Dissecting loci and genes controlling Sclerotinia disease resistance associated with flowering time in rapeseed

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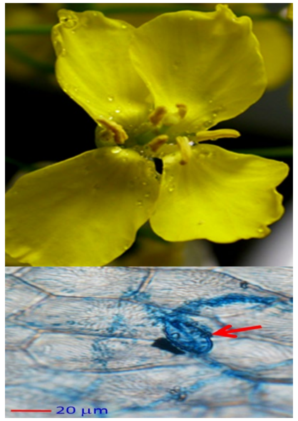
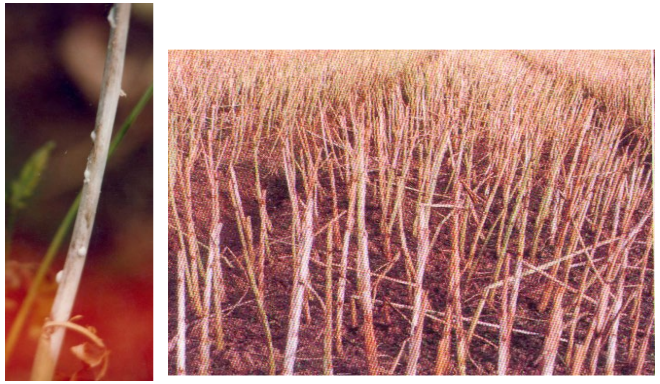
1. Background / overview

- *Sclerotinia sclerotiorum*-caused stem rot is a major disease in China and other regions of the world

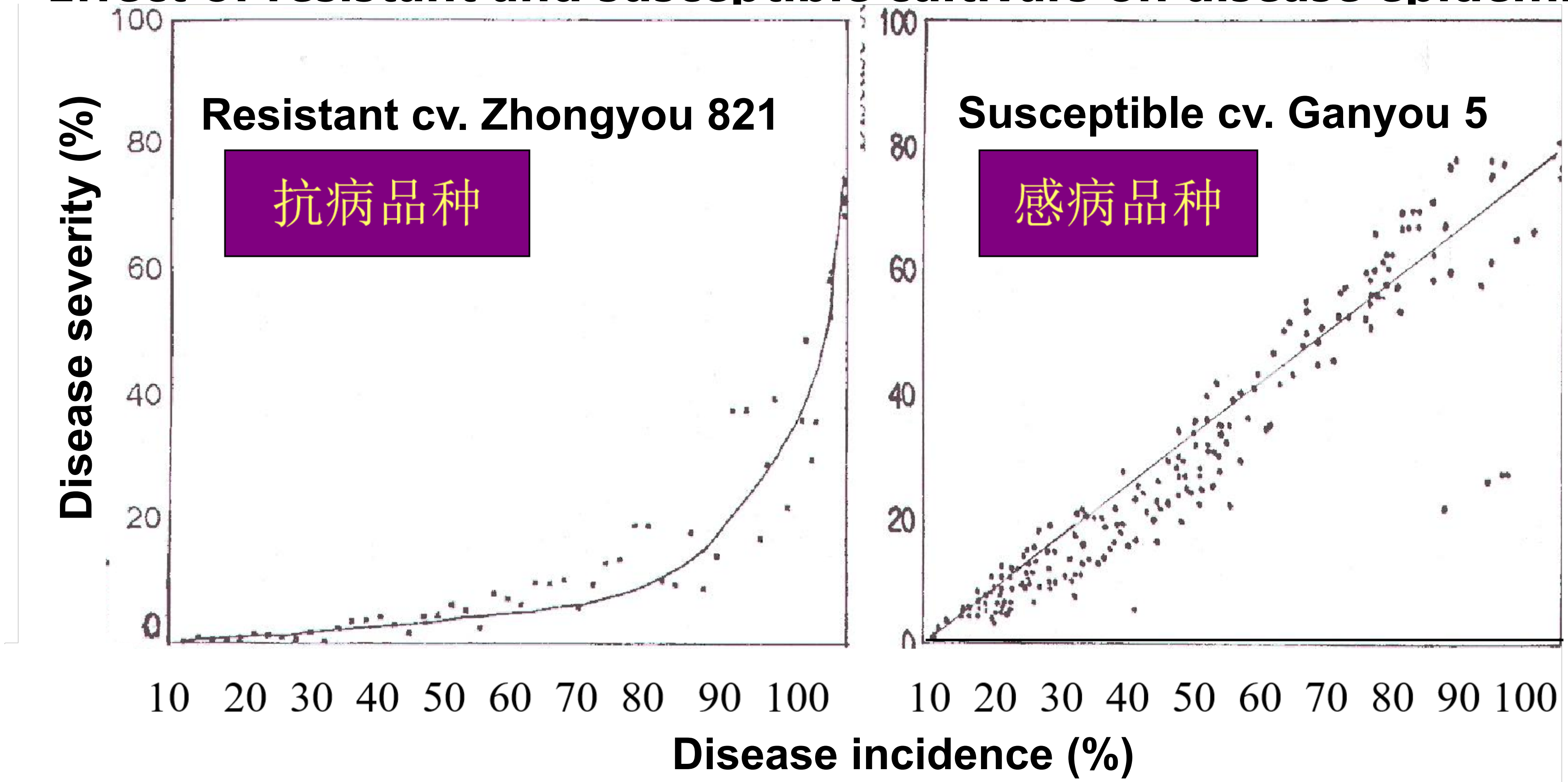
- Annual yield loss is 5% - 30%, equal to RMB ￥8 400 millions (US＄1 200M) in China





Resistant cultivars play a fundamental role in the disease control

Effect of resistant and susceptible cultivars on disease epidemic



Zhongyou 821 had a historic contribution to rapeseed production in 1980s-2000s in China

The consequence of high incidence but low severity:

1. low yield loss at harvest.

2. low pathogenic inoculum and thus reduce infection in subsequent seasons.

- Since 1995, my group has conducted varietal resistance identification of the China Rapeseed Variety Regional Test
- Prevention of high susceptible cultivars into market

ICS 65.020.01
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NY

中华人民共和国农业行业标准

NY/T 3068—2016

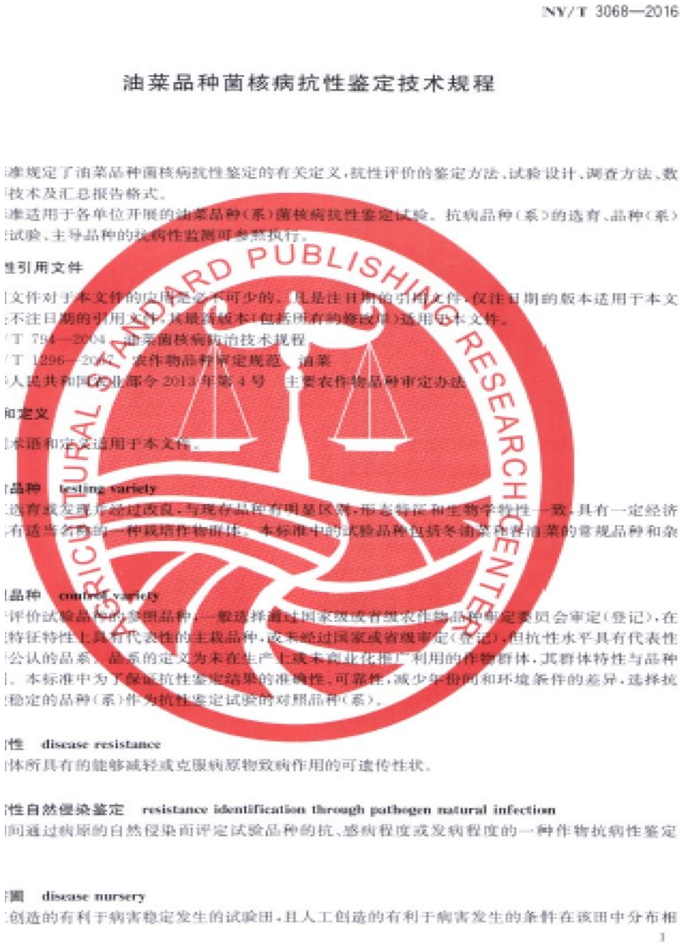
油菜品种菌核病抗性鉴定技术规程

Code of practice for identification of resistance to *Sclerotinia sclerotiorum* in oilseed rape

2016-12-23 发布　　2017-04-01 实施

中华人民共和国农业部 发布

Code of practice for identification of resistance to Ss in OSR



NY/T 3068—2016

油菜品种菌核病抗性鉴定技术规程

准规定了油菜品种菌核病抗性鉴定的有关定义、抗性评价的鉴定方法、试验设计、调查方法、数
技术及汇总报告格式。
准适用于各单位开展的油菜品种(系)菌核病抗性鉴定试验。抗病品种(系)的选育、品种(系)
试验、主导品种的抗病性监测可参照执行。

性引用文件

文件对于本文件的应用是必不可少的。凡是注日期的引用文件，仅注日期的版本适用于本文
不注日期的引用文件，其最新版本(包括所有的修改单)适用于本文件。
T 794—2004 油菜菌核病防治技术规程
T 1296—2007 农作物品种审定规范 油菜
人民共和国农业部令 2013 年第 4 号 主要农作物品种审定办法

和定义

术语和定义适用于本文件。

品种 testing variety

选育或发现并经过改良、与现有品种有明显区别、形态特征和生物学特性一致，具有一定经济
有适当名称的一种栽培作物群体。本标准中的试验品种包括冬油菜和春油菜的常规品种和杂

品种 control variety

评价试验品种的参照品种，一般选择通过国家级或省级农作物品种审定委员会审定(登记)，在
特征特性上具有代表性的主栽品种，或未经过国家或省级审定(登记)，但抗性水平具有代表性
公认的品系。品系的定义为未在生产上或未商业化推广利用的作物群体，其群体特性与品种
。本标准中为了保证抗性鉴定结果的准确性、可靠性，减少年份间和环境条件的差异，选择抗
稳定的品种(系)作为抗性鉴定试验的对照品种(系)。

性 disease resistance

体所具有的能够减轻或克服病原物致病作用的可遗传性状。

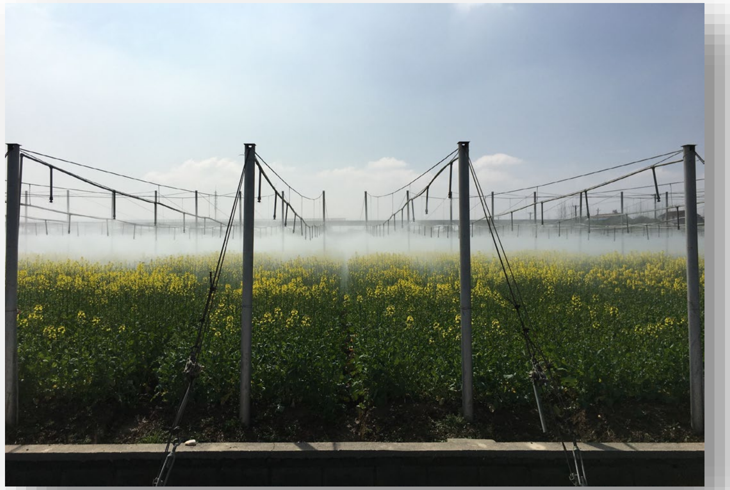
性自然侵染鉴定 resistance identification through pathogen natural infection

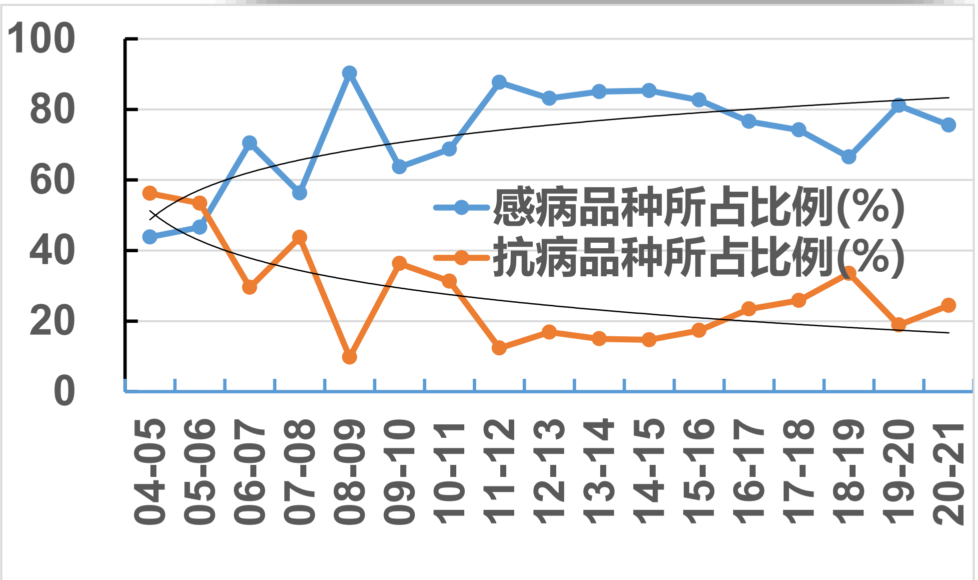
间通过病原的自然侵染而评定试验品种的抗、感病程度或发病程度的一种作物抗病性鉴定

圃 disease nursery

创造的有利于病害稳定发生的试验田，且人工创造的有利于病害发生的条件在该田中分布相

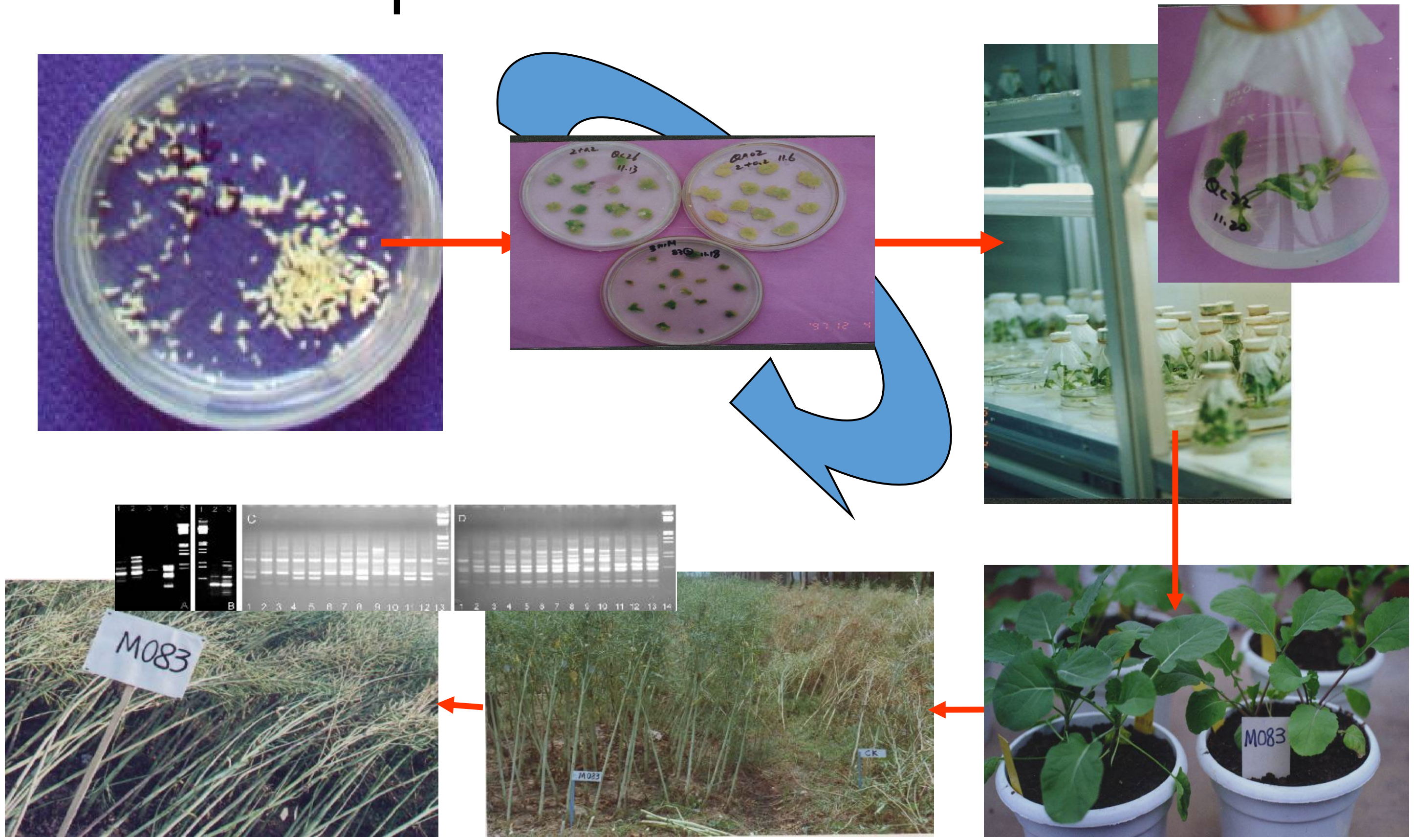
1





Development of resistant germplasm in my group

- Since 1990, we released 3 generation resistance lines to breeder as well as identification methods
- An examplar: resistance line M083



Liu et al. 2005. Plant Cell Reports 24:133-144

Ding et al. 2020. Plant Biotechnology Journal 18:1255-1270
Zhang et al. 2019. Journal of Integrated Plant Biology, 61 (1): 75-88
Li et al. 2019. International Journal of Molecular Science, 20:5949
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Cao et al.. 2016. Chinese J. Oil Crop Sci. 38 (1): 1-6
Cao et al. 2015. PLoS ONE 10(9):e0137414
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Xu Q et al. 2009. Analytica Chimica Acta 632:21-25
Dong et al. 2008. Planta, 228: 331-340
Zou et al. 2007. Phytochemical Analysis 18: 341-346
Liu et al. 2005. Plant Cell Reports, 24: 133-144
Liu et al. 1998. Journal of Plant Protection 25(1): 43-47
Liu et al. 1998. Acta Phytopathologica Sinica 28(1):33–37.
Zhou et al. 1994. Chinese J. Oil Crop Sci. 16 (Special issue): 57-61
Zhou et al. 1993. Chinese J. Oil Crop Sci. 15 (Special issue): 14-17

Resistance variety development

- **Zhongshuang 13: good resistance, high yield**

品种	田 间 长 势			菌核病发病(5月10日)		
	年前长势	春后长势及倒伏情况	发病株率(%)	病情指数	比ck +-数	比ck +-%
CP-06	叶小、色淡长势较弱	色淡、长势一般、无倒伏	1.58	1.14	-10.02	-89.79
CP-37	叶大、色深长势较强	色深、长势稳健、无倒伏	1	0.83	-10.33	-92.56
GDP-10	叶中等大小、色中长势中等	色中、长势旺、无倒伏	2.2	1.99	-9.17	-82.17
阳光2009	叶中等、色深长势较强	色深、长势稳健、90%斜倒	6.4	5.63	-5.53	-49.55
华油杂9号（ck）	叶大、色中、生长势旺	色中、长势旺、倒伏98%	12.37	11.16	0	0


非主要农作物品种
登记证书

登记编号：GPD 油菜(2022)420167

作物种类：油菜

品种名称：中双 13 号

申 请 者：中国农业科学院油料作物研究所

育 种 者：中国农业科学院油料作物研究所

品种来源：中油 821 × 中双 11 号

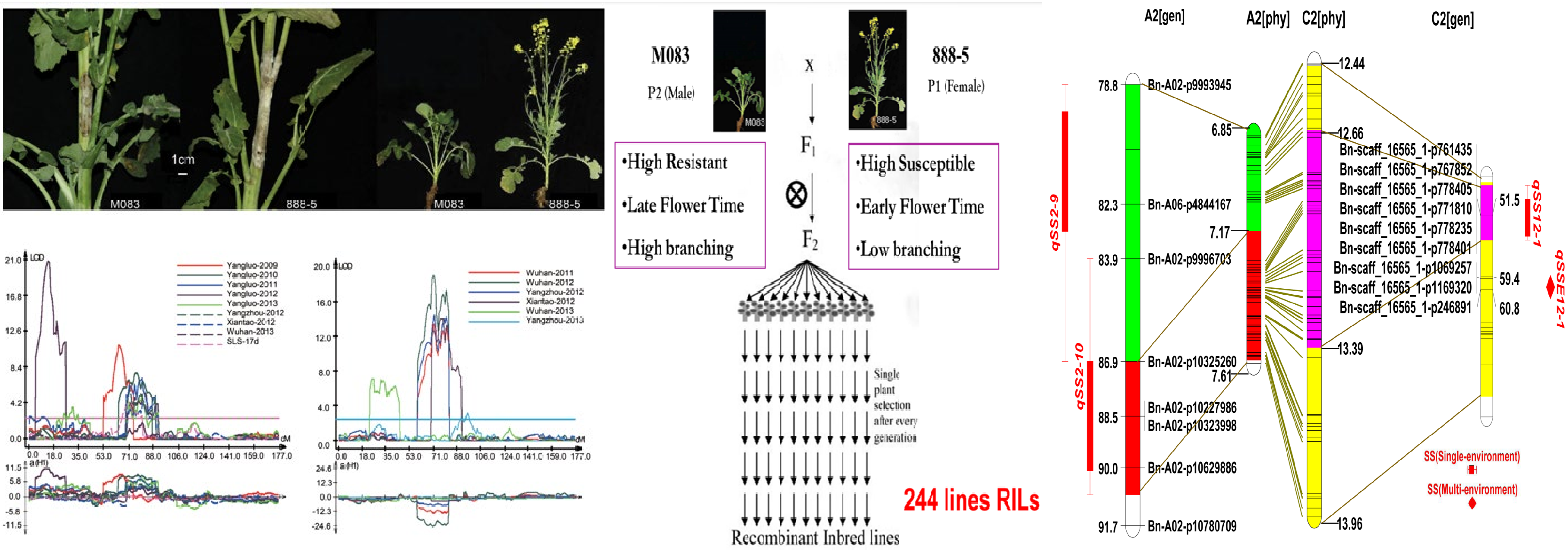
适宜种植区域及季节：适宜在四川、贵州、云南、重庆、陕西汉中和安康地区油菜主产区秋播种植。

中华人民共和国农业农村部

二〇二二年八月十八日

2. Progress in resistance loci/gene studies in my group

QTLs of Ss resistance (SsR) and flowering time

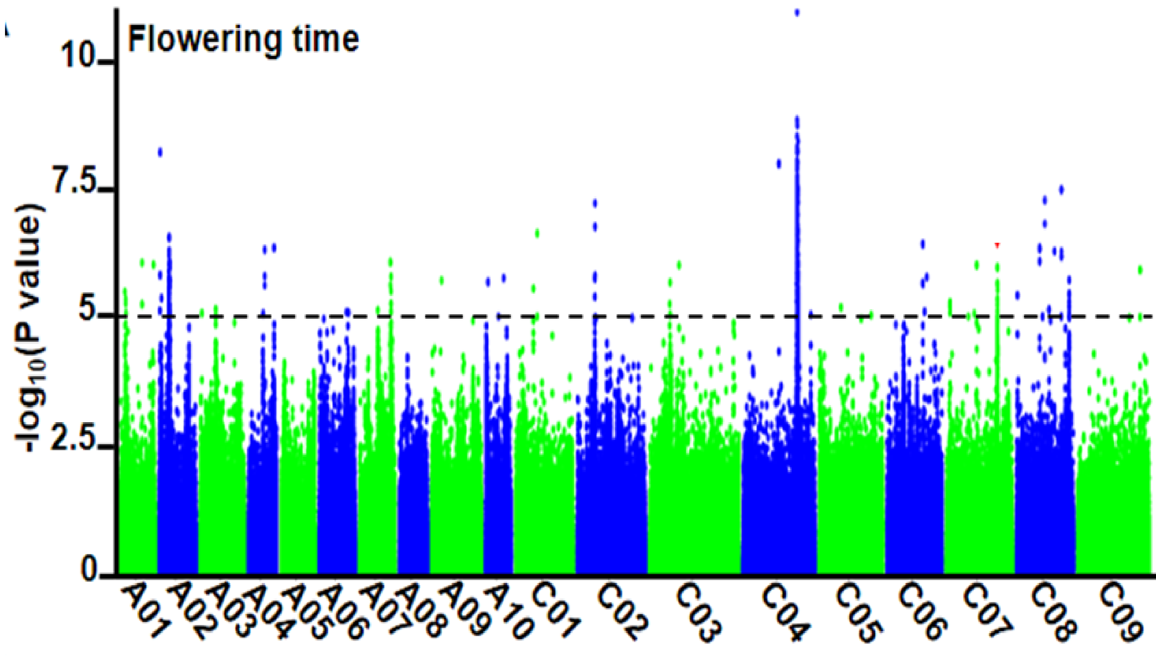


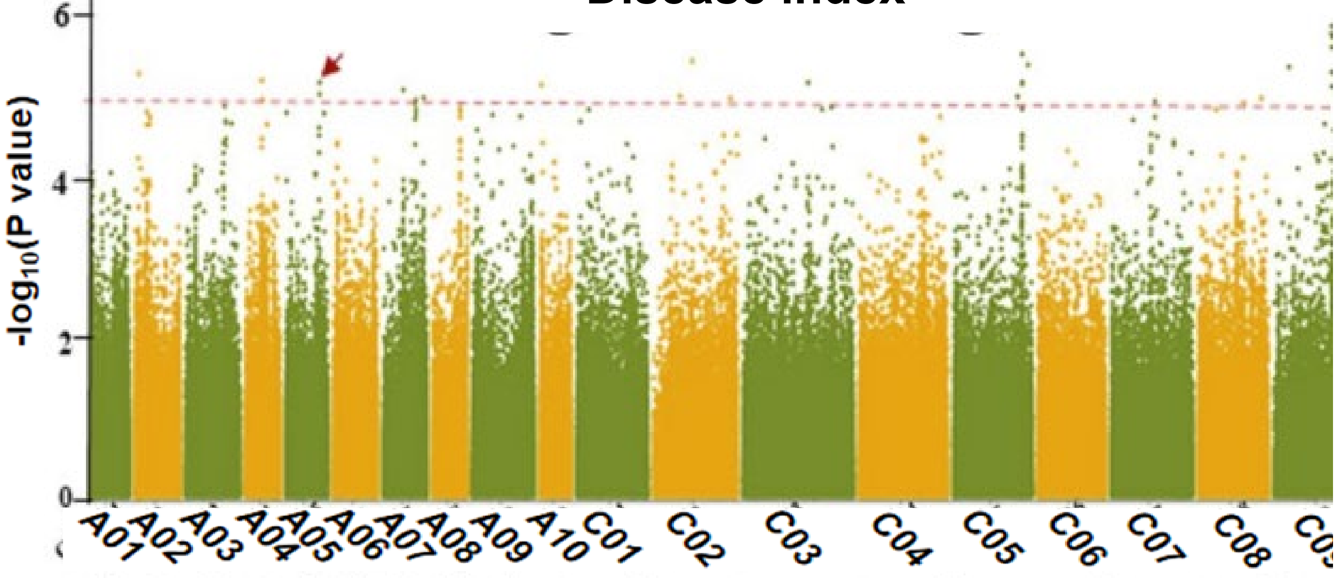
- **RIL population derived from a cross between M083 (**Liu et al. 2005. Plant Cell Reports 24:133-144), one of the most resistant line (Roger Rimmer, Personal Comm), and **highly susceptible one with early flowering.**
- **11 major QTLs for Ss resistance, 8 major QTLs for flowering time; 6 overlapping QTLs**

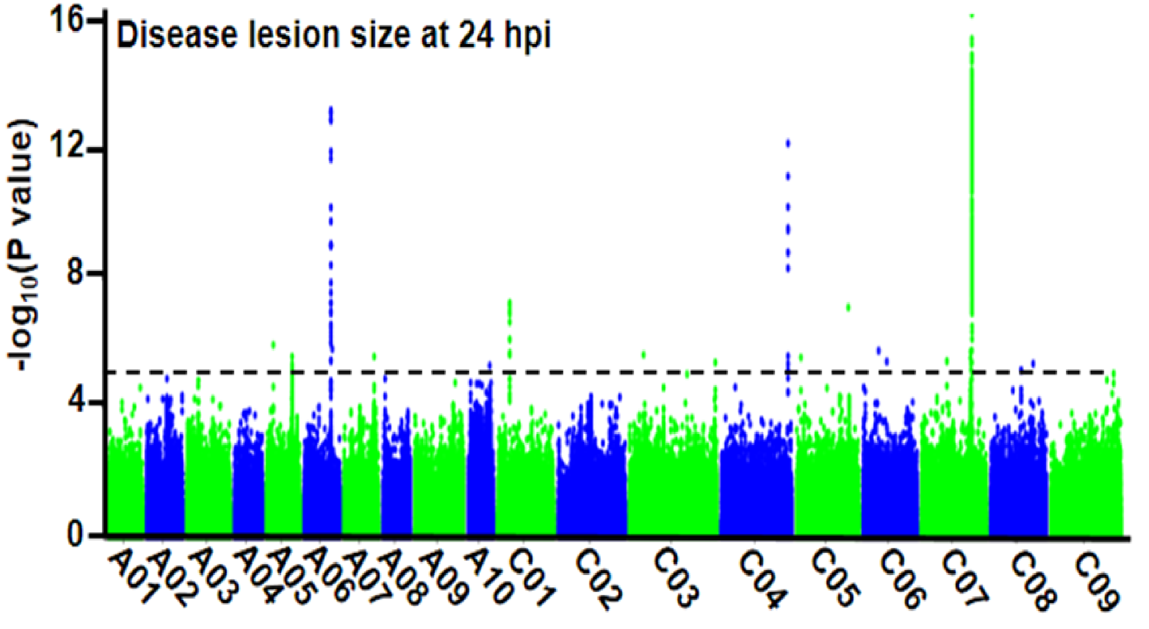
Zhang et al. 2019. Journal of Integrative Plant Biology, 61: 75-88

GWAS

- 21 significantly GWAS loci for Ss resistance
- 25 significantly GWAS loci for Flowering Time
- 12 loci are overlapped
- Further， some of loci supported by QTLs from RIL population
- A pipeline developed for gene exclusion from QTL and GWAS loci (partly to see JIPB 61:75-88, 2019)







Example #1: a pleiotropic major QTL and its causal gene on A02 detected from both RIL and GWAS populations

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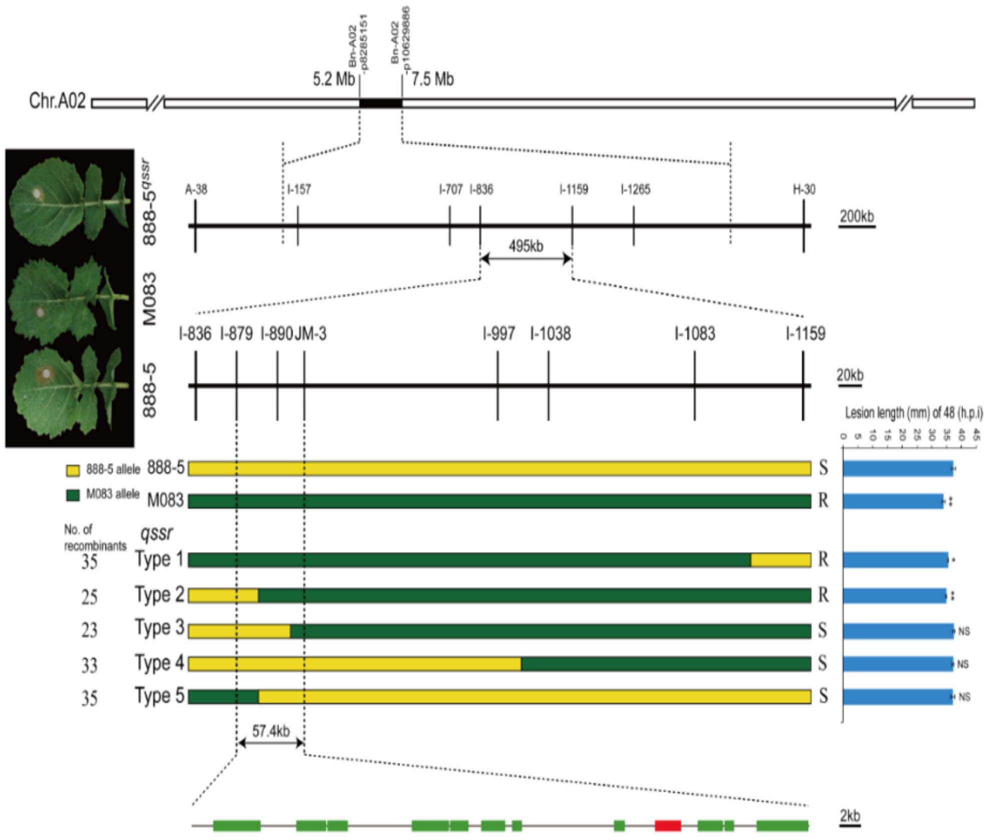
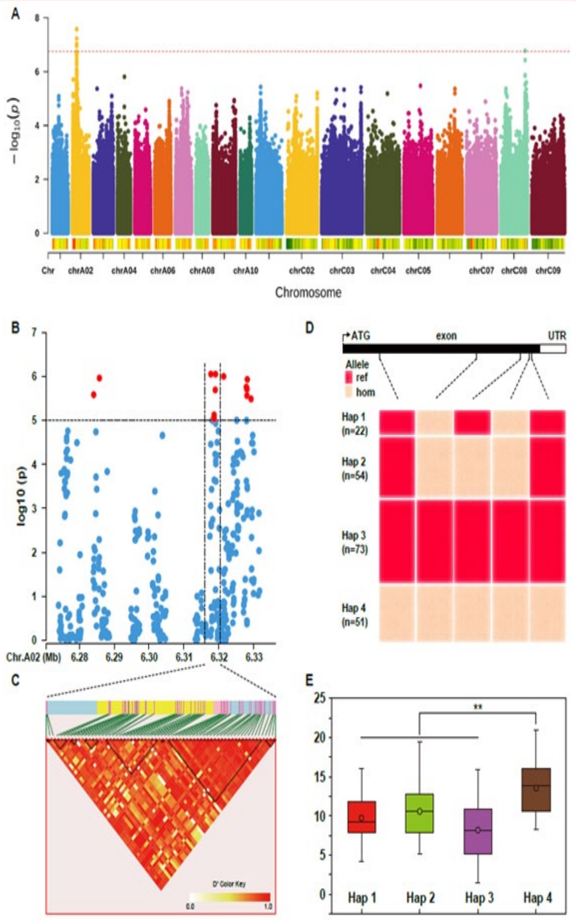
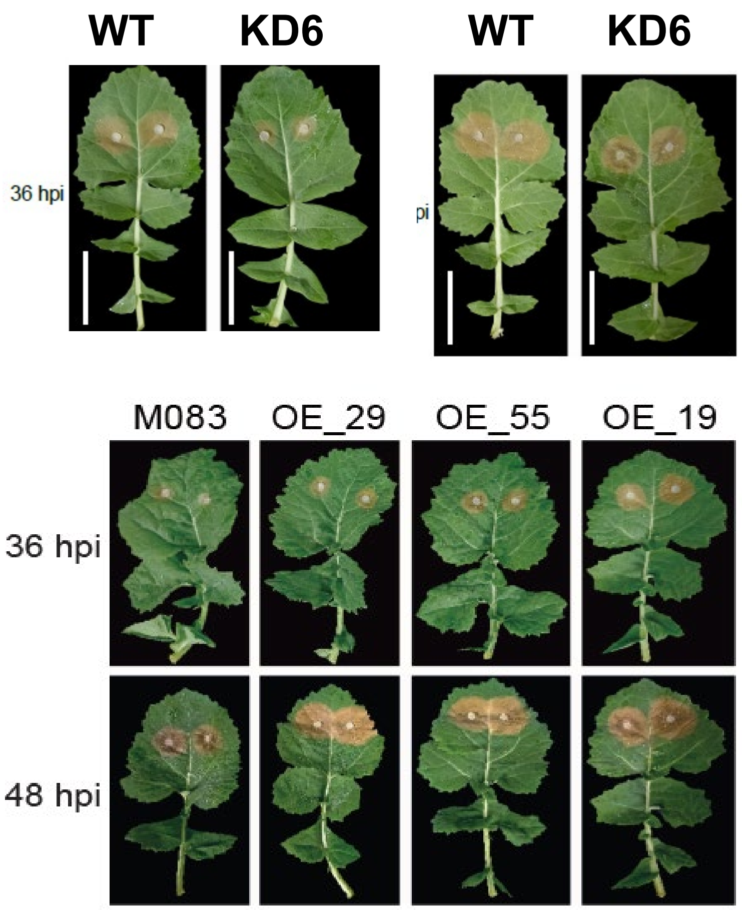
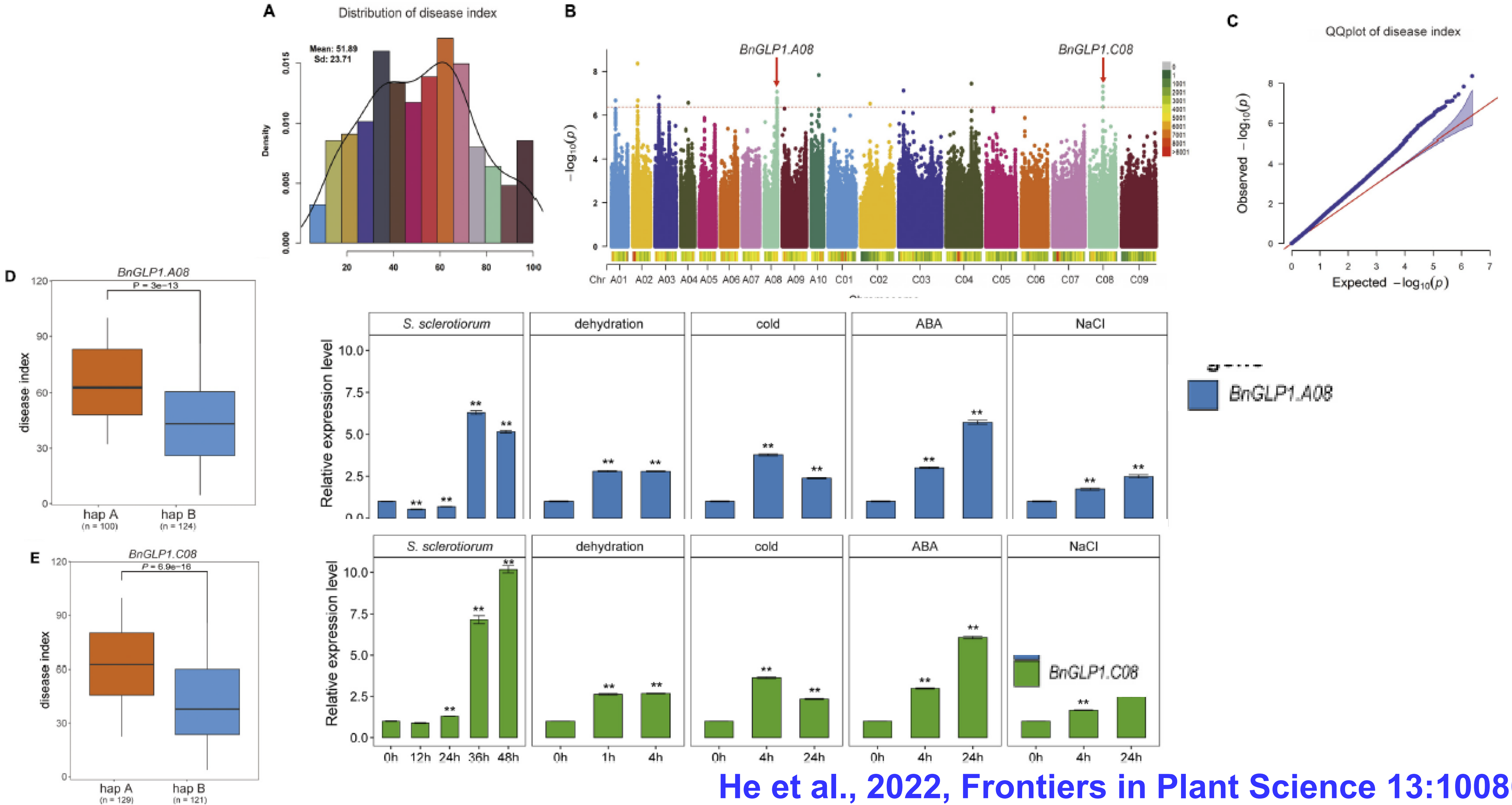






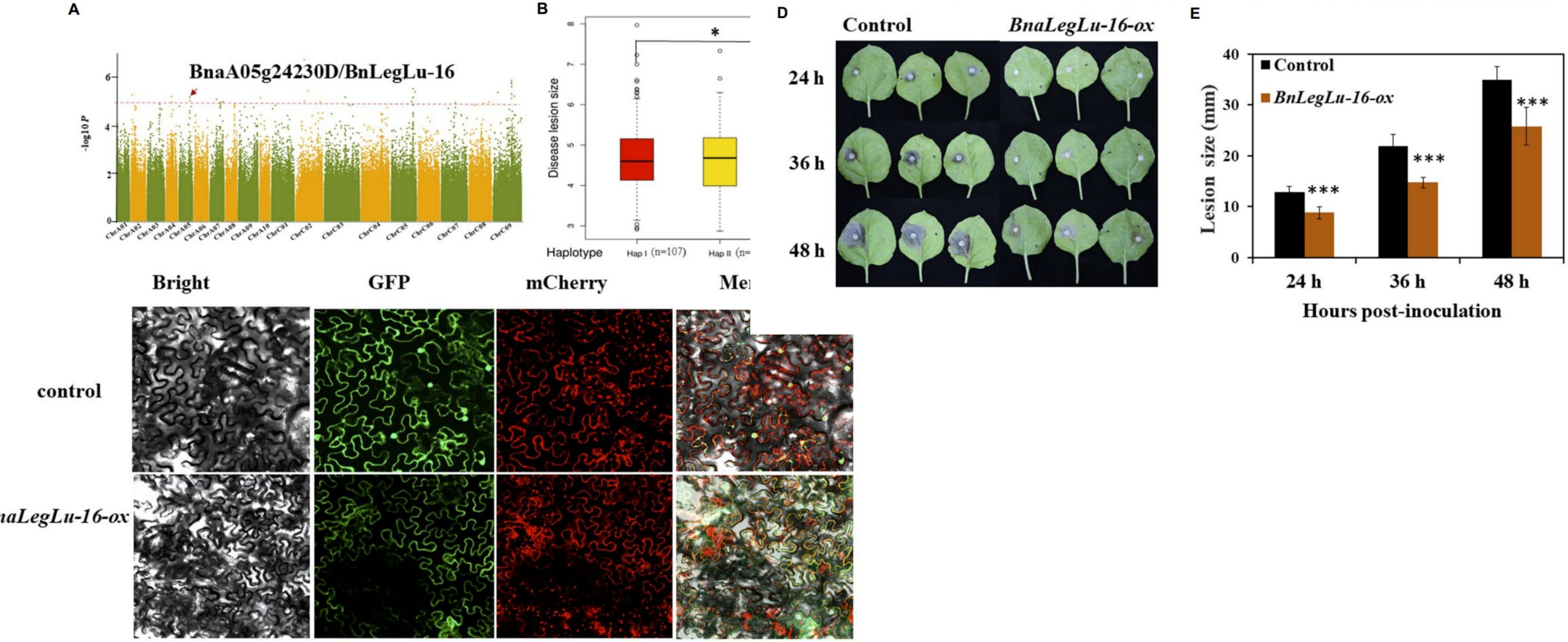
- From RIL development to now, we spent 17 years to map and clone Ss resistance genes.
- Through three rounds of mapping, each round with selfed populations to identify SsR, we found major *qSsR*-6 on A02 tightly links *FT* gene.
- gene editing and overexpression showed that a gene *Clv* regulates SsR and flowering time.

Example #2: a cupin_1 protein involves in regulation of Ss resistance



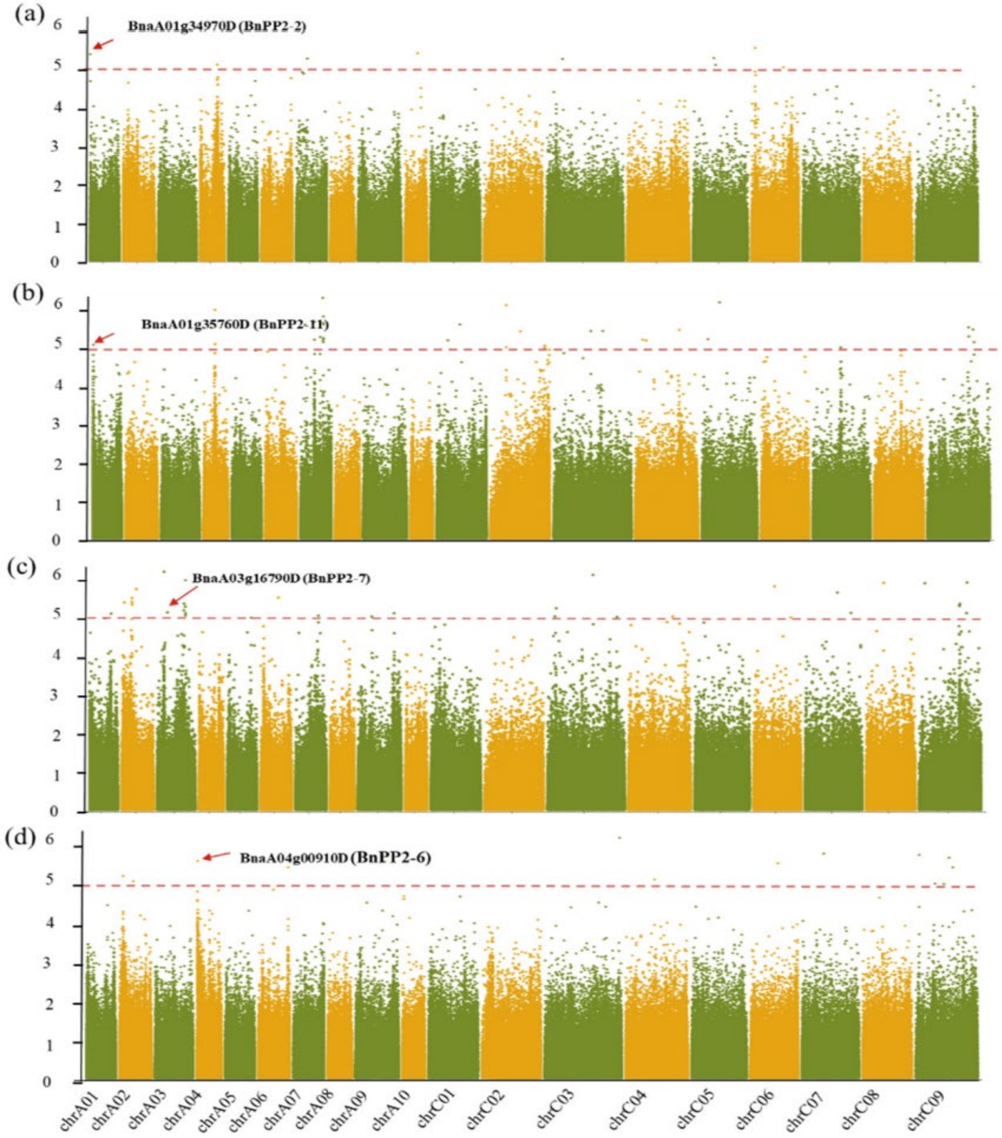
He et al., 2022, Frontiers in Plant Science 13:1008665.

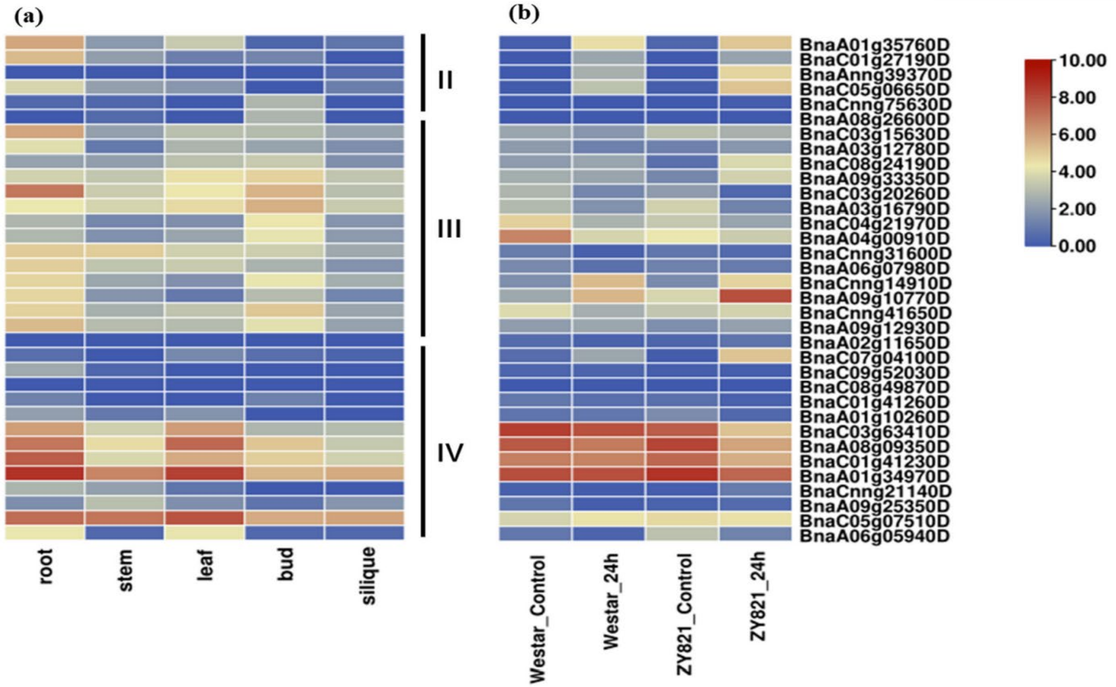
Example #3: a lectin-like protein LegLus-16 positively regulates Ss resistance

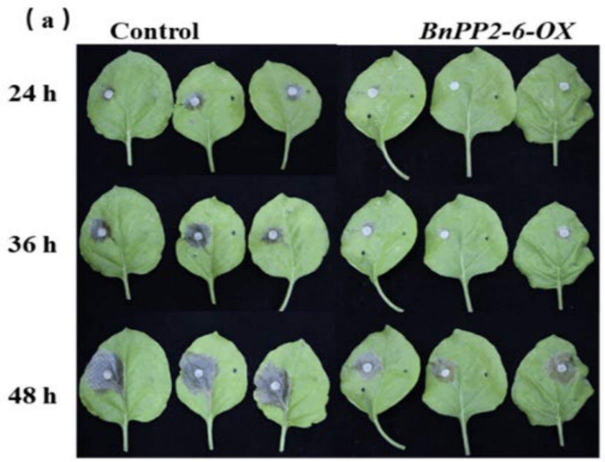


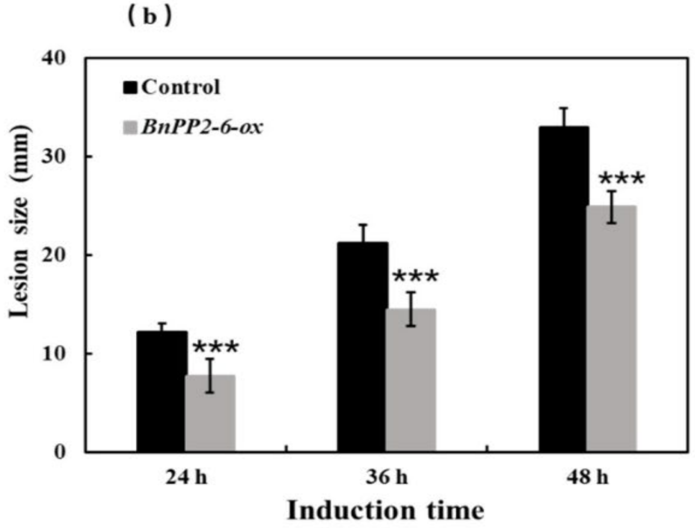
Zuo et al., 2022, Frontiers in Plant Science 13:963263

Example #4: a Phloem Protein 2 Gene *BnPP2* positively regulates Ss resistance







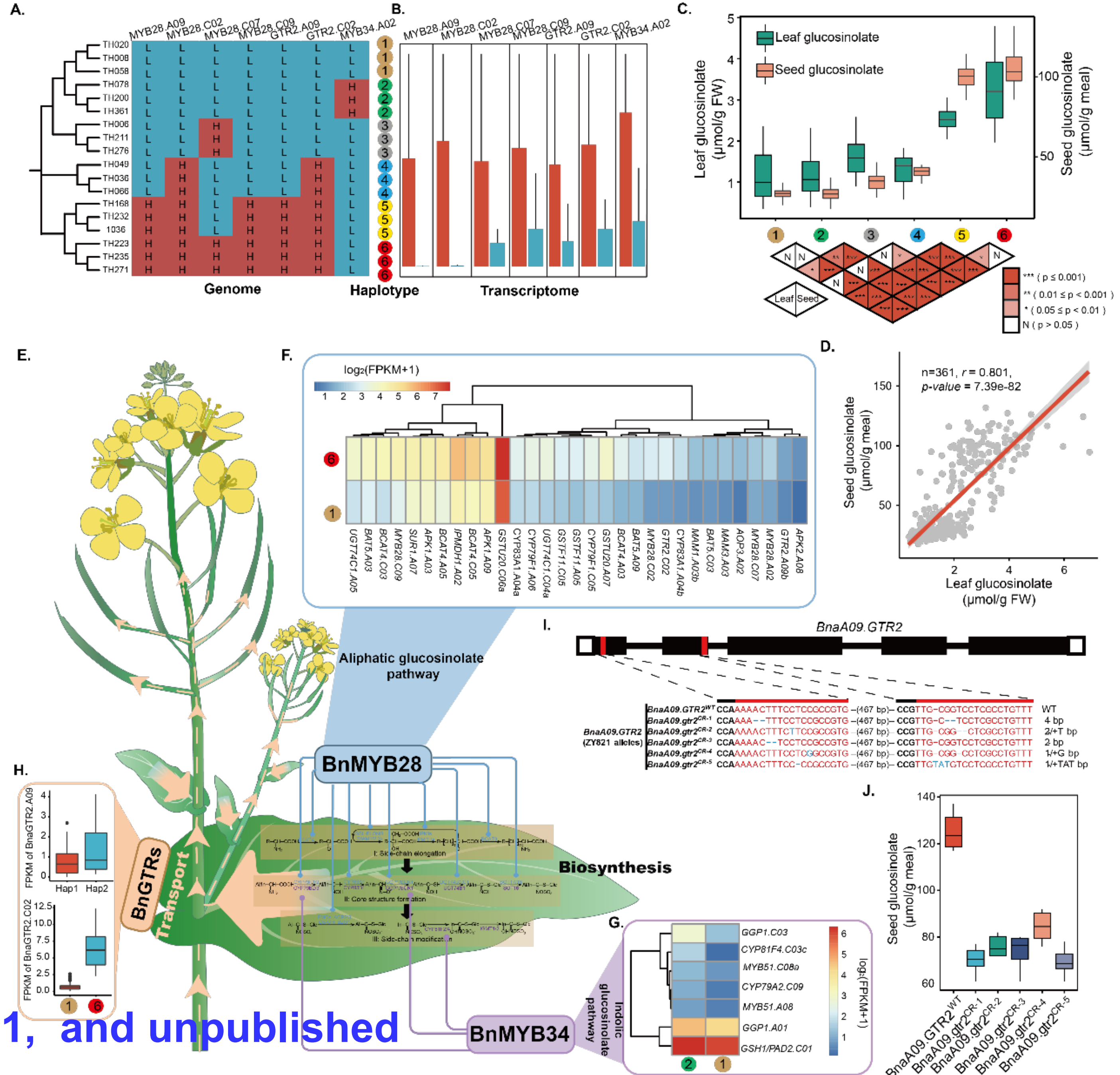


Zuo et al., 2022, Intl J. Mol. Sci. 23(7):3934. doi: 10.3390/ijms23073934

Example #5: gene editing for high glucosinolates in leaves & low in seeds

QTL and GWAS mapping:

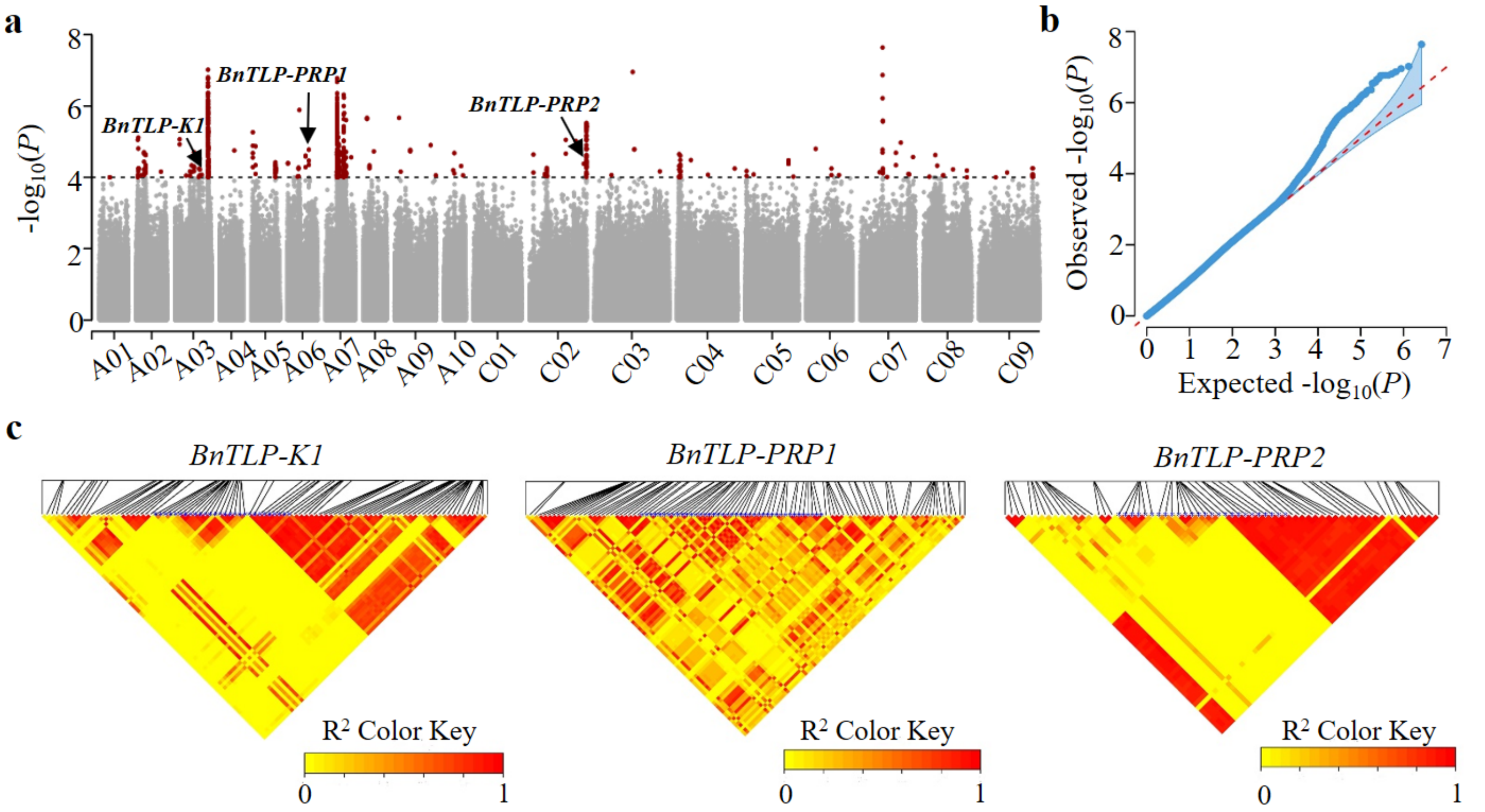
- a complete set of haplotypes
- gene editing created high glucosinolates in leaves & low in seeds
- higher resistance to insect and Ss.

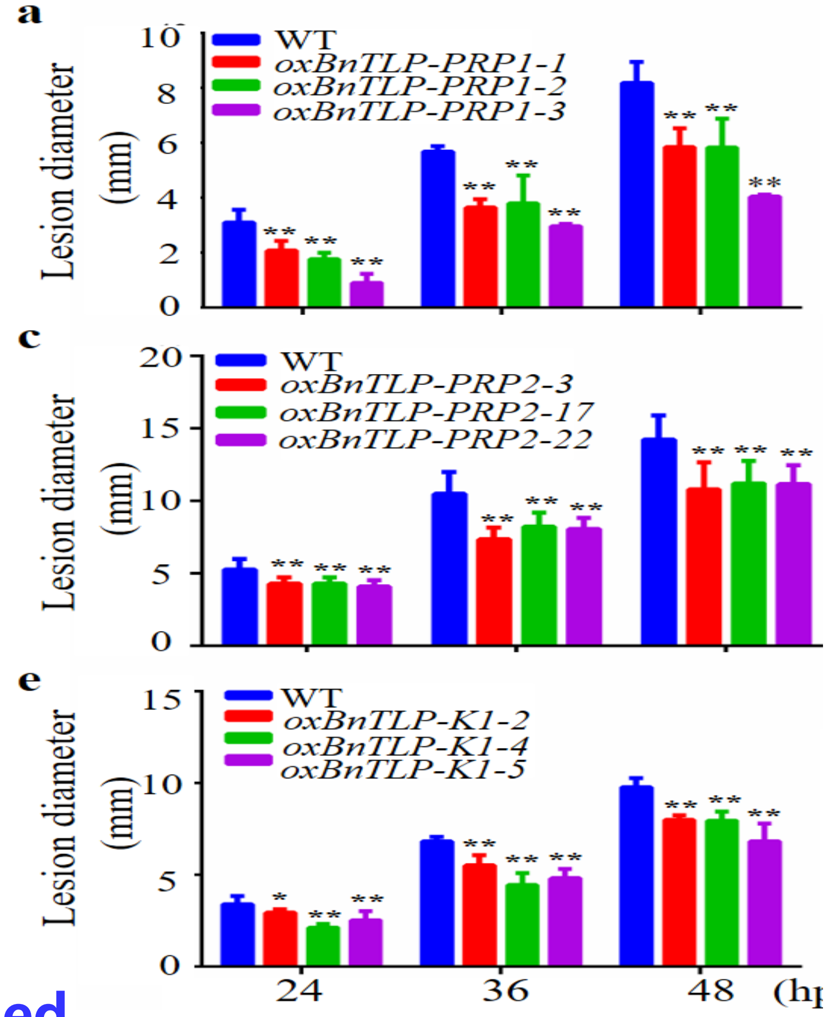


He et al. Plant Physiology, 2022, 188:1848–51, and unpublished

Example #6: A fused gene *TLP-PRP* increases Ss resistance

- A fused gene *BnTLP-PRP* from thaumatin-like protein (TLP) gene and an antimicrobial polypeptide (*PRP1*) gene was identified by GWAS, and haplotype analysis showed it increases resistance.
- Over-expression in B. napus further increases resistance





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3. Detailed molecular mechansims of the association of Ss resistance with flowering time

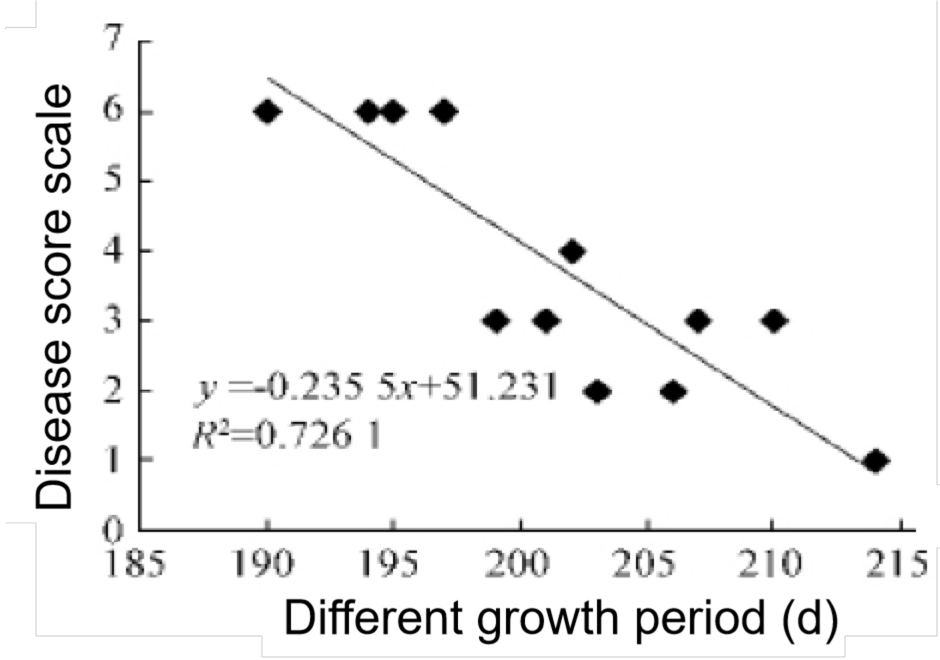
Phenotypic relationship between Ss resistance and flowering time

- Different flowering time by sowing at different dates

  Yu Q., B. Zhou, L. Zhou and S. Liu. 1994. Chinese J. Oil Crop Sci. 16 (Special issue): 43-45.

- >6000 germplasm showed negative correlation between SsR and flowering time

- such negative correlation also exists in other host crops like soybean and sunflower

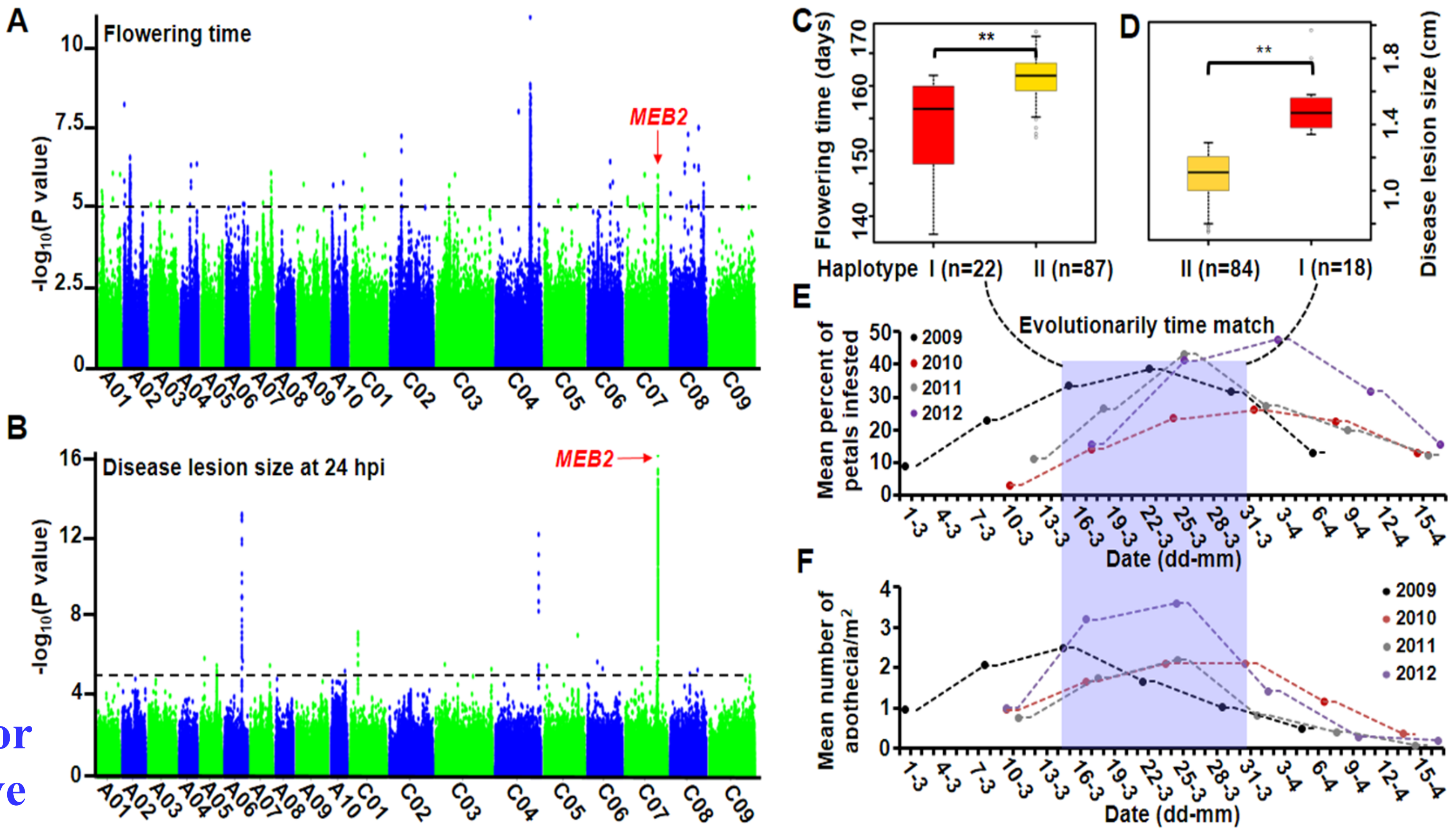


What is its molecular mechanism and whether it is a consequence of co-evolution remain unknown

MEB2 negatively regulates Ss resistance and positively FT

MEB2 = Membrane Protein of Endoplasmic Reticulum Body2 (Yamada et al., 2013; Zhu et al., 2016)

- ***BnMEB2***, a gene with Fe tolerance and Ss resistance identified in our previous study (Zhu et al. 2016), locates in a GWAS locus.
- It is also in a FT locus.



the pathogen hijacks early flowering for petal infection and the host susceptibility for subsequent infection on leave and stems

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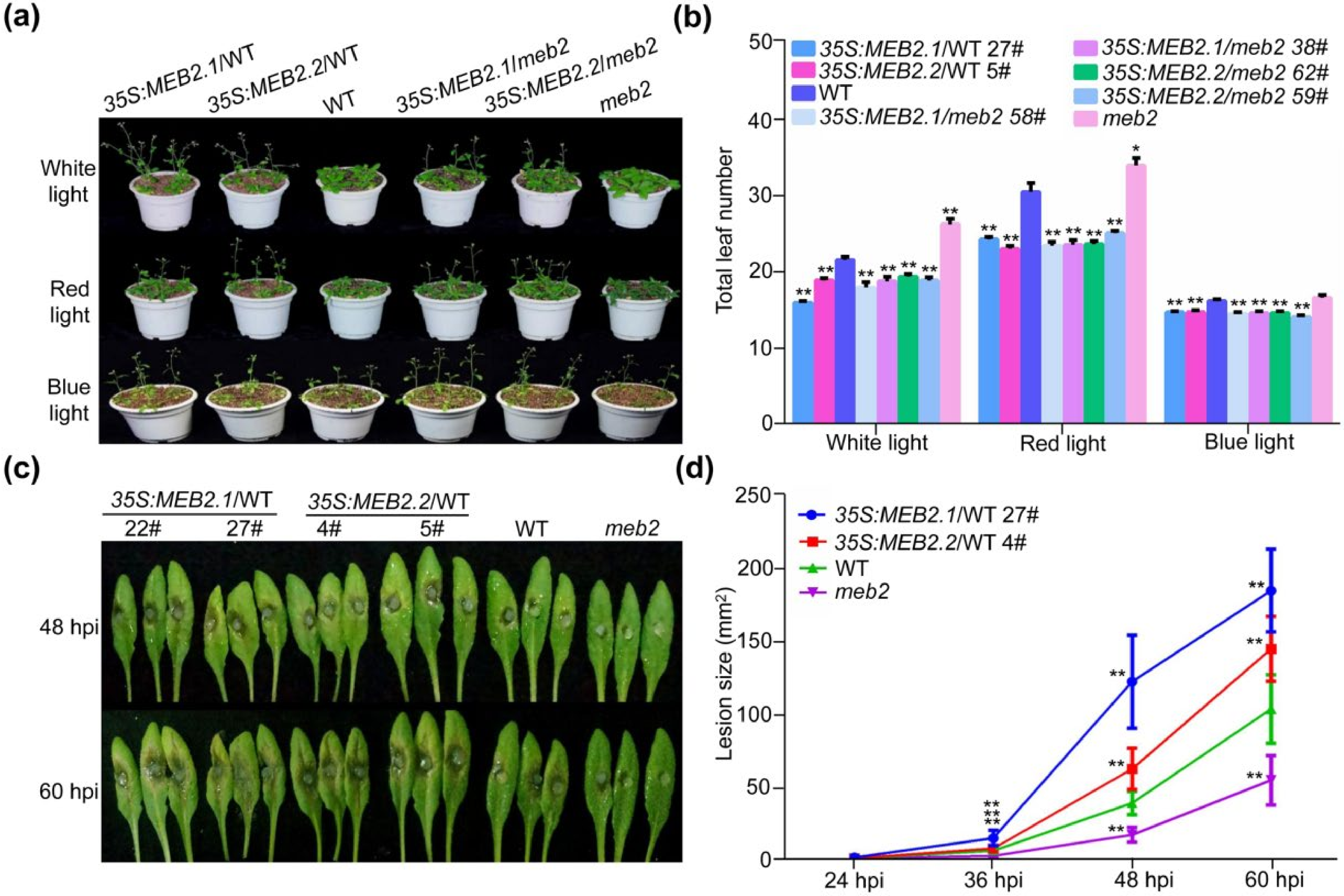
Further transgenics in Arabidopsis validated MEB2 function in negatively regulating Ss resistance and positively FT



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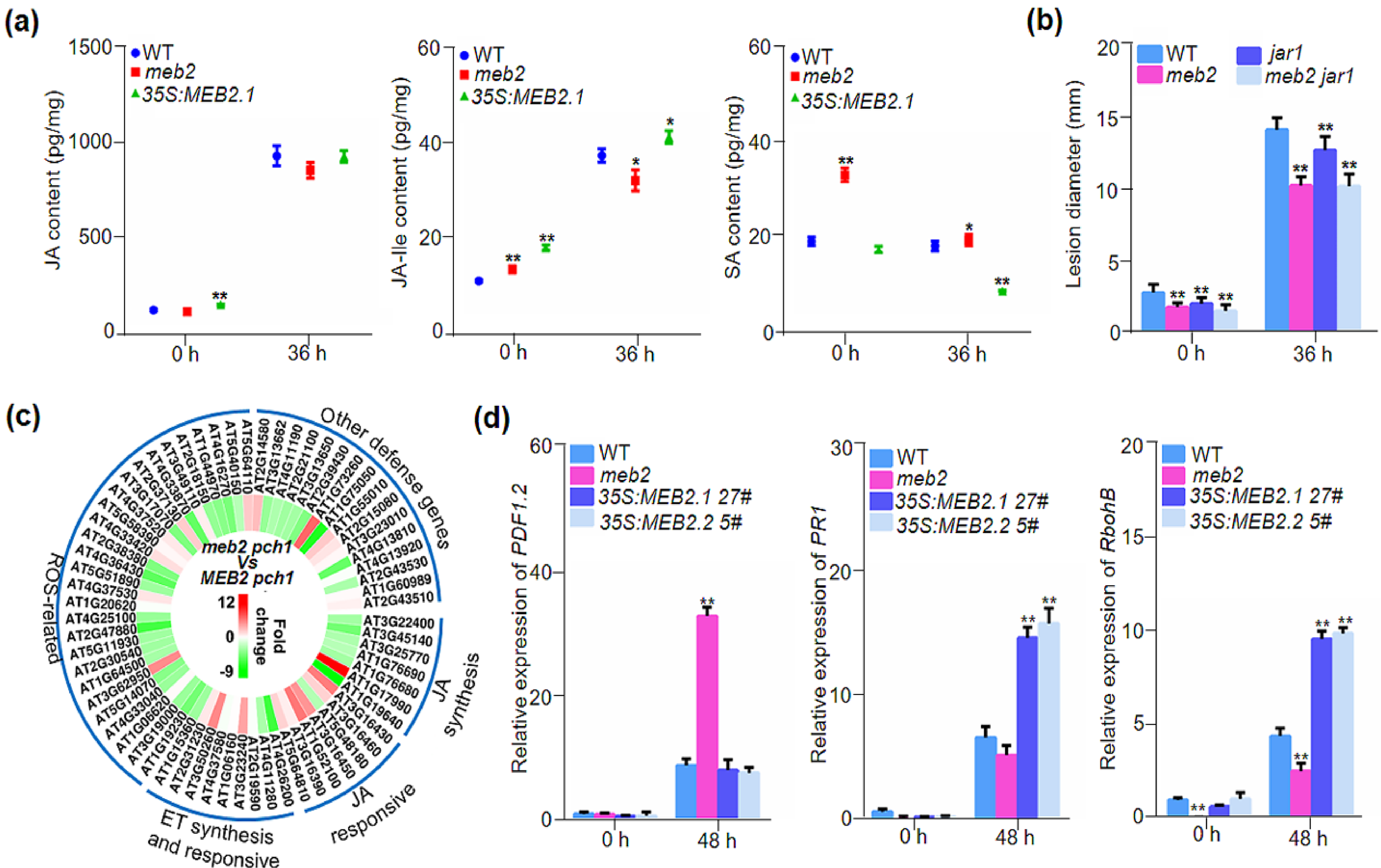
MEB2 promotes biosynthesis of jasmonic acid (JA) and JA-Ile and is epistatic to *JAR1* to regulate resistance



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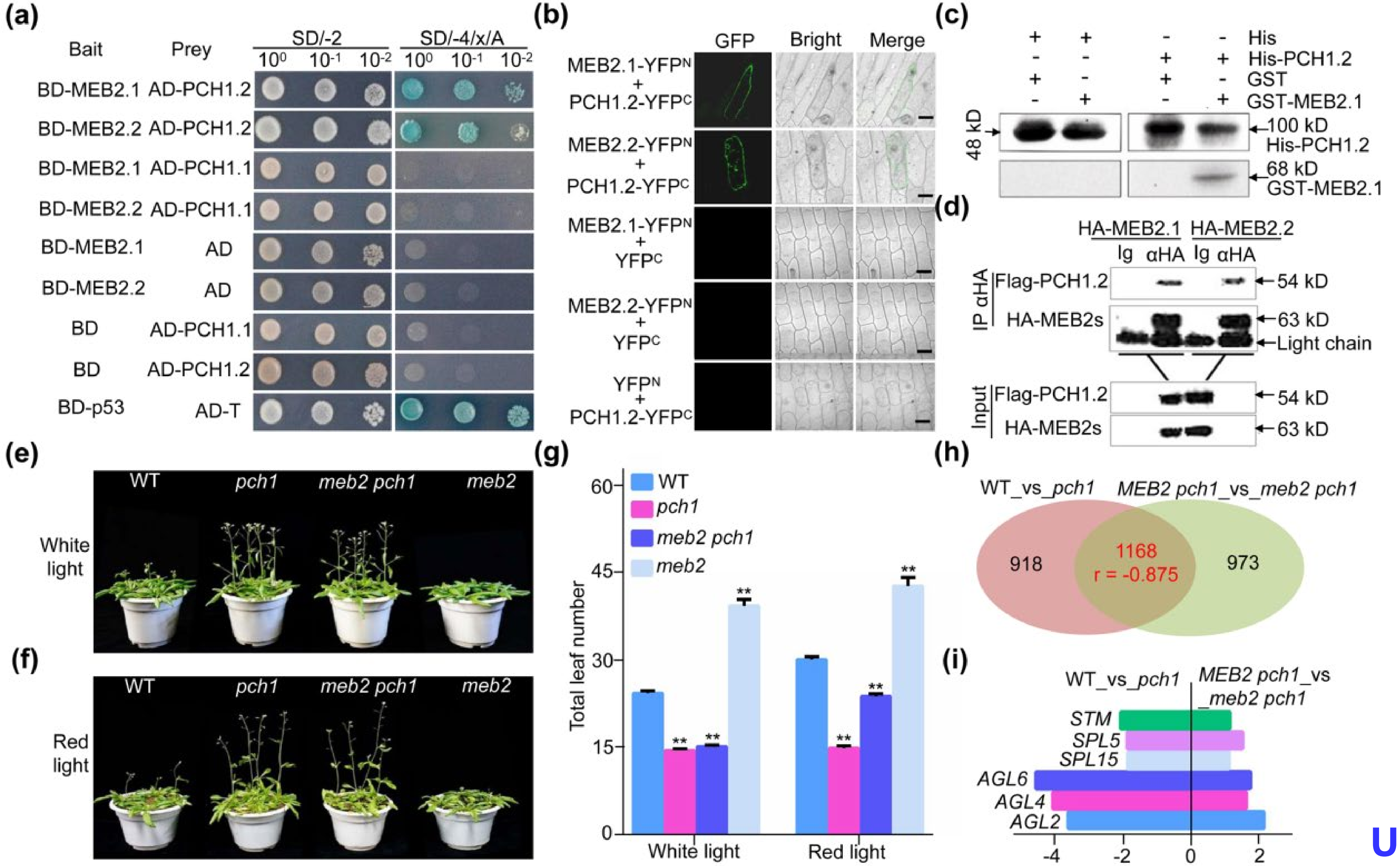
MEB2 and PCH1.2 directly interact and reversely regulate a set of genes to control flowering (PCH1 = Photoperiodic Control of Hypocotyl1)



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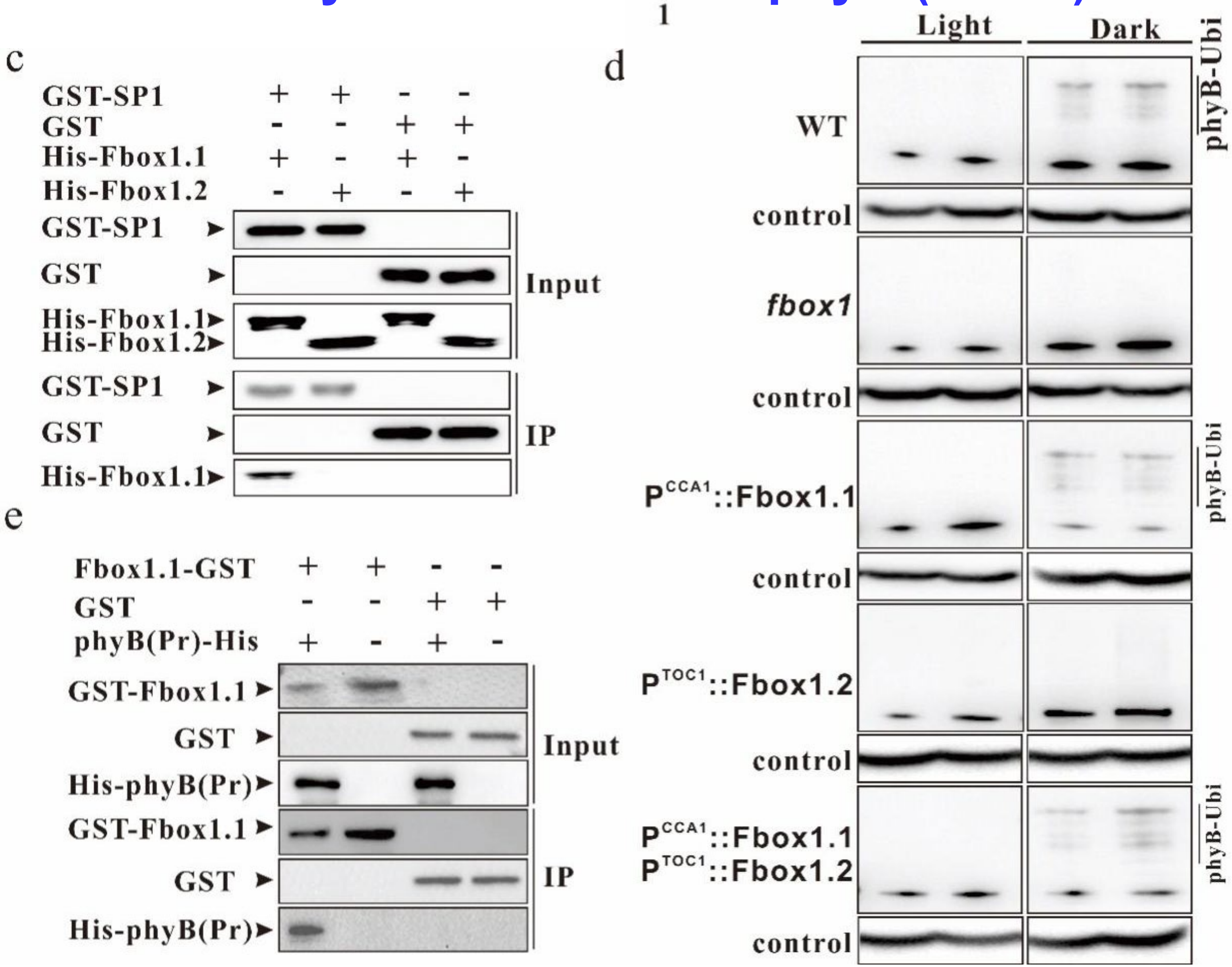
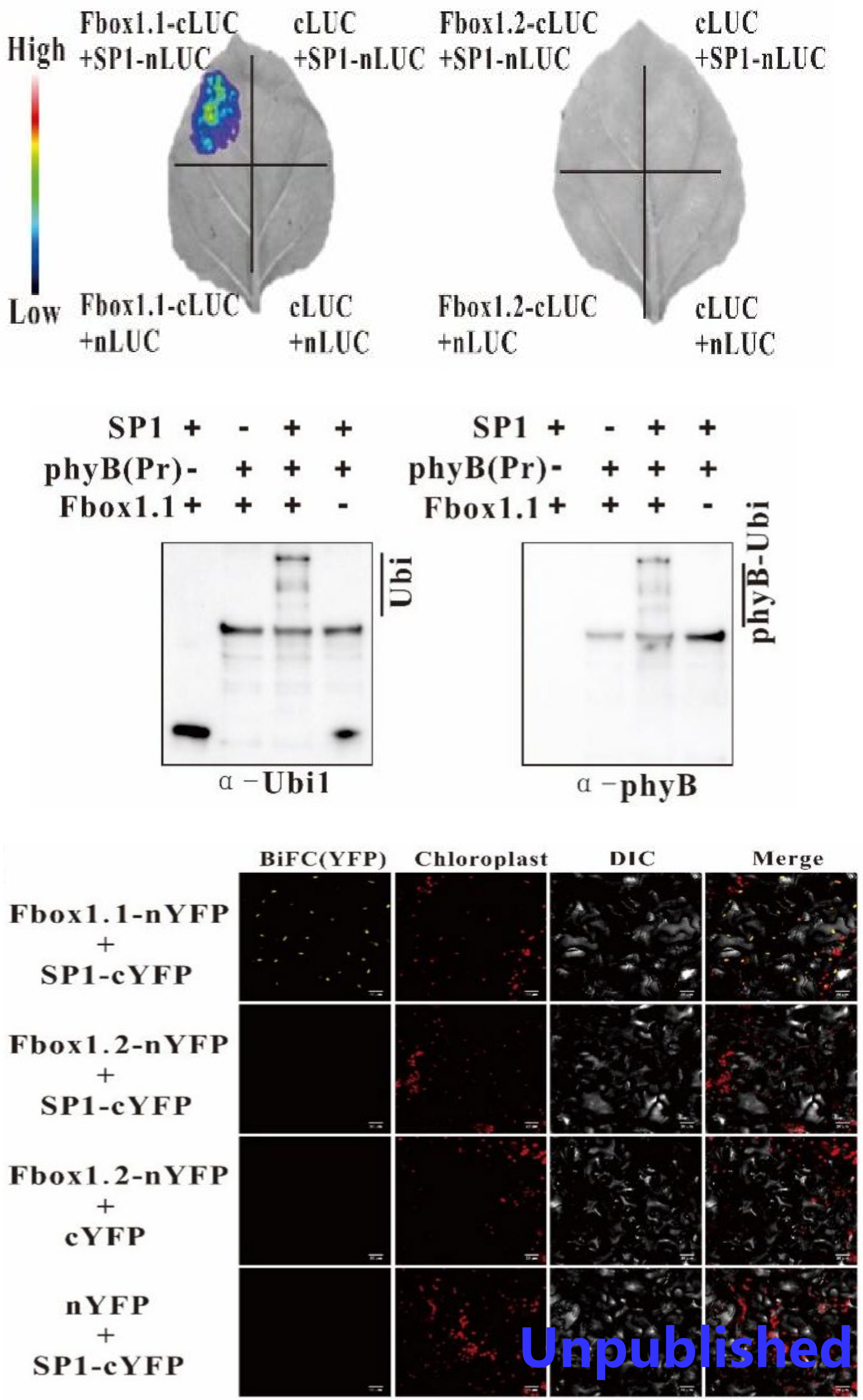
PCH1.1 (Fbox1.1) interacts with Fbox1.2, SP1 and phyB (PFT1.1+PFT1.2+SP1+phyB) to regulate circadian rhythm turn-over of phyB (Pfr/Pr)





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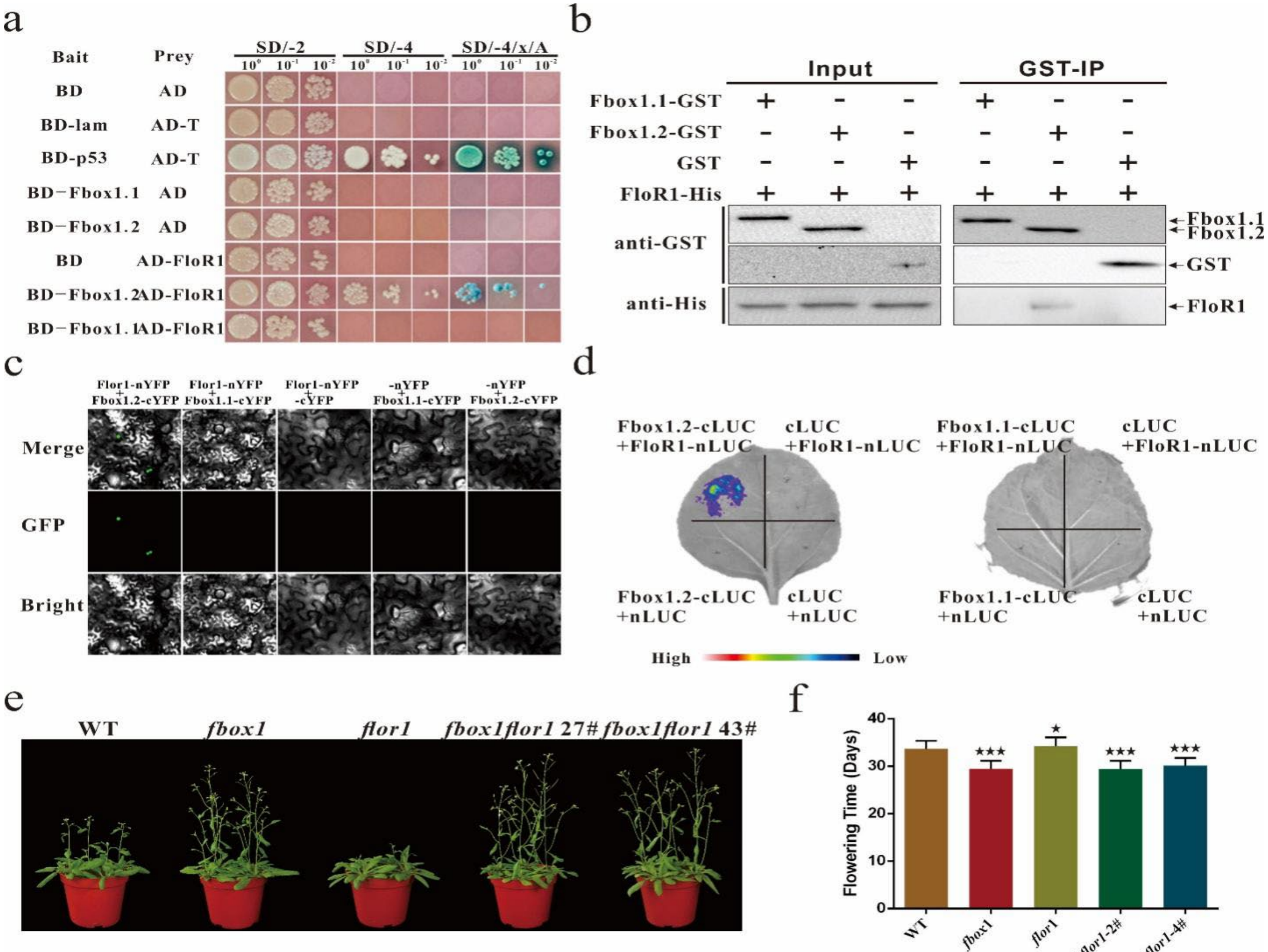
Fbox1.2, but not Fbox1.1, interacts with FloR1 *in vitro* and *in vivo* to regulate flowering time (FloR1 is a flower-specific leucine-rich repeat protein)



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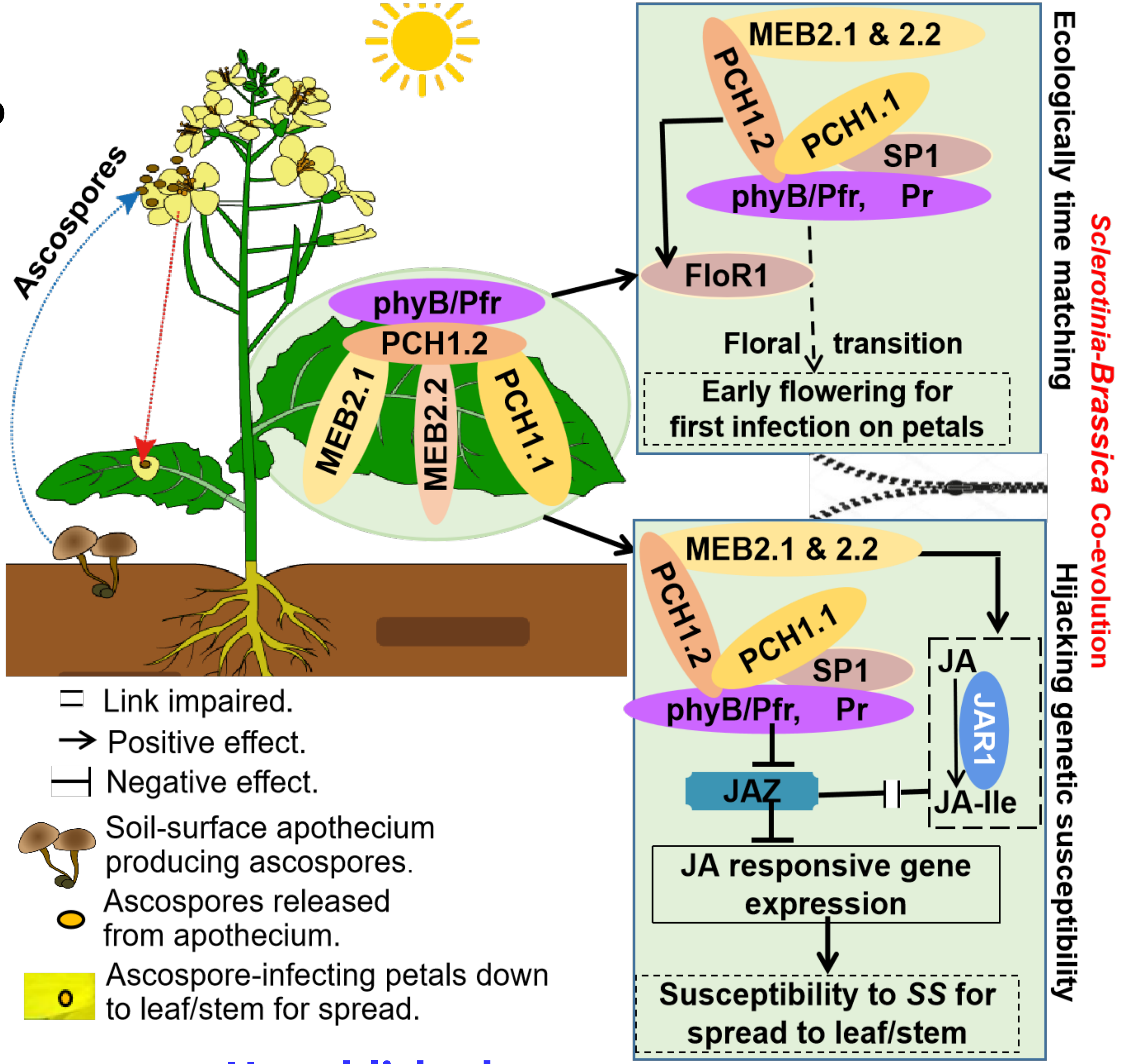
A proposed model explaining two coevolution layers corresponding to two key steps of *Sclerotinia* disease cycle by promoting flowering and increasing disease susceptibility:

- pleiotropic gene networking promotes flowering to enable time match of flowering to ascospore release for infection of petals as nutrient media
- the networking through phyB(Pfr/Pr), possibly through suppressing JAZ/MYC, increases susceptibility of leaves and stems

Interestingly, this network is wired by functionally differentiated alternative splice transcripts



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Thanks!

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- ✓ group members
- ✓ Collaborators