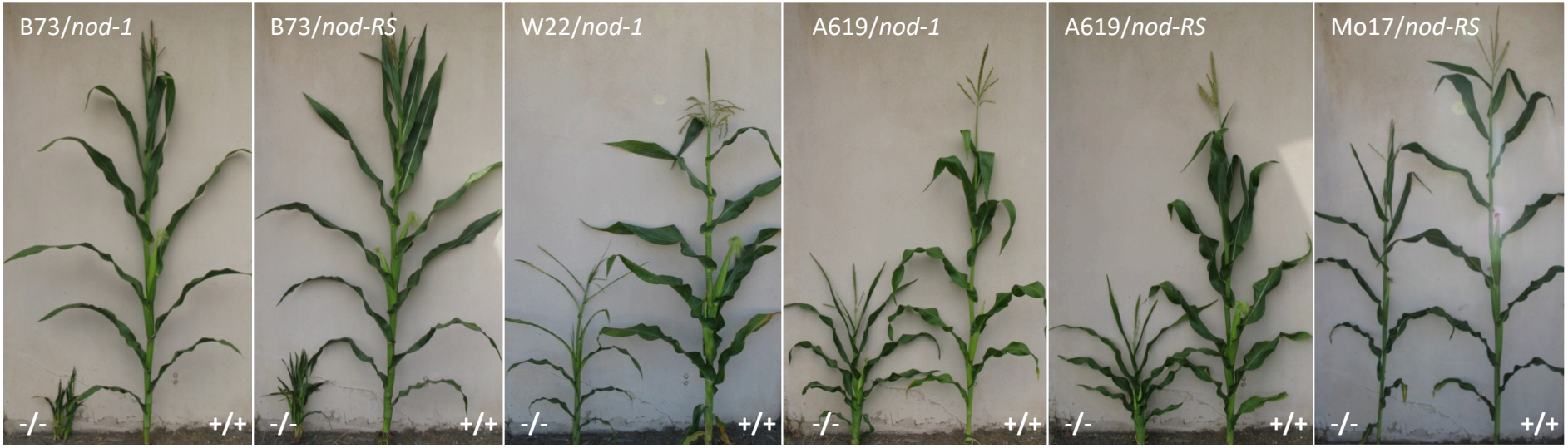
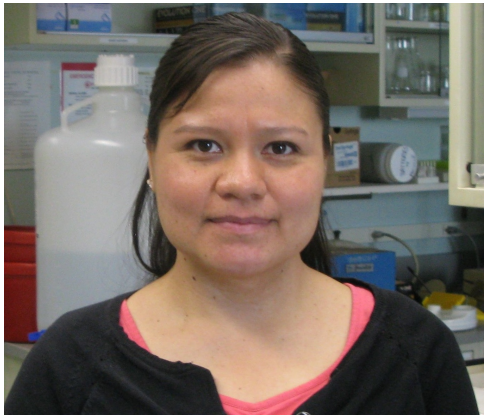
The phenotype of *nod* is dependent on inbred background



Marisa Rosa

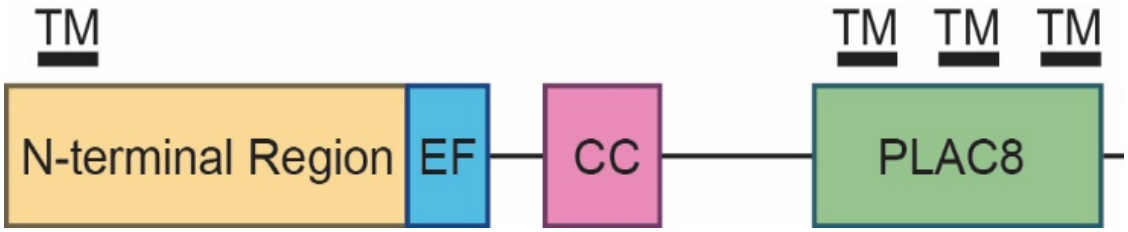


Jazmin Abraham-Juarez

narrow odd dwarf (*nod*)

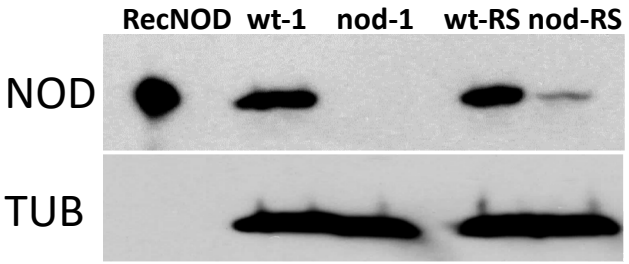
Short internodes, ears ok, tassel sparse, leaves narrow

narrow odd dwarf (nod)
cell number regulator13 (fw2.2 family)
aka maize MCA (mid-complementing activity)



Implicated in Ca^{2+} signaling

2 alleles



Rosa et al., 2017

to understand NOD function we carried out immunoprecipitation using the NOD antibody followed by mass-spec

In every experiment NOD was found in wild-type precipitation and not in mutant precipitation

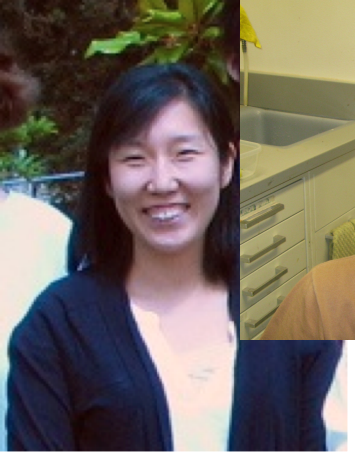
Categories include Calcium related, plasma membrane localized and kinases

Gene ID	Protein name	CoIP Replicate and Y2H	Subcellular localization	Mass spectral count WT	Mass spectral count *nod-1*	Identificatio probability
GRMZM2G027821	**NOD** / CNR13	1,2,3,Y2H	PM	17	0	100%
GRMZM2G134382	Liguleless Narrow	3	PM	4	0	100%
GRMZM2G107444	Membrane steroid-binding protein 1	1	PM	4	0	100%
GRMZM2G134668	Calnexin 1	1	PM, ER	3	0	99%
GRMZM2G358059	Calreticulin 1	1	PM, ER	2	0	99%
GRMZM2G360867	RABE1C, RAB GTPase homolog 8A, GTP binding	1	PM, Cyt	2	0	99%
GRMZM2G075150	Exocyst complex component, SEC15A	2,3	PM, Cyt	7	0	100%
GRMZM2G126019	Dynamin-related protein 1C, GTP binding	1	PM	6	0	100%
GRMZM2G181151	Vacuolar ATPase subunit B	1	PM	16	0	100%
GRMZM2G023073	Stomatin like protein 1, SPFH/Band 7/PHB domain-containing protein	1,2	PM	6	0	100%
GRMZM2G402417	Jasmonate induced protein	1,2	Cyt	8	0	100%
GRMZM2G451483	Ftsh4 ATP-dependent zinc metalloprotease	1,3,Y2H	PM	17	0	100%
GRMZM2G164224	Vesicle-associated protein 1, (BSK1) BR-signaling kinase 1	3,Y2H	PM	16	0	100%
GRMZM2G084540	KH domain RNA-binding protein	1,Y2H	Cyt	4	0	99%
GRMZM2G062373	USP domain, Universal Stress Protein	1,Y2H	Cyt, PM	2	0	99%
GRMZM2G058560	Serine/threonine-protein phosphatase 2A activator, PTPA	Y2H	Cyt			

Calcium binding

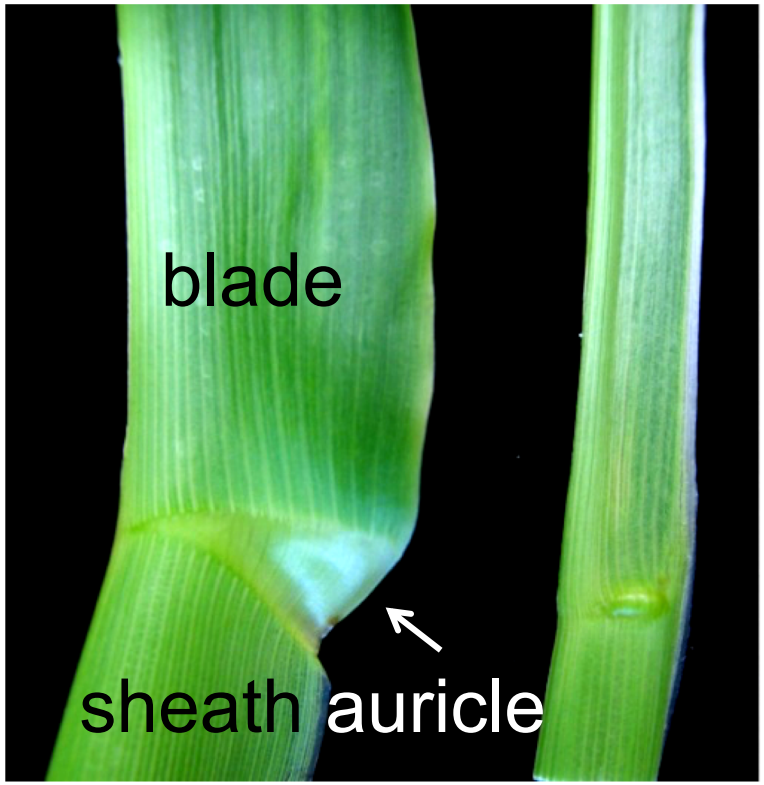
Brassinosteroid signaling and immune response

Membrane trafficking

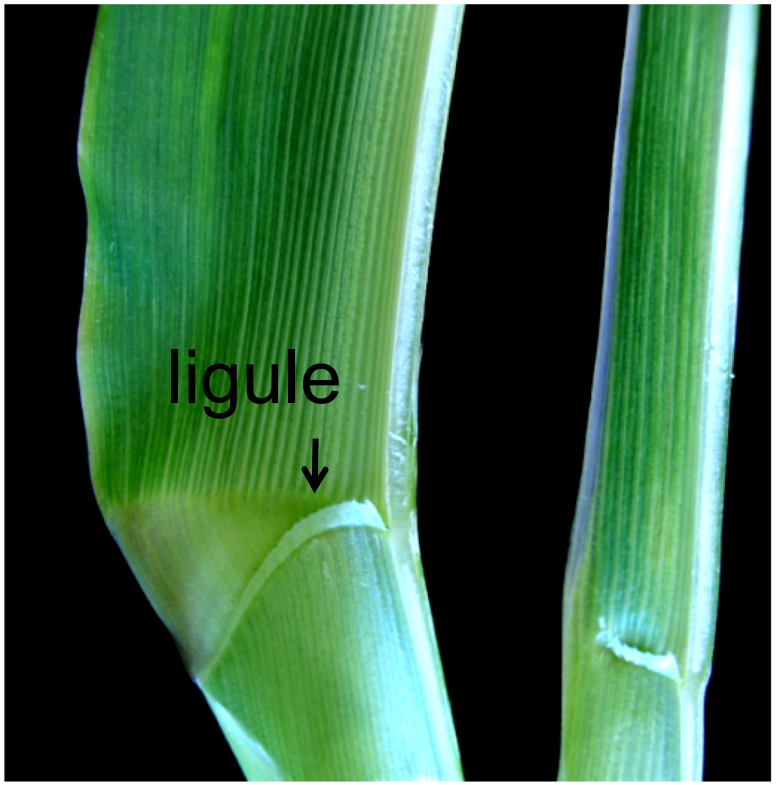


Lgn-R/+

Narrow leaves, reduced ligule, upright tassel branches

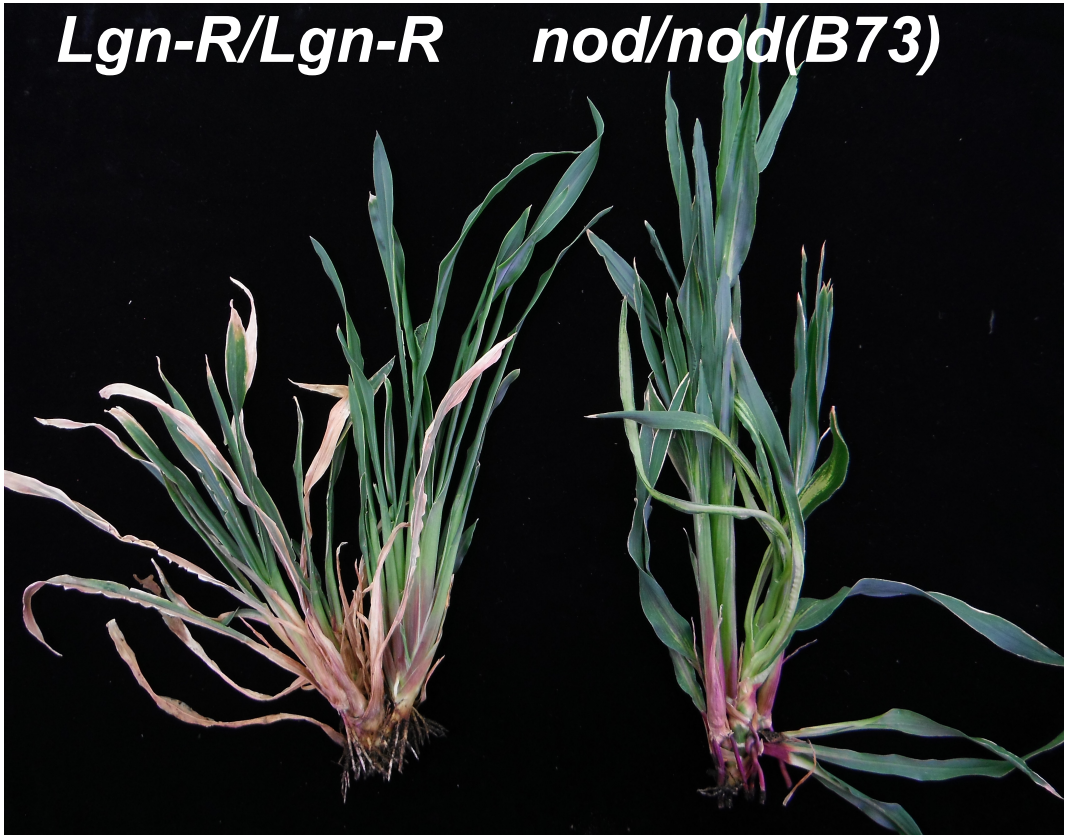


Wild-type *Lgn-R/+*



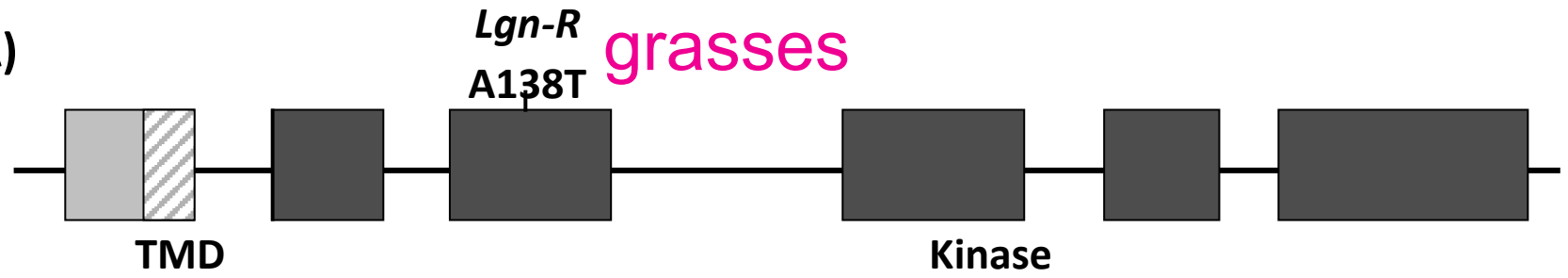
Wild-type *Lgn-R/+*

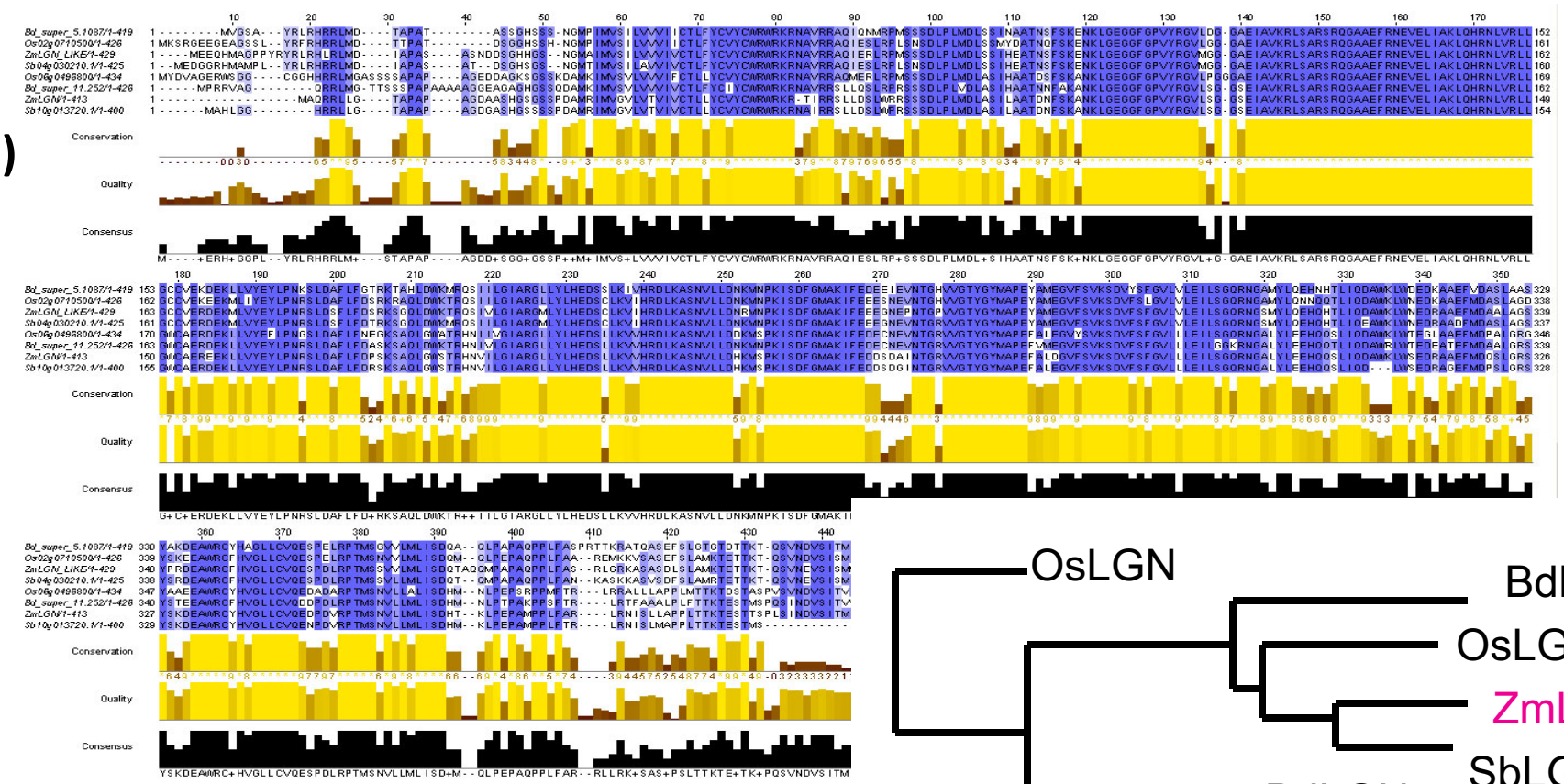
Lgn-R homozygote looks similar to *nod* homozygotes when they are both in B73



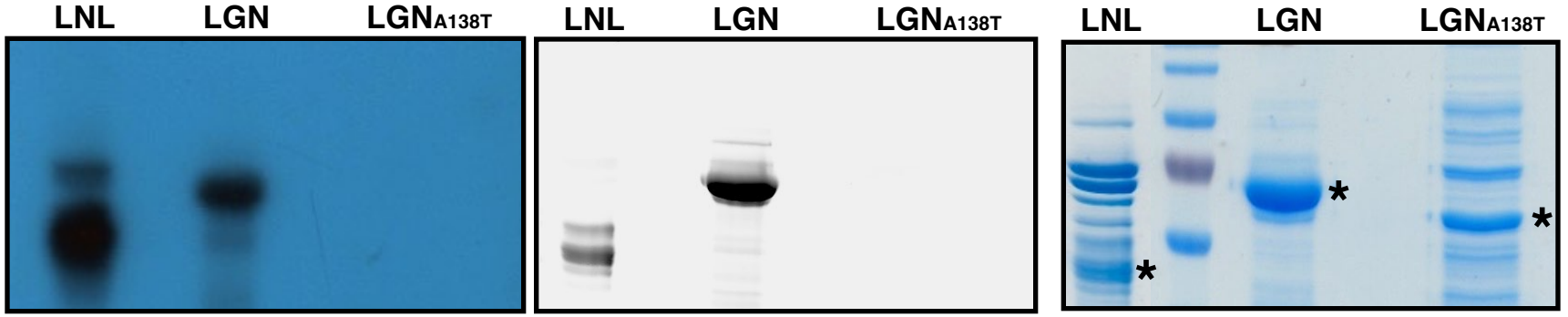
Plants at maturity

LGN genes are duplicated in the grasses





LGN and the duplicate, LNL, encode kinases. The mutant is kinase defective



Radioactive assay ProQ Diamond staining Coomassie staining

Recombinant proteins:

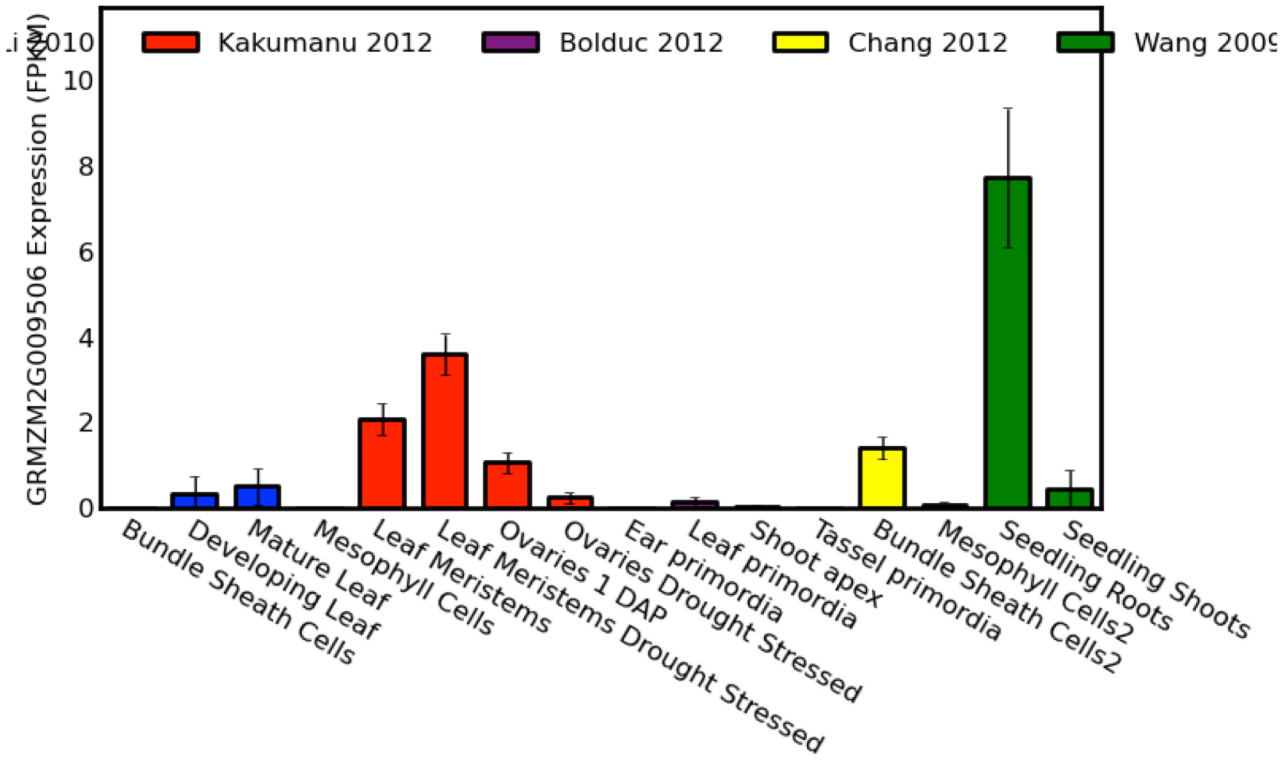
LNL - Liguleless narrow like
LGN - Liguleless narrow
LGN$_{A138T}$ - Liguleless narrow A138T

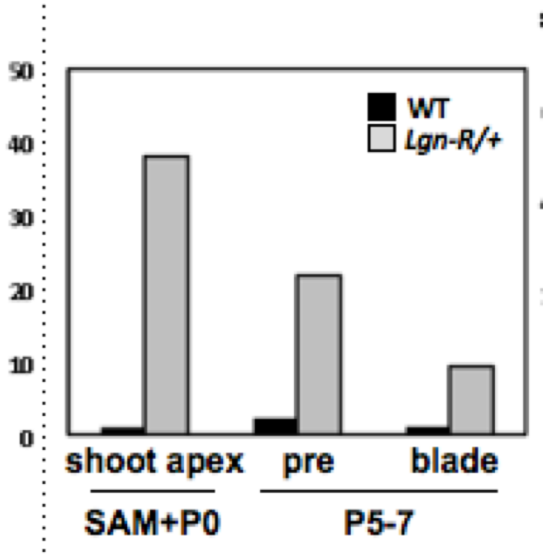
The dominant LGN-R (A138T) may block phosphorylation by the normal copy

Lgn-R phenotype is suppressed in Mo17

B73 *Lgn-R*/+ (B73) Mo17 *Lgn-R*/+ (Mo17)

Duplicate gene is normally expressed at low levels in roots and not in shoots, but is expressed at high levels in *Lgn-R* mutants

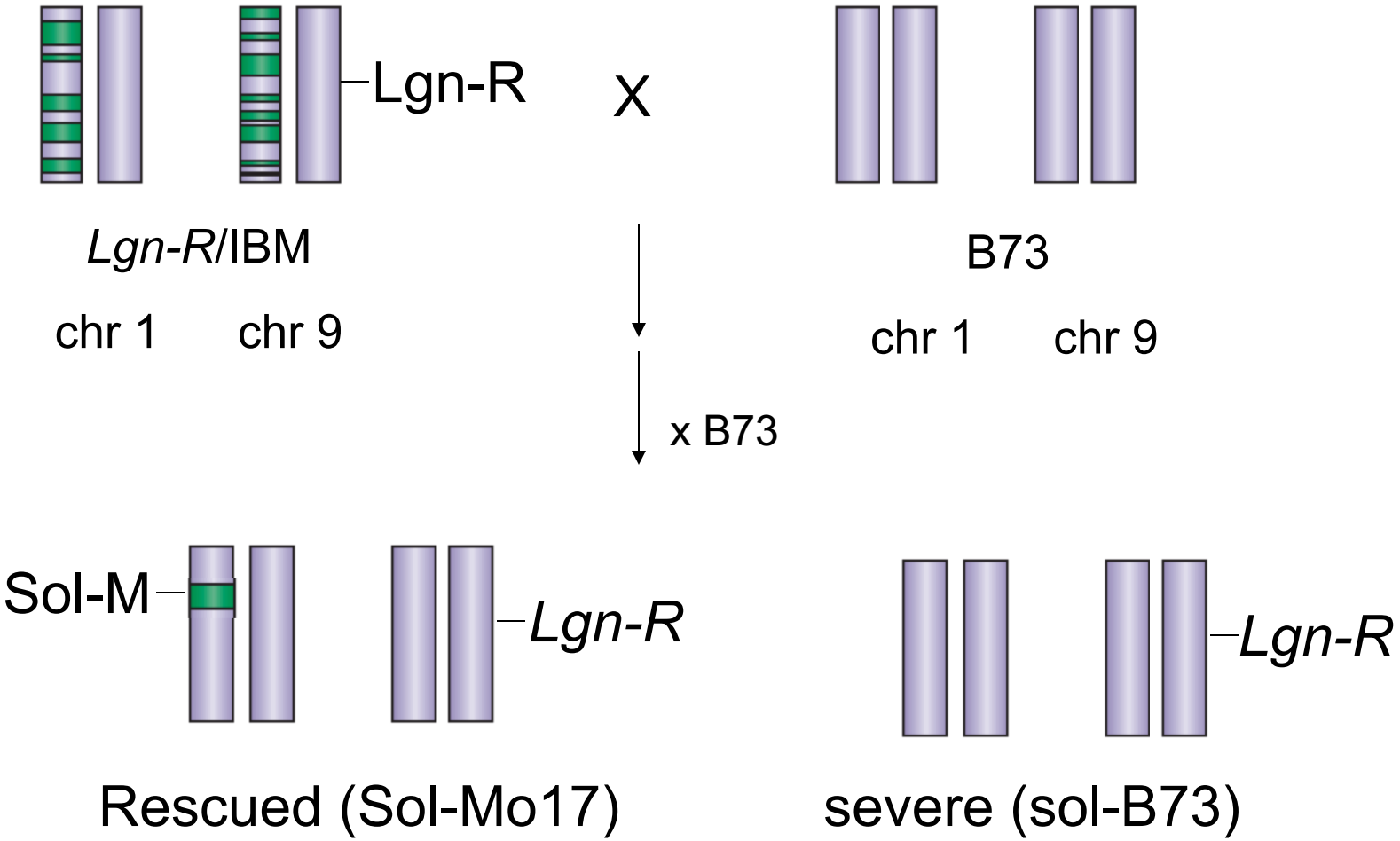




Moon et al 2013

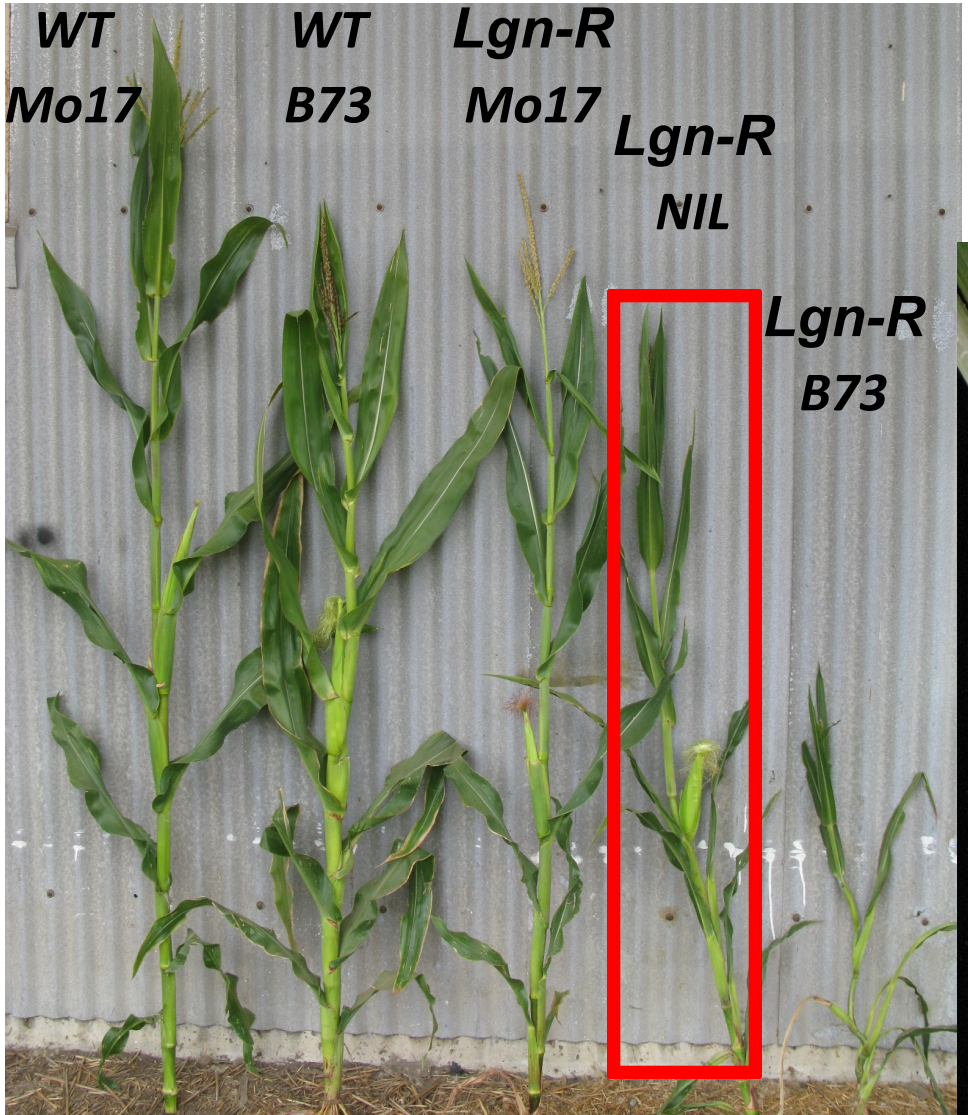
QTeller

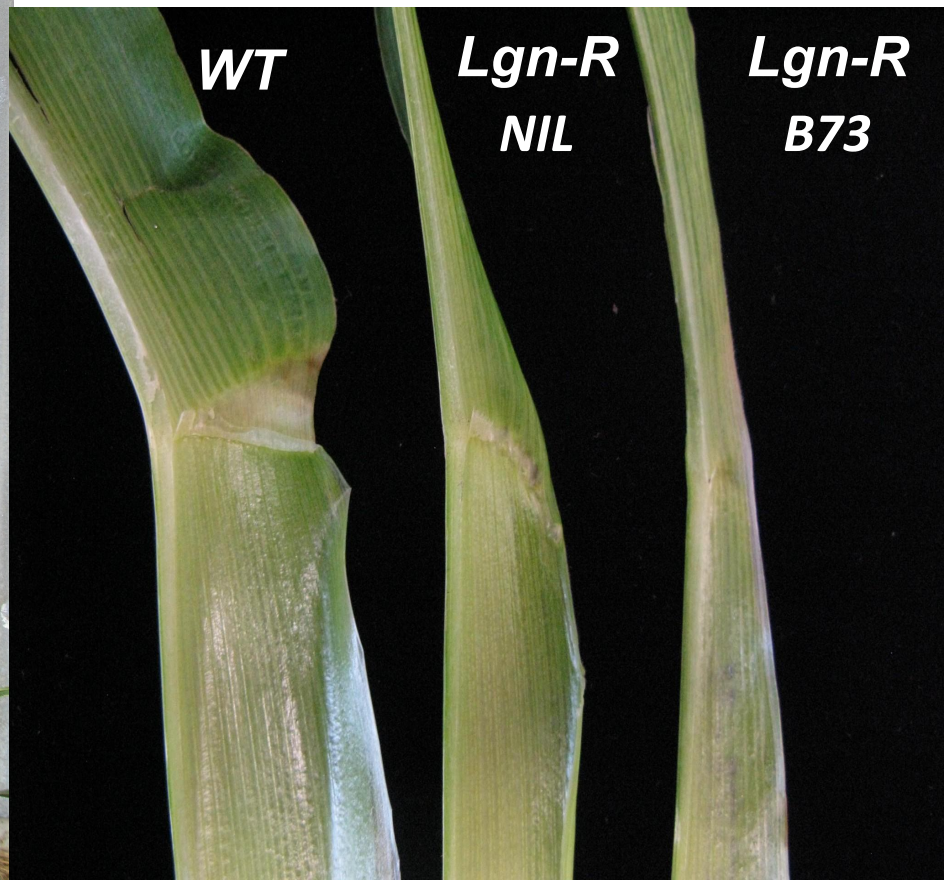
Used recombinant inbreds to identify a major QTL, then backcrossed to develop a near isogenic line (NIL)



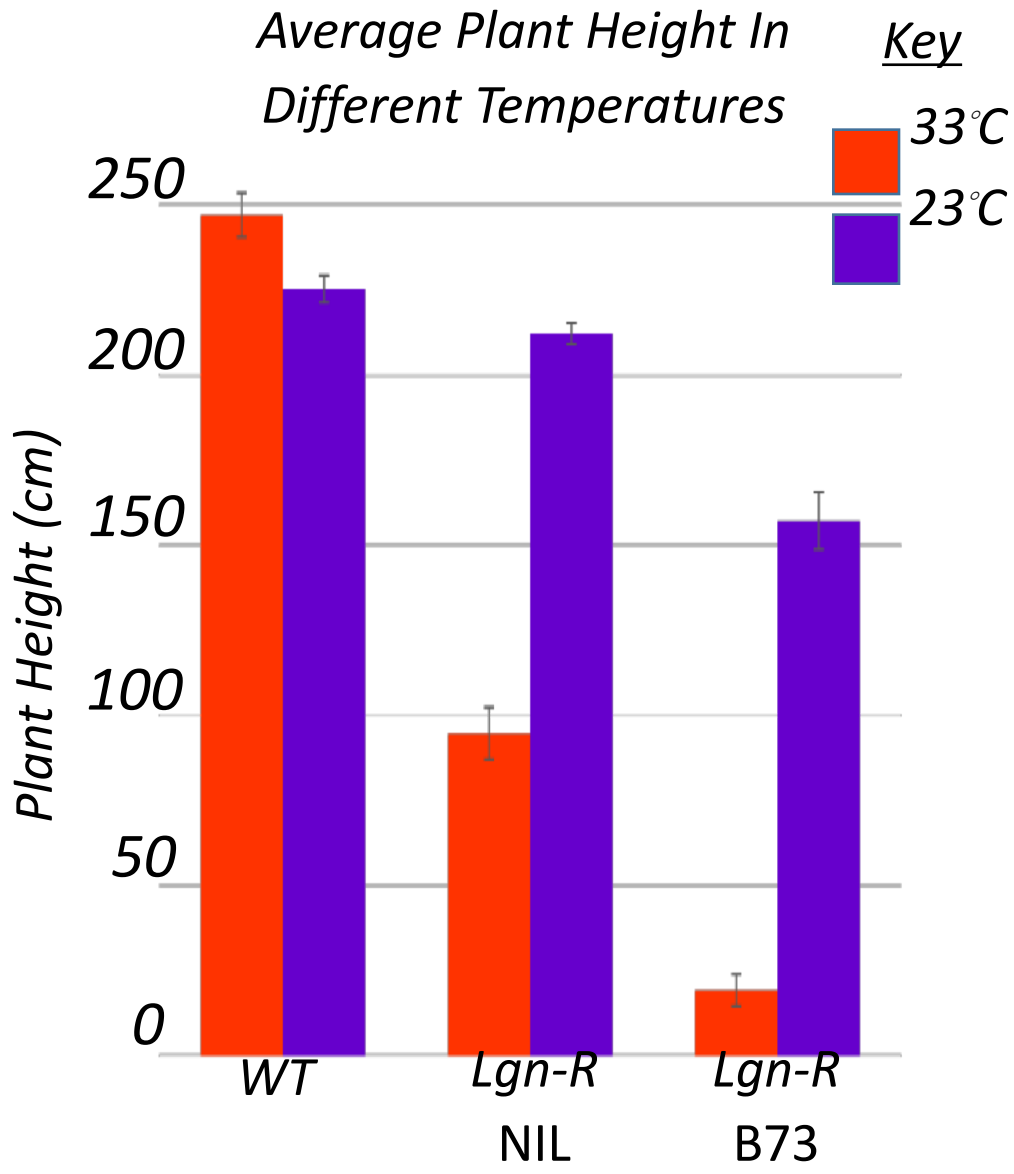
Sympathy for the ligule - Sol

The NIL (Sol) Partially Rescues the *Lgn-R*/+ Phenotype



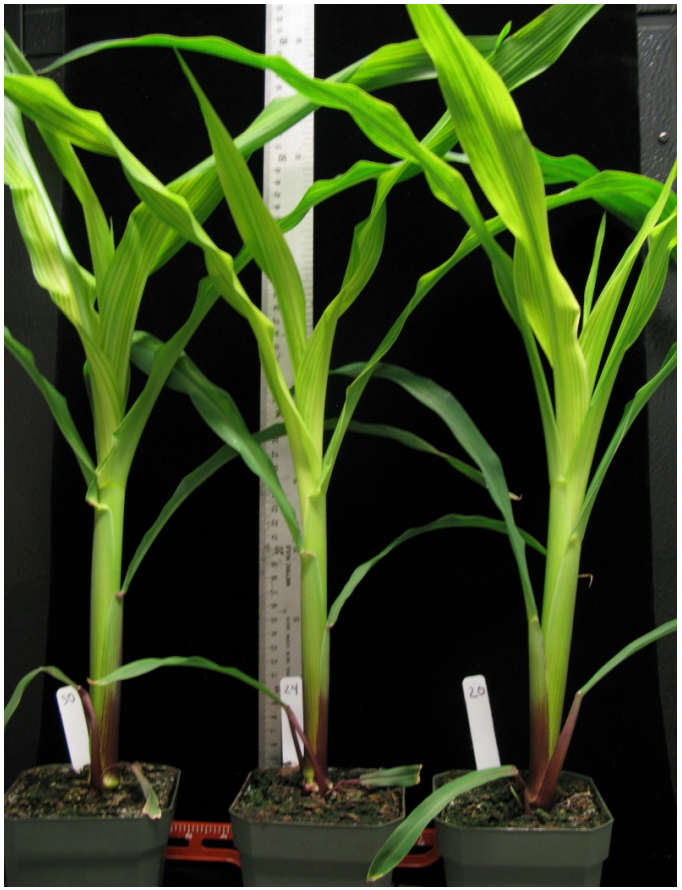


Lgn-R Plants Show a Genotype by Environment Effect



Lgn-R mutants suffer at higher temperatures in growth chamber

Cool temp (11C˚ night - 21C˚ day)

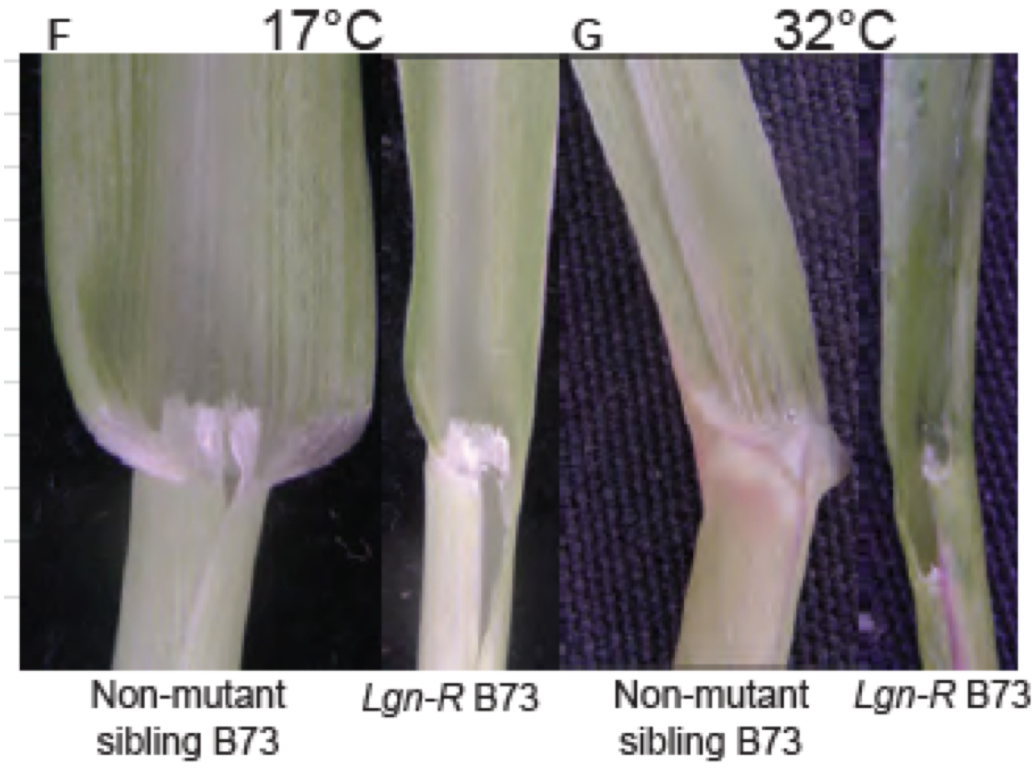


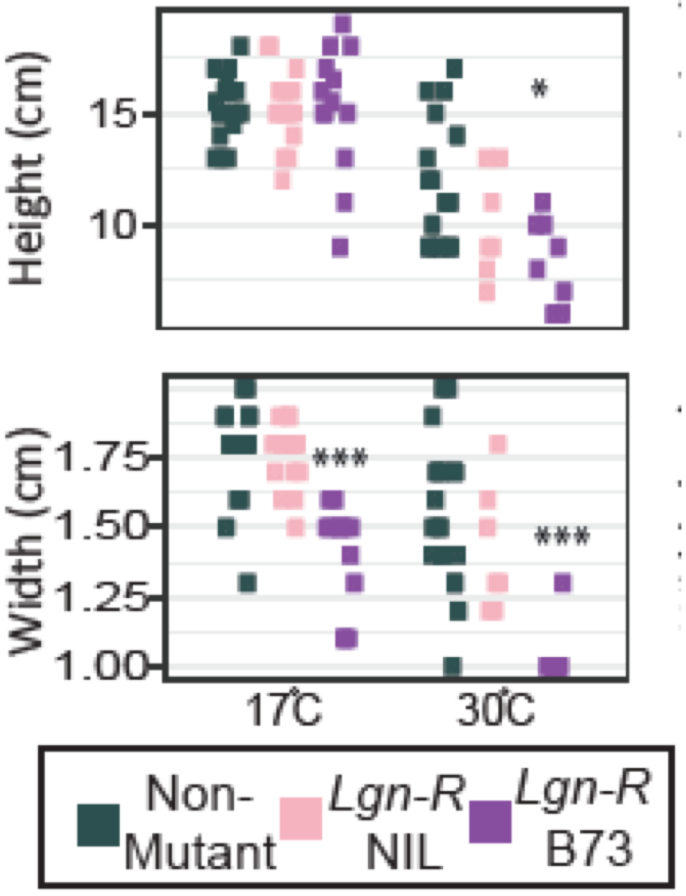
WT *Lgn-R* NIL *Lgn-R* B73

Warm temp (15C˚ night -32C˚ day)

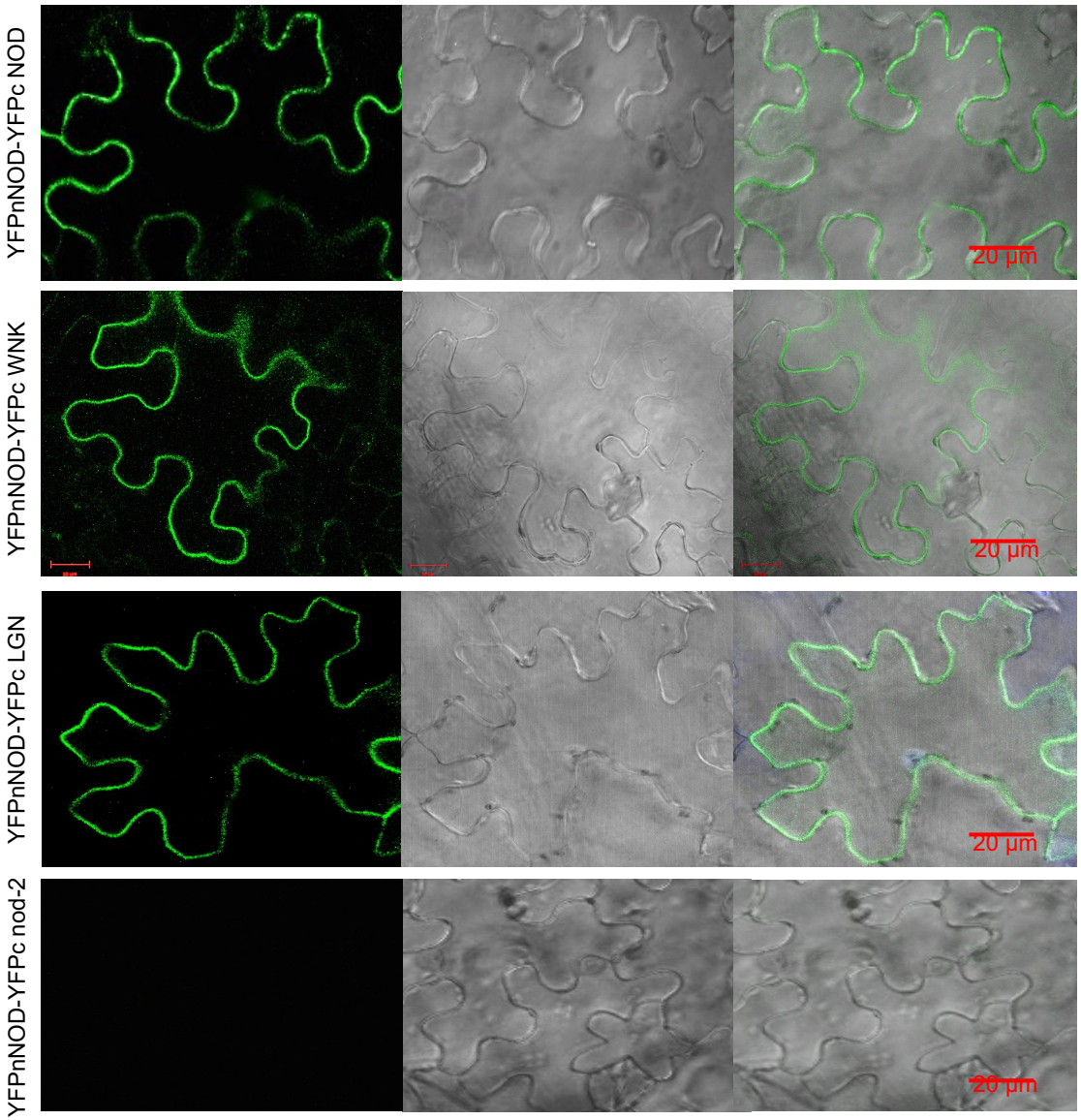
WT *Lgn-R* NIL *Lgn-R* B73

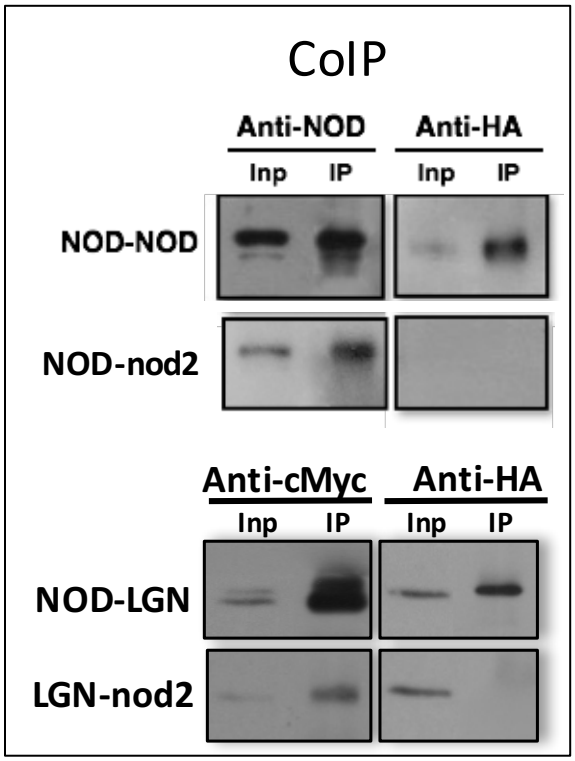
In contrast, cool temperatures make *Lgn-R* more like normal



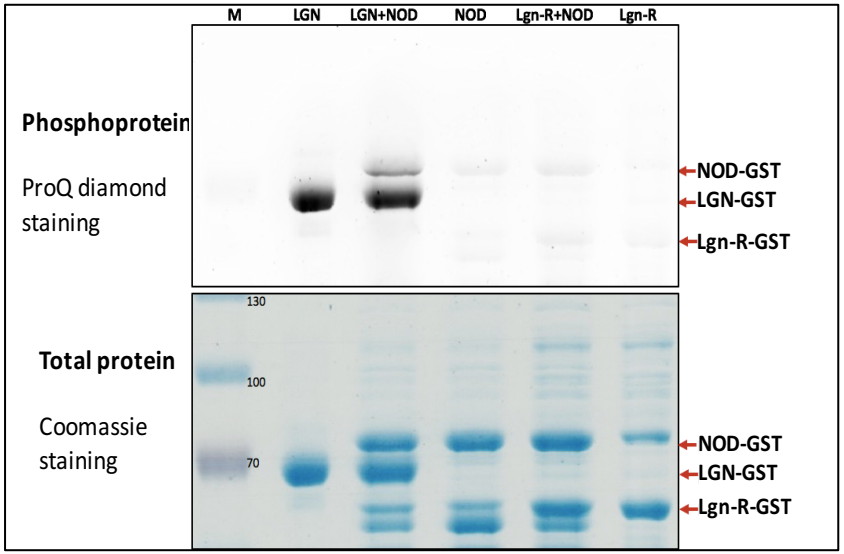


BiFC shows that NOD interacts with itself and with LGN, but not with the mutant version

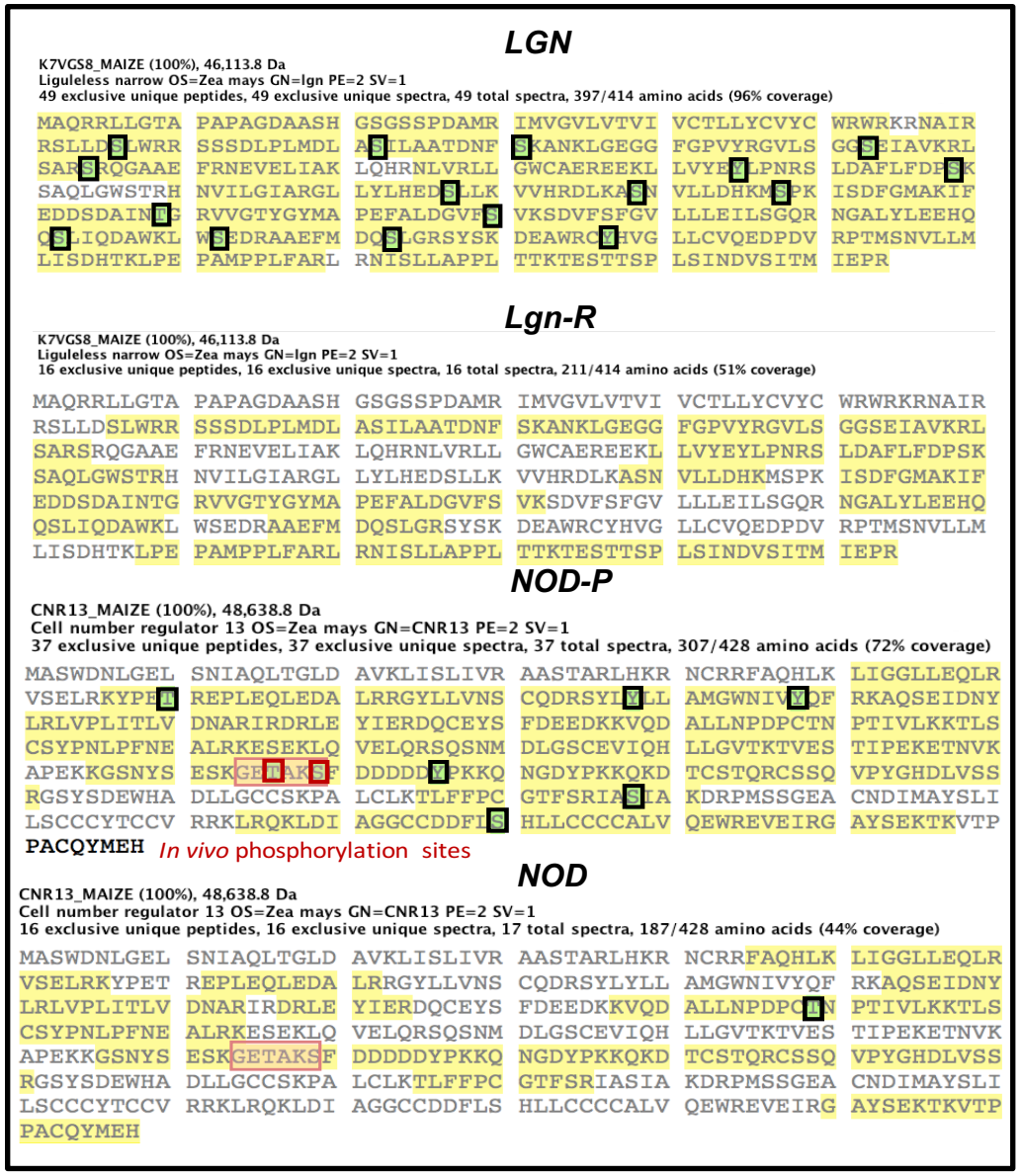




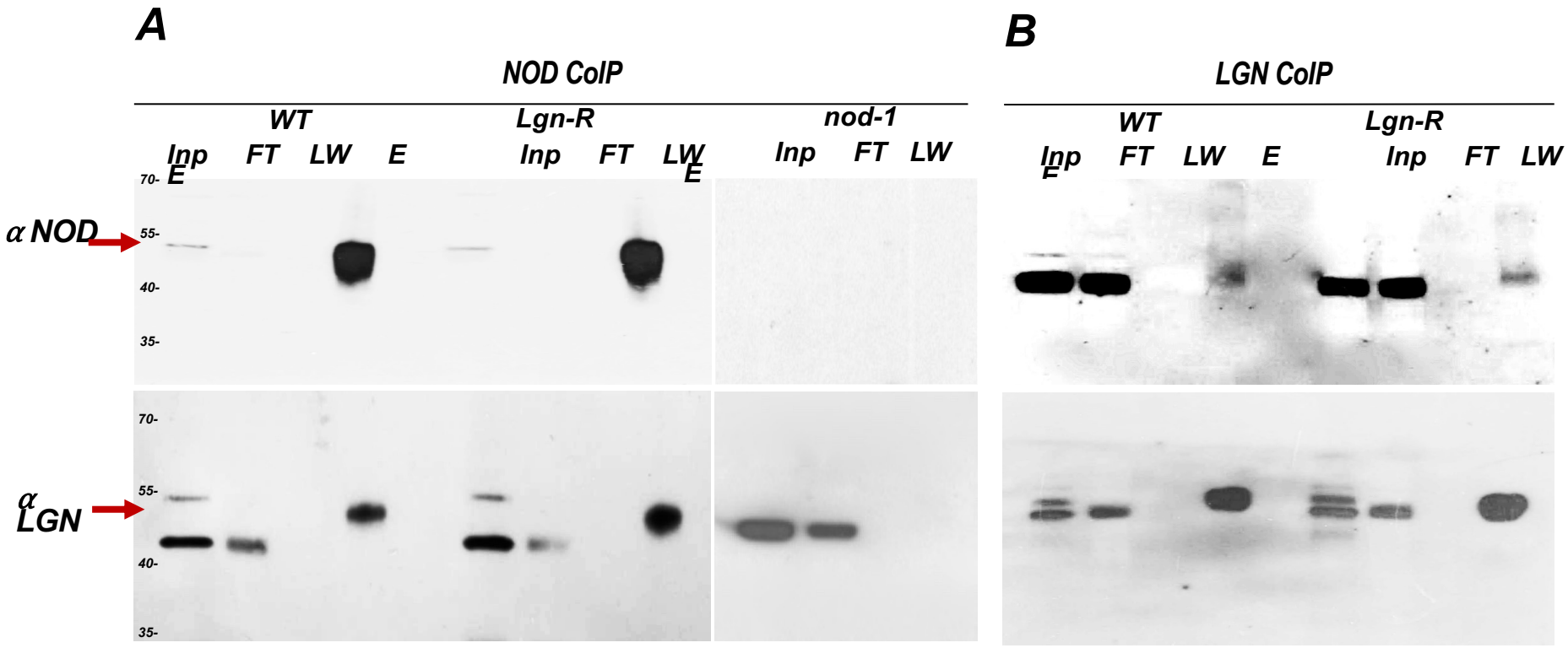
LGN phosphorylates NOD in vitro



Similar phospho sites as found in wild-type protein preps



NOD Co-IP pulls down LGN and LGN Co-IP pulls down NOD



Asked what does double mutant look like

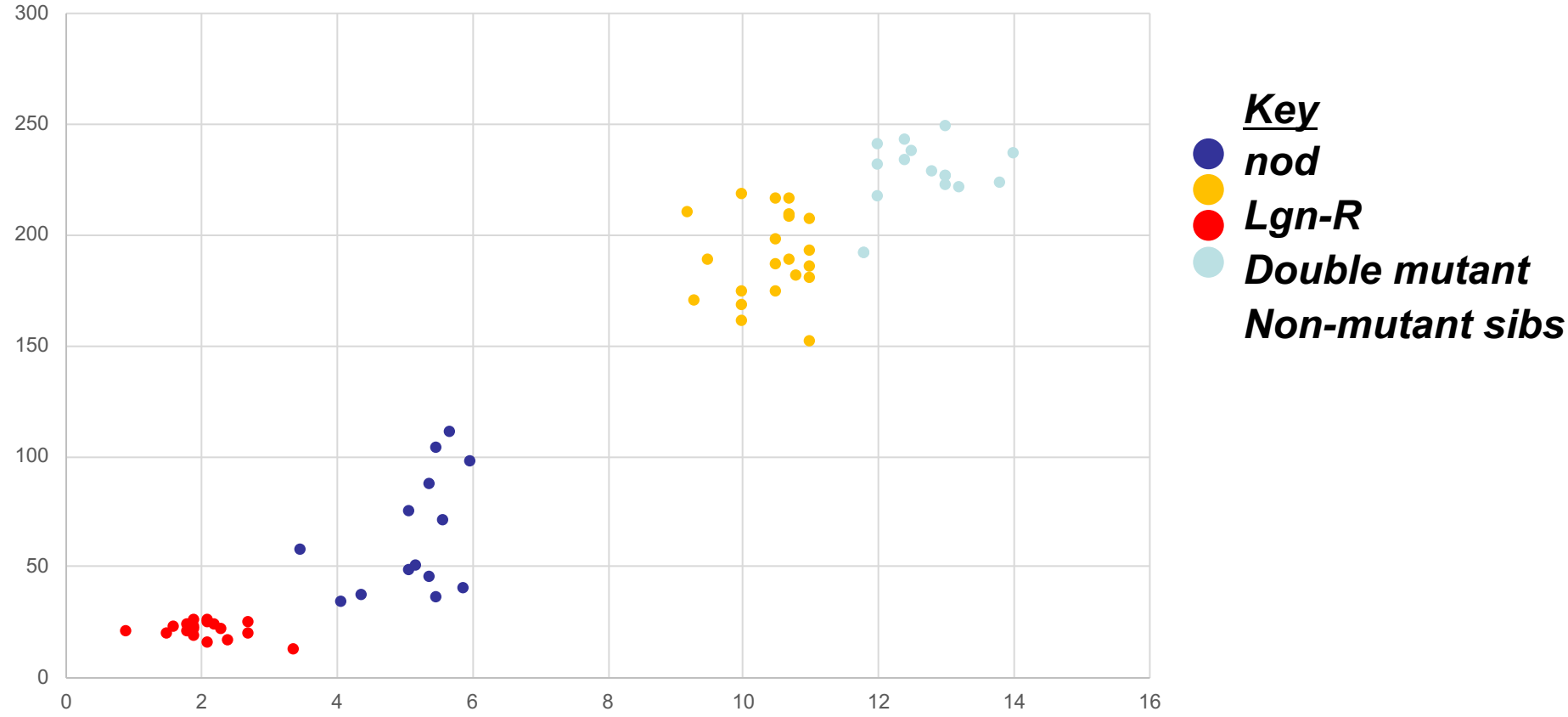
- Carried out crosses in A619 where *nod* has a mild phenotype (*a* narrow odd dwarf) and *Lgn-R* is nearly normal

The double in A619 looks like *Lgn-R* homozygote or *nod* homozygote in B73

Results suggest that they have different rescuing modifiers and in double mutant, the modifiers are ineffective

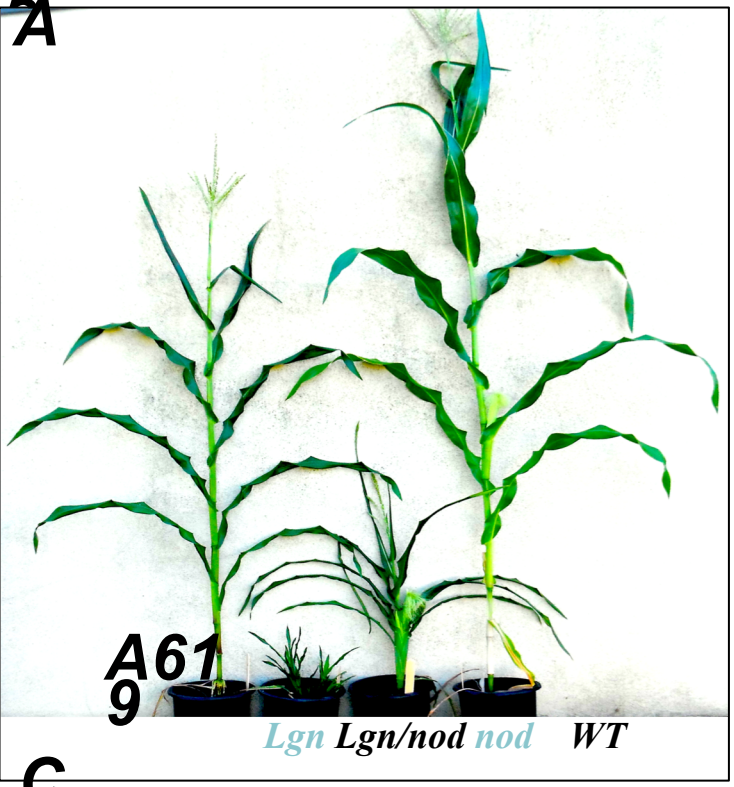
Lgn-R double *nod* normal

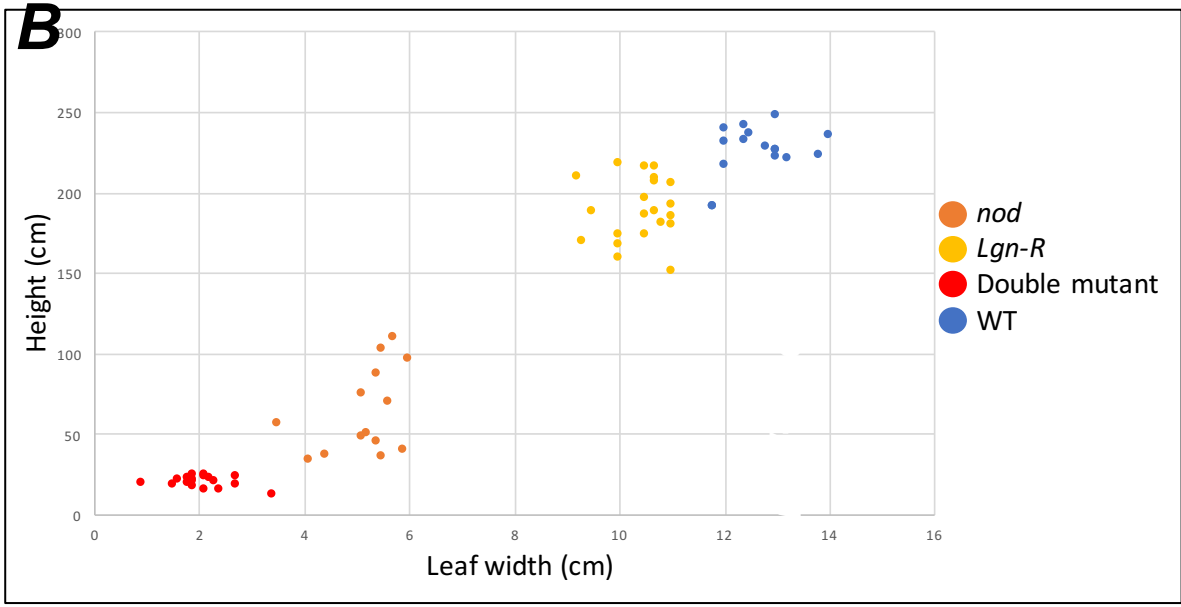
Lgn-R/nod double mutant population



Plotted height and leaf width

Figure 5

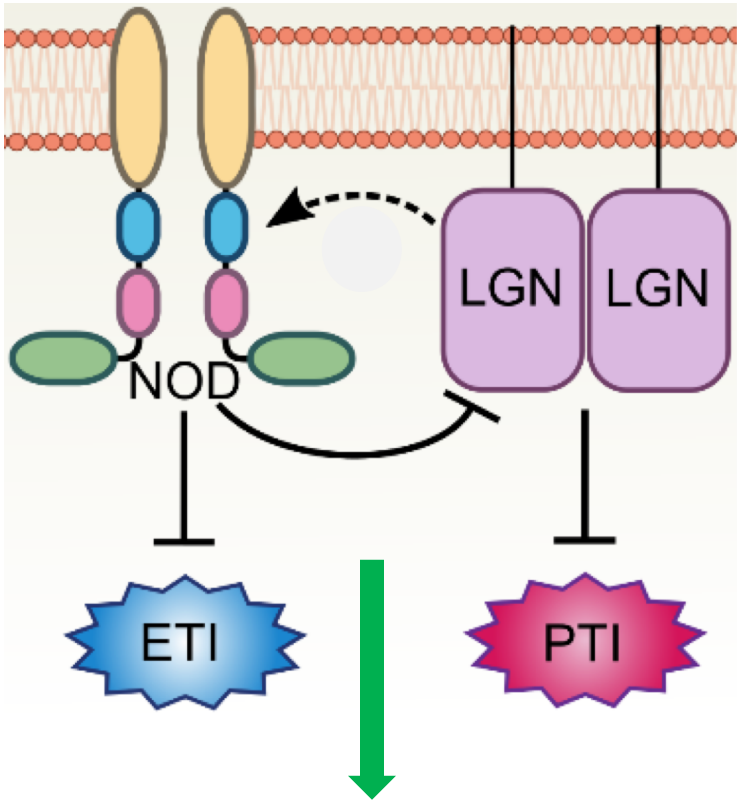

A
A619
Lgn Lgn/nod nod WT


B
Height (cm)
Leaf width (cm)
0
50
100
150
200
250
300
0
2
4
6
8
10
12
14
16
nod
Lgn-R
Double mutant
WT


C
Lgn Lgn/nod nod WT

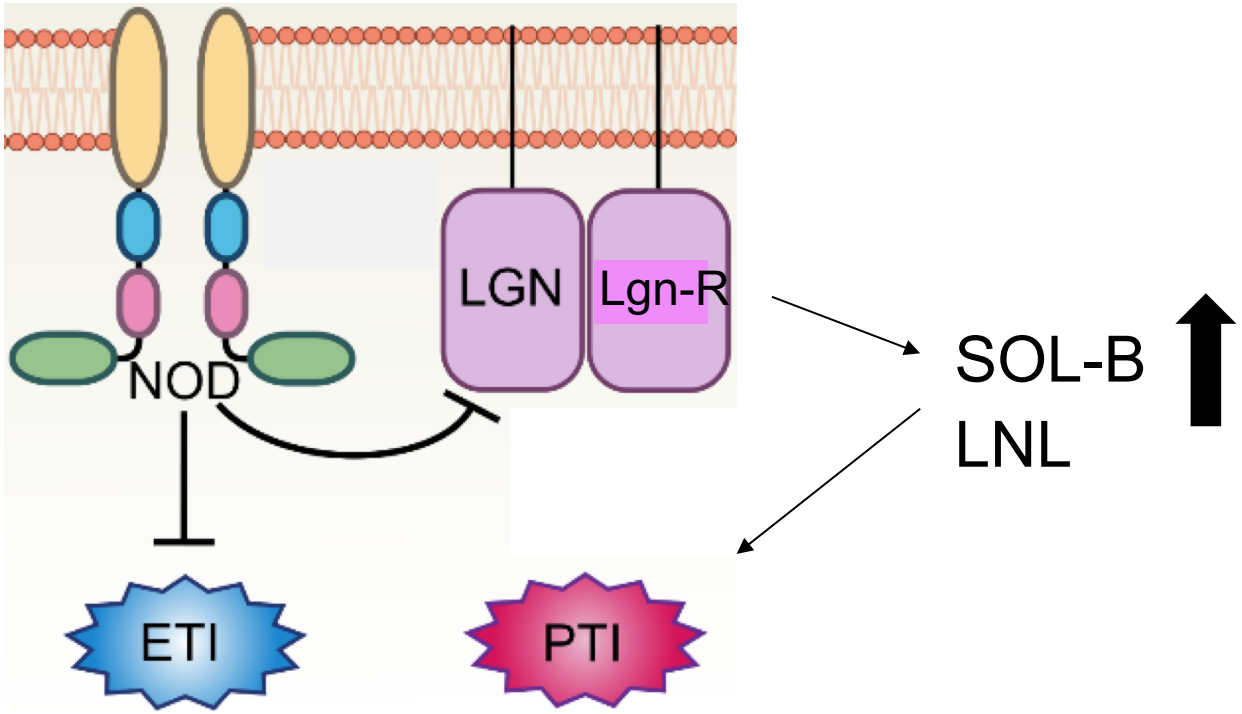

D


E
WT
Lgn-R/+
Lgn-R/Lgn-R
Lgn-R/+; nod
nod

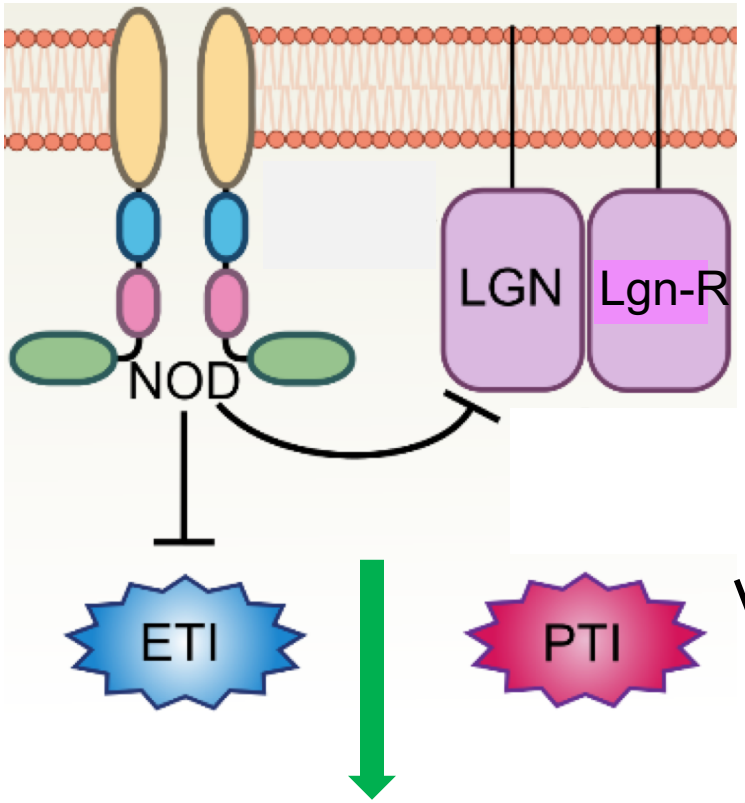


Normal leaf development

Propose that LGN and NOD have dual functions – promote leaf development and keep immunity in check



In *Lgn-R* mutant in B73, failure to phosphorylate targets, Sol-B transcripts up, PTI increases, abnormal leaf development



SOL-B

SOL-M

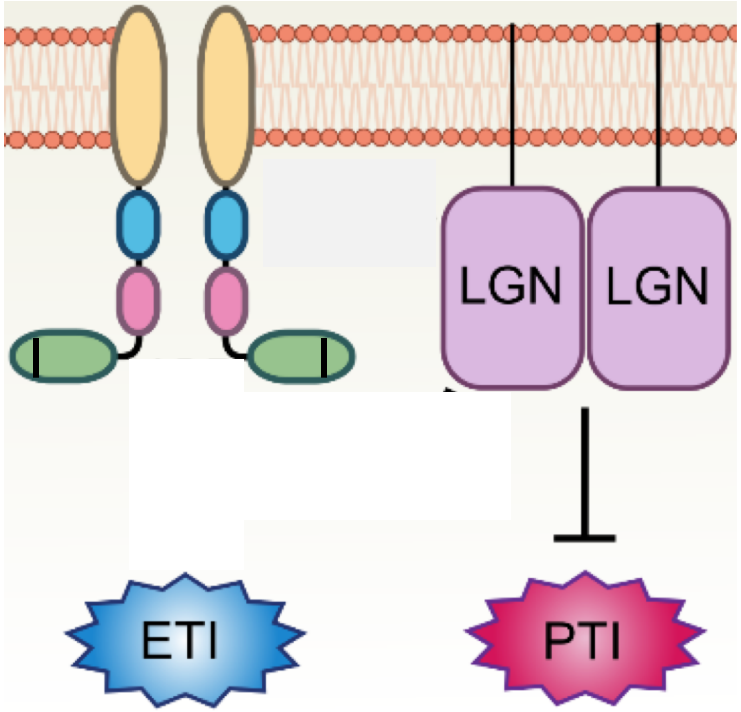
Normal leaf development

Even with 1 copy of Sol-M, SOL-B is reduced, leaf is more normal and PTI is reduced

Suggests that SOL-M may interact with different proteins than SOL-B

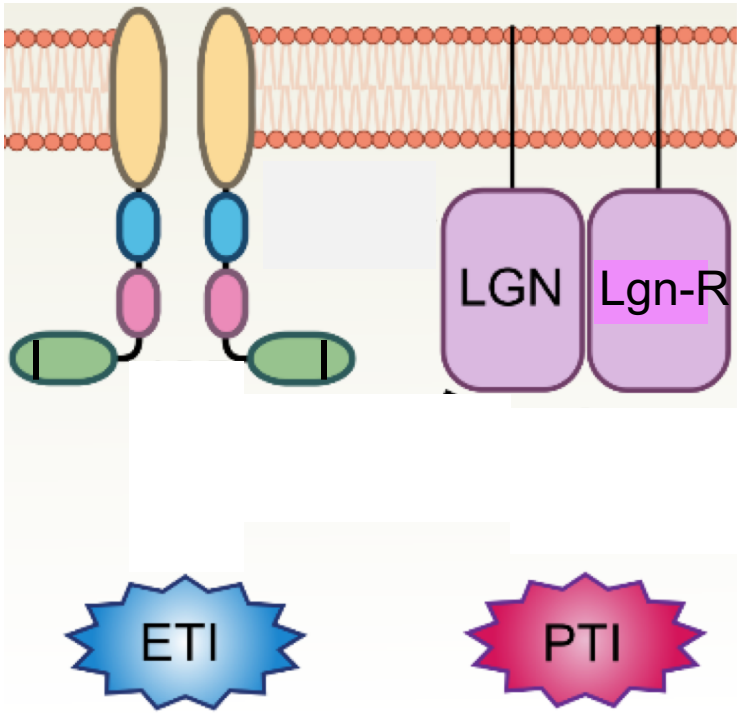
In nod mutant,

ETI is activated but LGN still keeps brakes on PTI



In double mutant mutant,

ETI and PTI are activated and modifiers not able to rescue plant



Take away lesson

Cross your mutants to different inbreds

You never know what you might discover


Alyssa
Jazmin
Marisa
Sam

Present lab members

China Lunde	Ts5 and JA
George Chuck	tillers
Zhaobin Dong	tillers
Angus Vajk	sex determination
Alyssa Anderson	LGN and SOL
Jake Brunkard	**cell biology**
Annis Richardson	leaf development
Sam Leiboff	sorghum, maize networks
Martin Alexander	tassel development
Brianna Haining	iron uptake in Setaria

Recent members

Jazmin Abraham Juarez	proteomics
Mike Lewis	lg1, lg2, wab
Marisa Rosa	nod
Katsutoshi Tsuda	KNOX and BEL

Collaborators

Leaf collaborators: Anne Sylvester, Mike Scanlon, Robyn Johnston, Rico Coen, Pat Brown

Inflorescence collaborators: Erik Vollbrecht, Dave Jackson, Jose Dinneny, Andrea Eveland, Torbert Rocheford

Funding

ARS - positional cloning

NSF & BBSRC - leaf modeling

NSF - leaf & inflorescence

The double in A619 looks like *Lgn-R* homozygote or *nod* homozygote in B73

Results suggest that they have different rescuing modifiers and in double mutant, the modifiers are ineffective

Lgn-R double *nod* normal