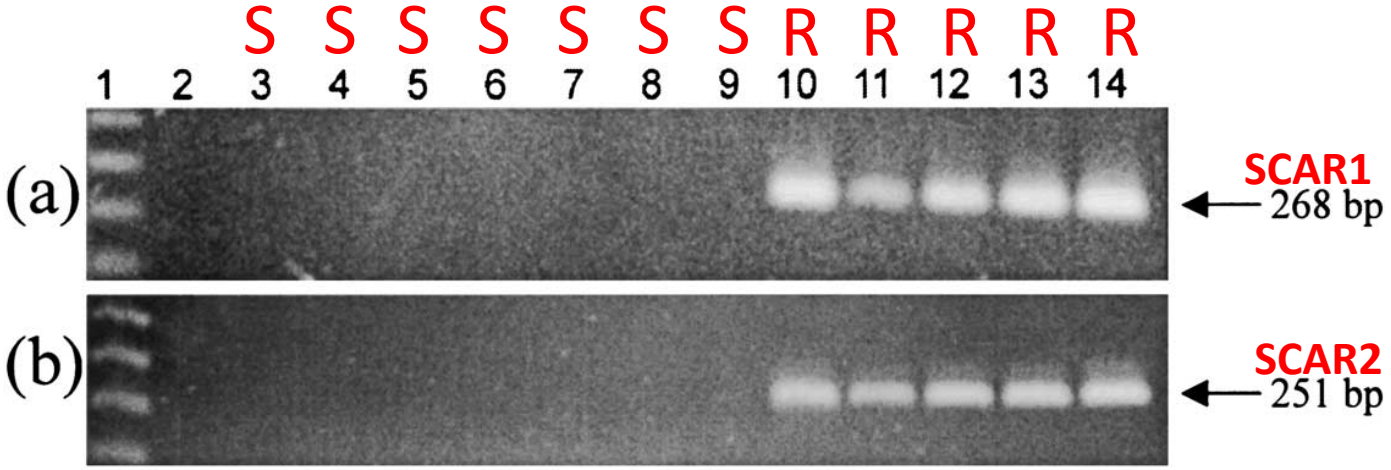
- Tobacco blue mold can be effectively controlled by chemical treatment.
- Given the economic cost and possible negative environmental effects of long-term fungicide application, utilization of host resistance to this disease would be a preferred strategy for blue mold control in tobacco.
- No any natural resistant variety has been identified in cultivated tobacco.

Resistance to blue mold

- Several *Nicotiana* species were reported to contain the blue mold resistance.
- The resistance originated from *N. debneyi* was introgressed into cultivated tobacco (Milla *et al*, 2005)
- The molecular makers linked to this resistance gene to blue mold should be very valuable for tobacco breeding.

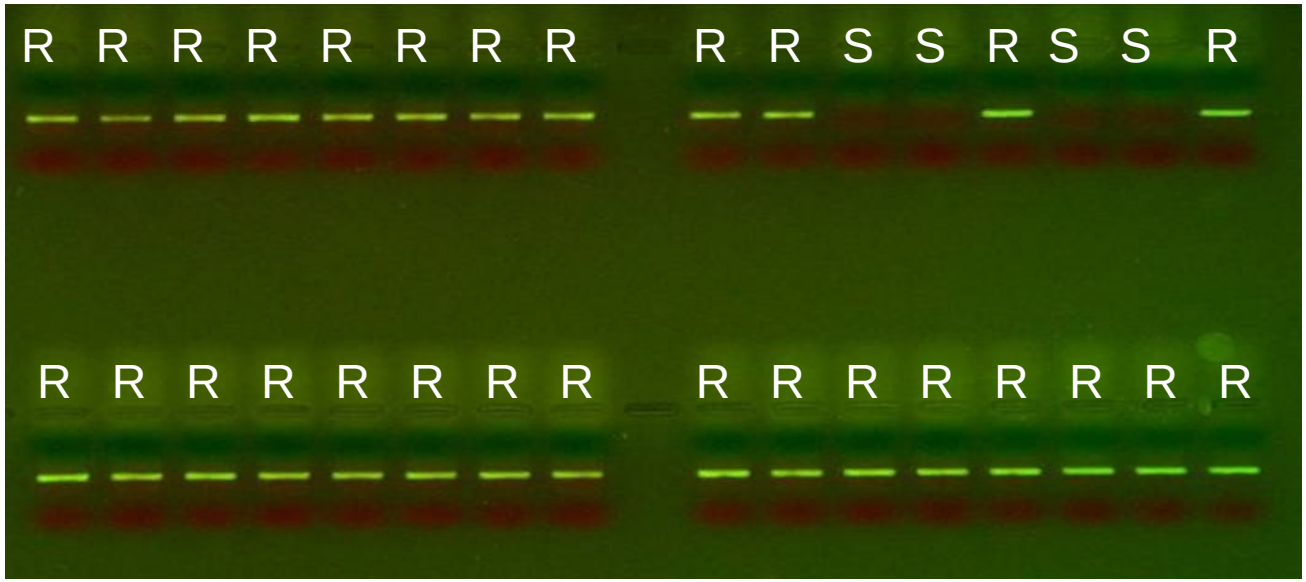
Resistance to blue mold

- Two flanking SCAR markers were identified to be linked with resistance to blue mold (Milla *et al*, 2005)



Lane 1 is molecular size maker, Lane 2 is water, Lane 3-9 are blue mold susceptible varieties, Lane 10-14 are blue mold resistant lines(Milla *et al*, 2005)

SCAR marker for blue mold



Screening of different breeding lines

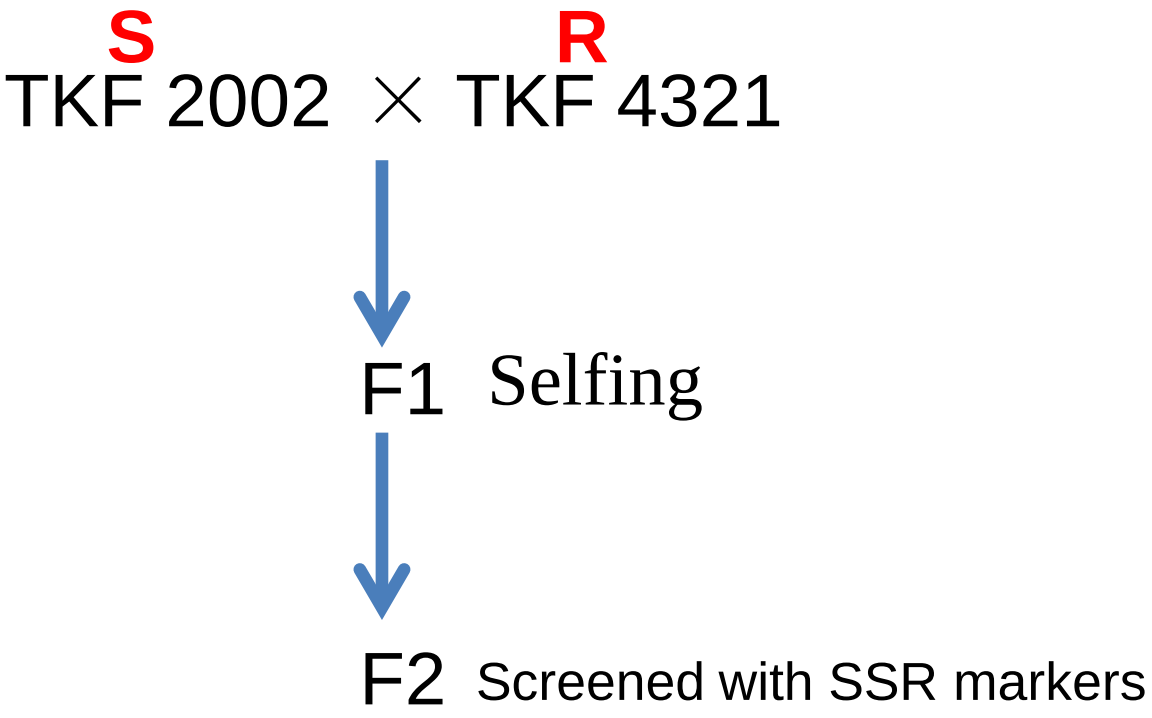
Resistance to blue mold

- These markers are dominant, therefore，homozygous resistant plants and heterozygous plants cannot be distinguished.
- Their chromosomal locations are also unknown to us.
- Genetic mapping of disease resistance to blue mold is necessary for improvement on selection efficiency.

Gene mapping of blue mold resistance

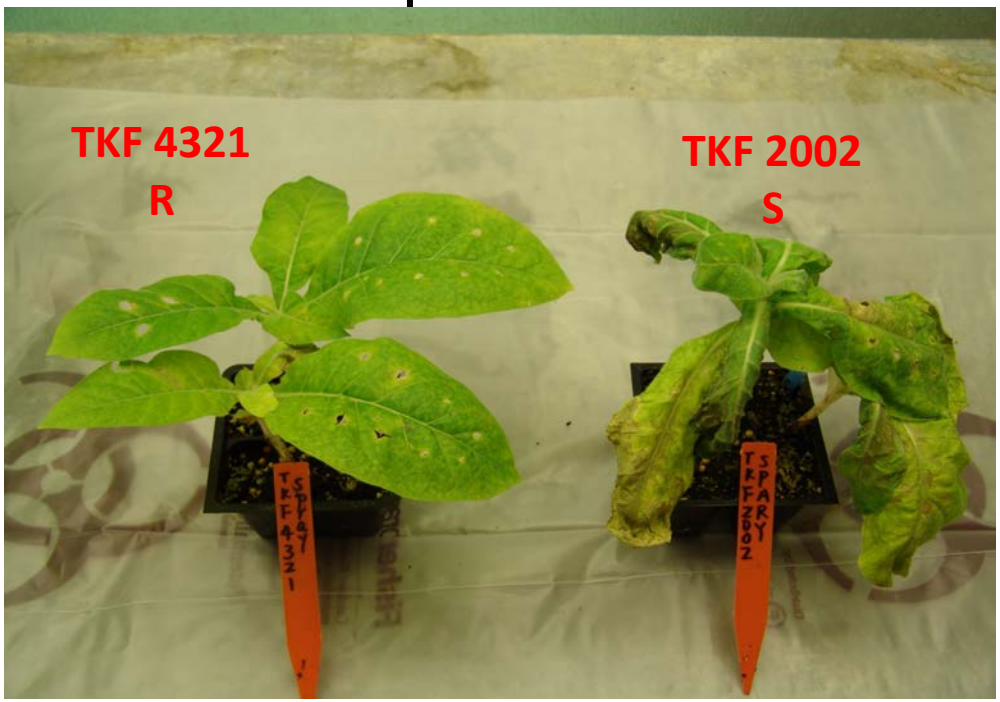
•>500 F2 plants from TKF2002 (S) × TKF4321 (R) were inoculated with blue mold pathogen.

•>250 SSR markers were screened.

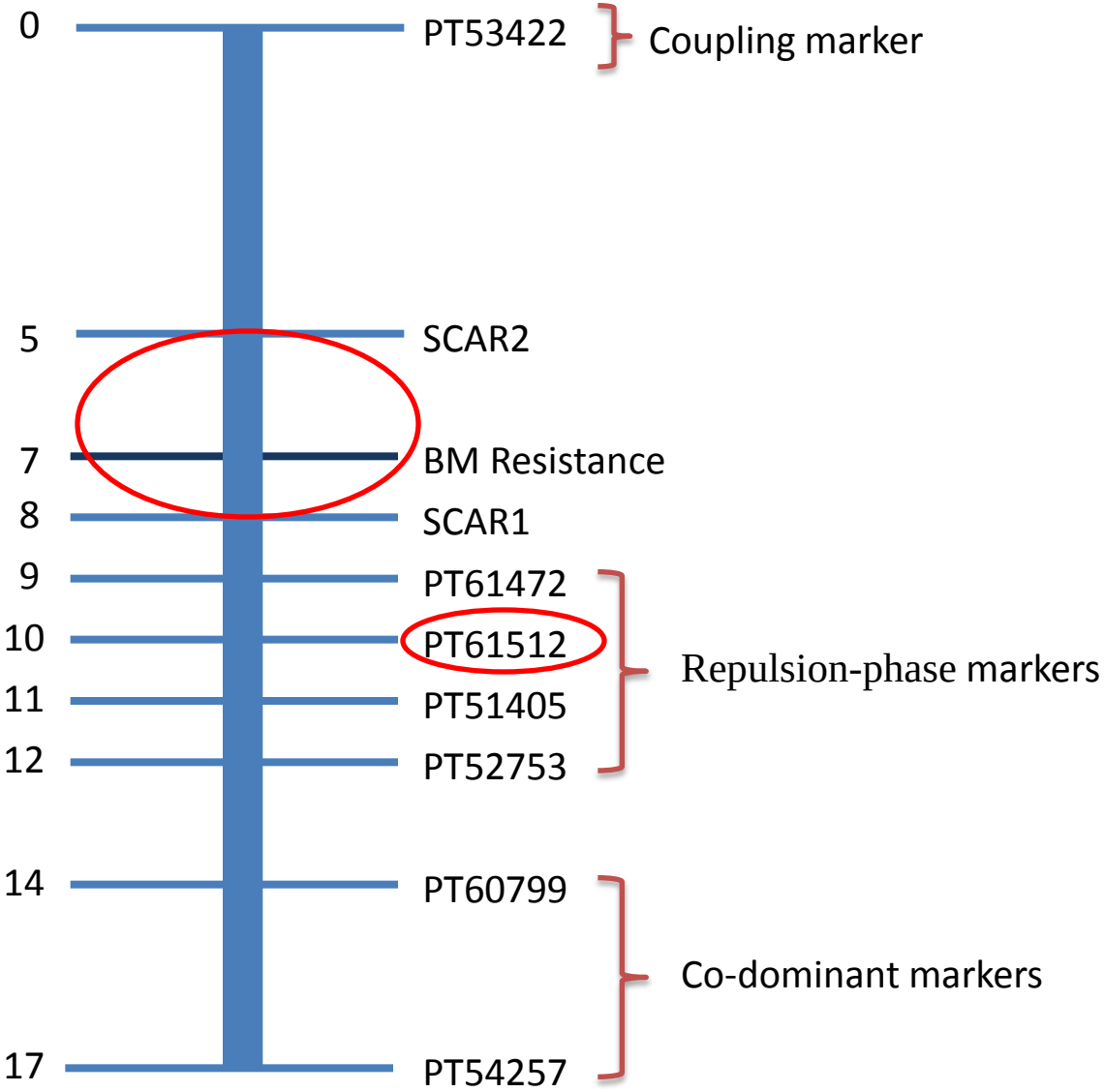


Blue mold inoculation

- Inoculated by spraying sporangial suspension.
- Placed in the pre-moistened plastic tubs overnight .
- Moved onto shelves in a growth chamber.
- Scoring the plant reactions (1 week) after inoculation.
- Double checked the plant reactions.

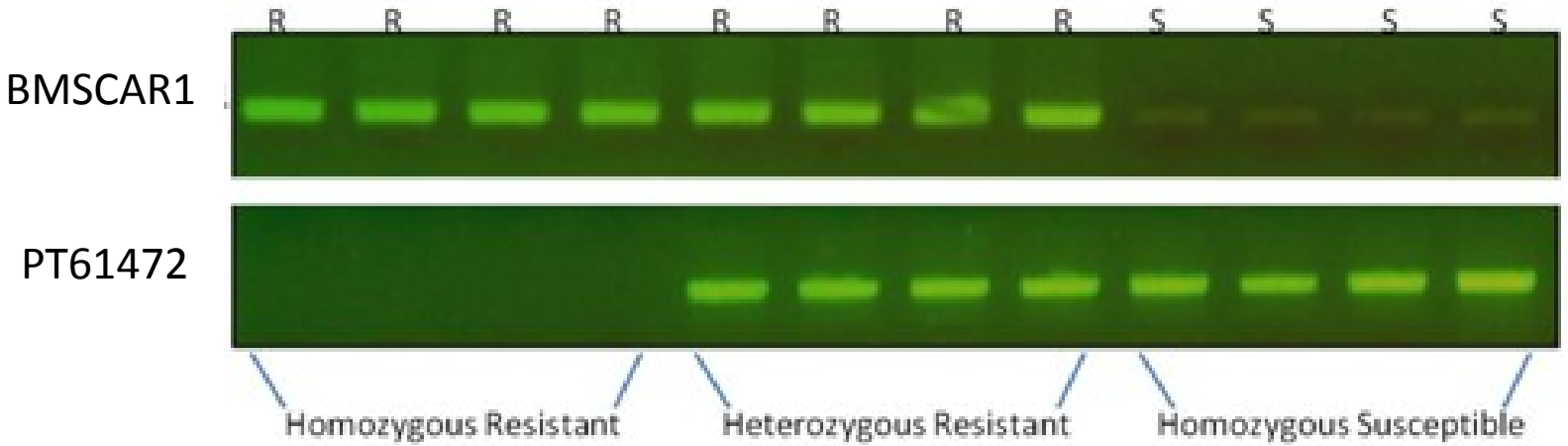


Genetic mapping of BM resistance



The thin blue line indicated linkage group 7; markers we screened were listed on the right side and genetic distance on the left side of chromosome.

Repulsion-phase marker PT61472



Using BMSCAR1 and PT61472 to screen a segregating population.

1. Missing of BMSCAR1: homozygous susceptible plants
2. Missing of PT61472 : homozygous resistance to BM
3. Showing both BMSCAR1 and PT61472 : heterozygous plants

Summary

•A genetic map was constructed based on the combination frequency among markers

•One repulsion-phase marker PT61472 was identified to be closely linked with BM resistance

•Combined BMSCAR1 with the repulsion marker PT61472, homozygous and heterozygous resistant plants can be easily distinguished.

Acknowledgements

•**BAT (** British American Tobacco**)**

KTRDC (Kentucky Tobacco Research & Development Center)

Dr. David Zaitlin

J. T. Hall

Glen Weinberger

Thanks, Questions?