Resistance Mechanisms and Management of *Gibberella zeae* to Benzimidazole Fungicide Carbendazim and a Novel Fungicide Phenamacril (JS399-19)

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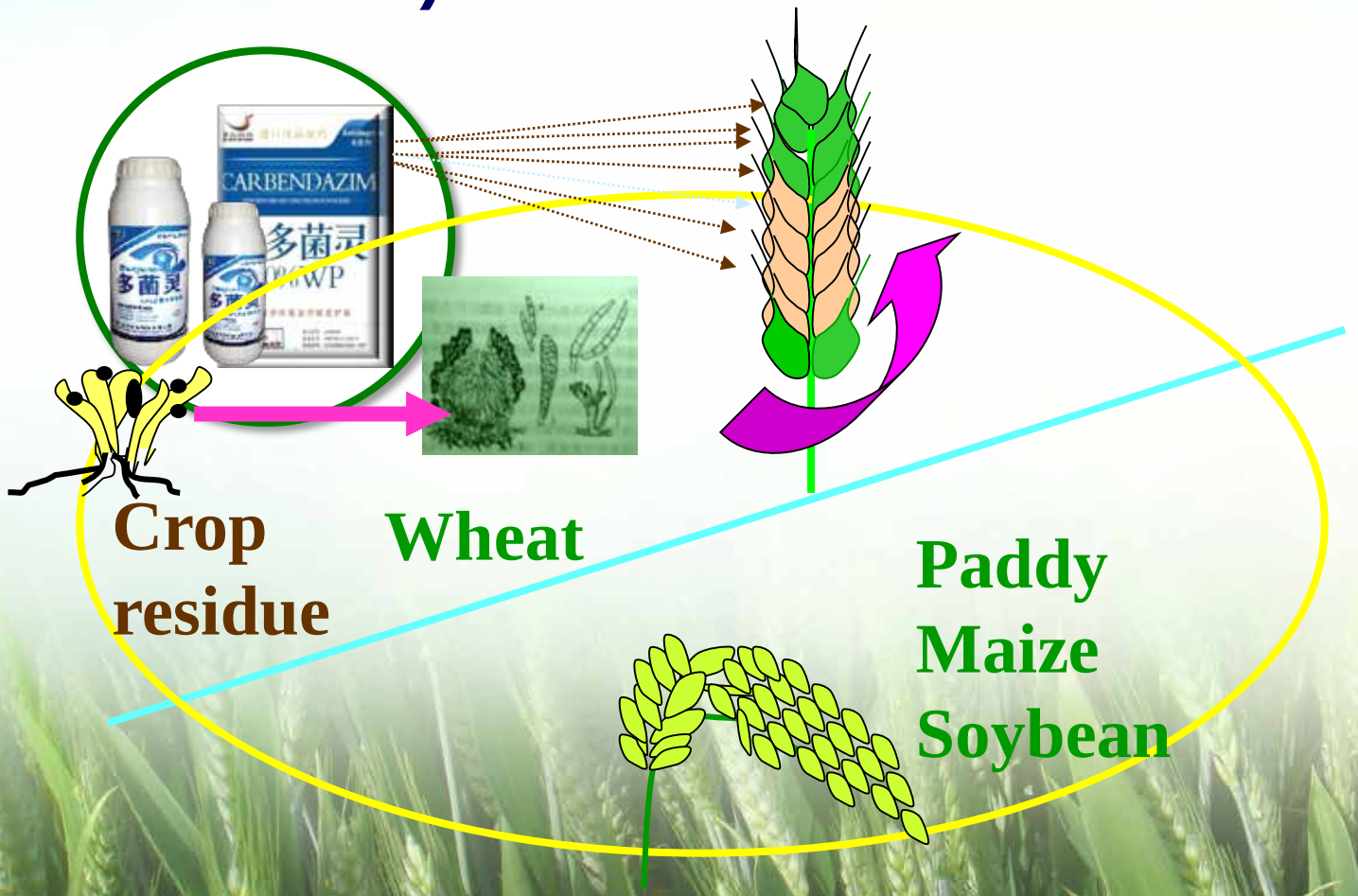
7/12/2014

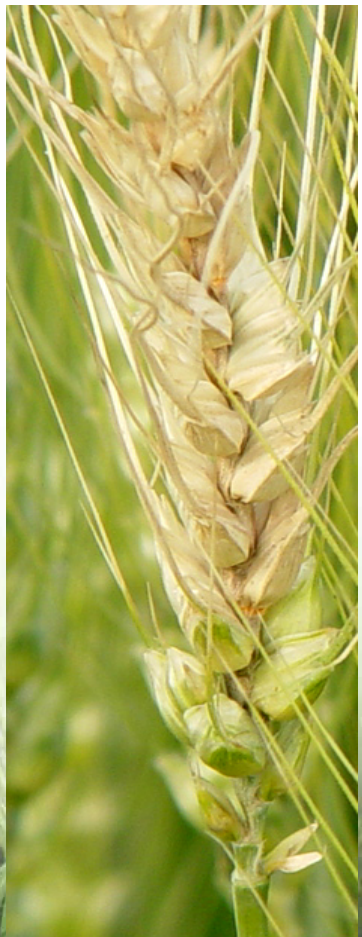
- **Background**
- **Mechanism of MBC-resistance in *G. zeae***
- **Interaction between b_1- and b_2-tubulin**
- **Difference in bio-property of point mutation and gene deletion**
- **Resistance management of *G. zeae***
- **Mechanism of JS399-19(Phenamacril)-resistance in *G. zeae***
- **Summary**

Background

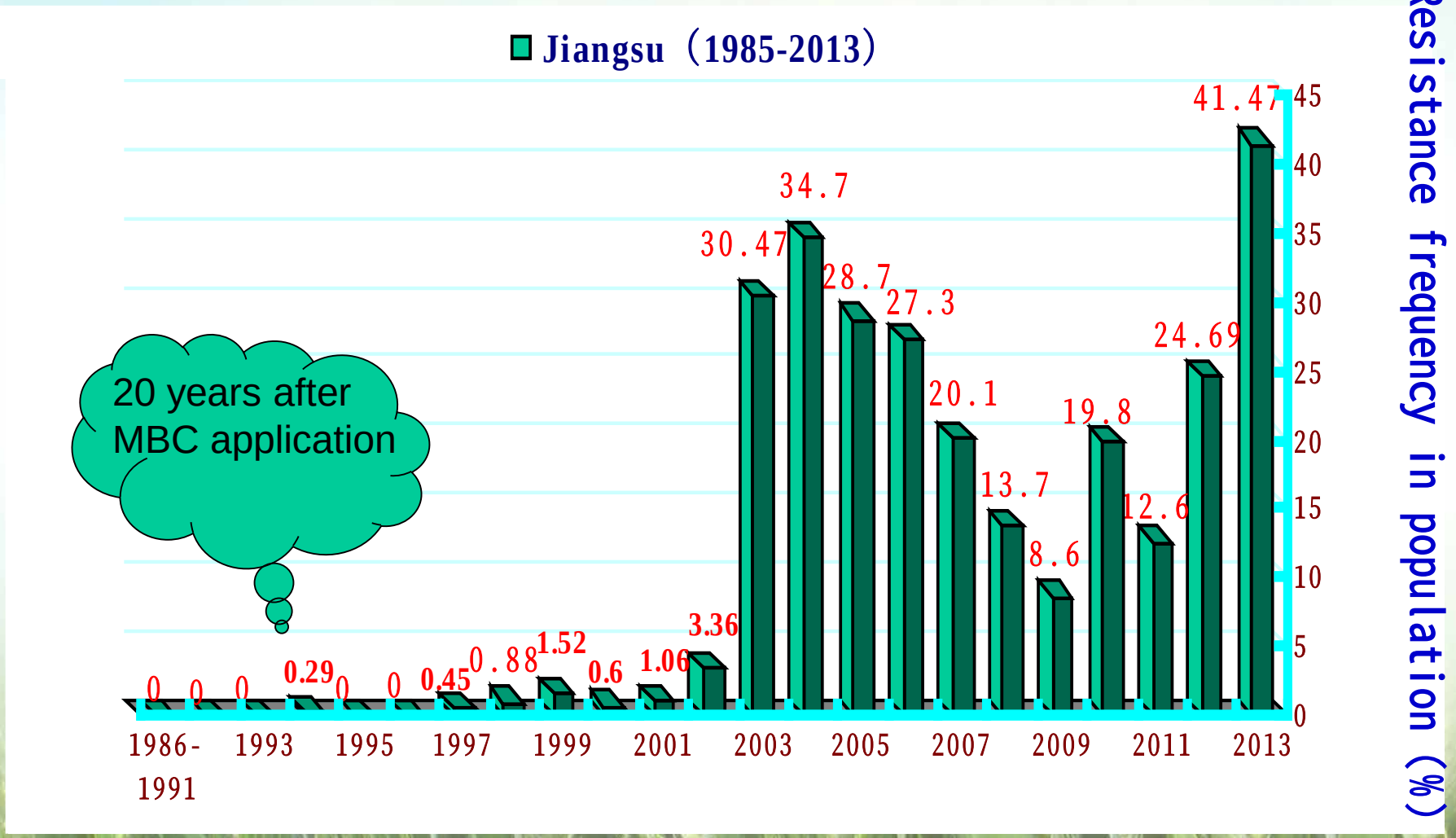
- **Wheat is sowed about 24million ha. in China every year**
- **FHB (scab) mainly occurs in Yangtze River valley, eastern coast and extends to Northwest**

- **No immunological or high-resistance wheat cultivar available**
- **Carbendazim (MBC, Methyl benzimidazole carbamate) has been used since the 1970s**

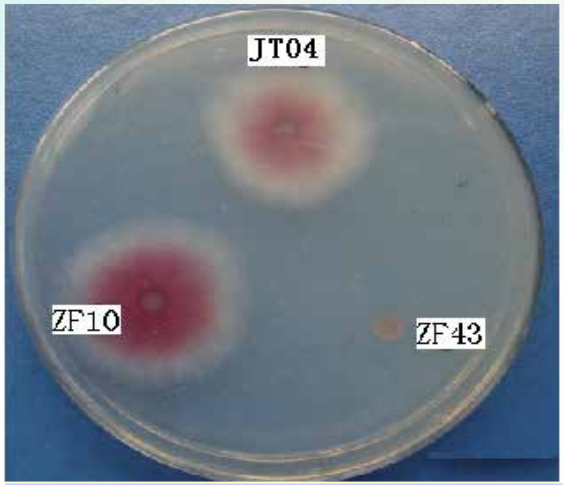




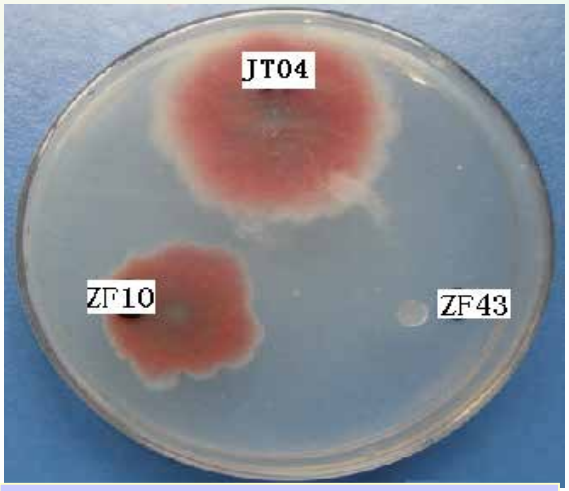
Development of MBC-Resistance Population



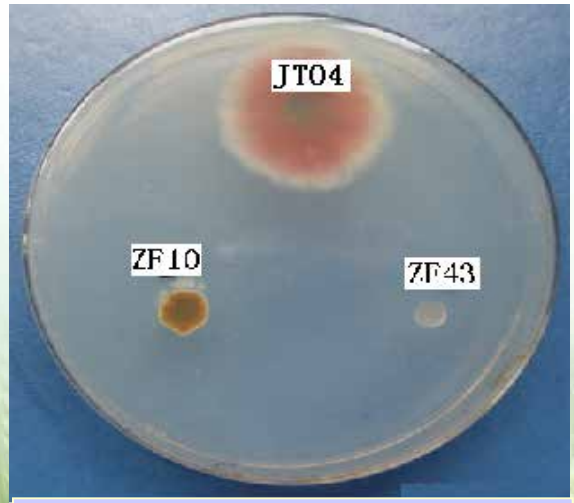
Phenotypes of Resistance



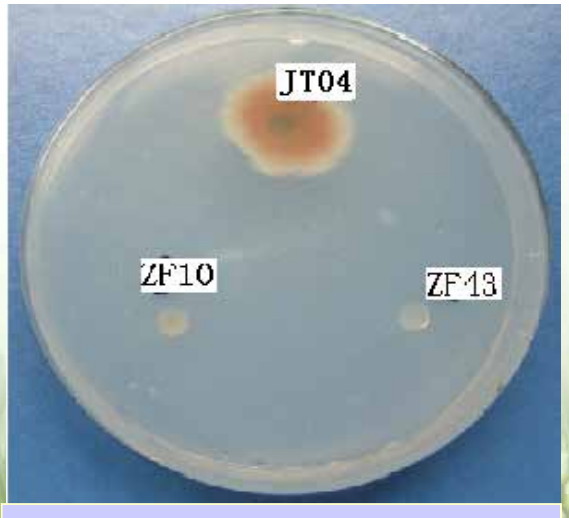
1.4 μ g/ml of MBC



10 μg/ml of MBC



50 μg/ml of MBC



100μg/ml of MBC

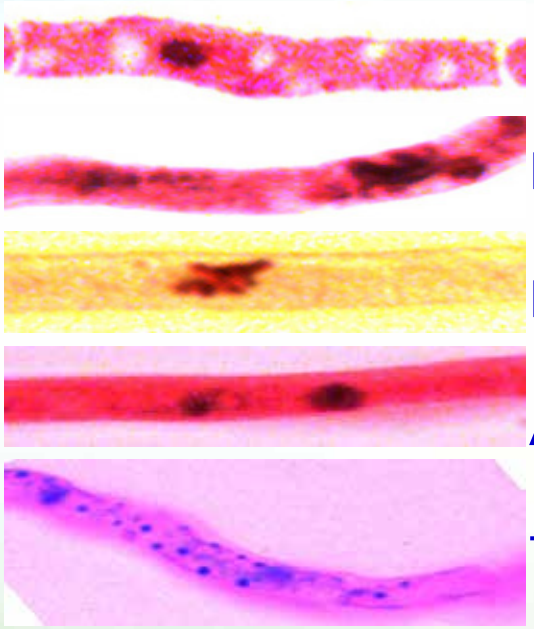
Mechanism of MBC-Resistance

Previous Result at Our Group

- **Frequency of MBC-resistance mutation in *G. zeae* is lower than that in other fungi.**
- **Carbendazim-resistance level is almost moderate.**
- **No change in the b-tubulin of MBC-resistant**
- **Resistance was mediated by one major gene by the analysis of genetics crosses.**

Observation of Mitosis Procedure

Wild-type sensitive



Interphase

Prophase

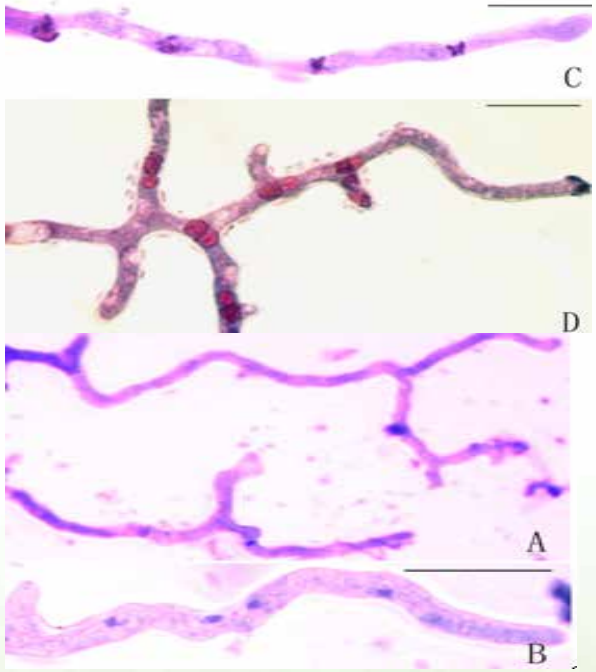
Metaphase

Anaphase

Treated by MBC

Carbendazim-resistant

MBC treat



30min

60min

6h

12h

It implied that the resistance is still involved in the gene related to mitosis

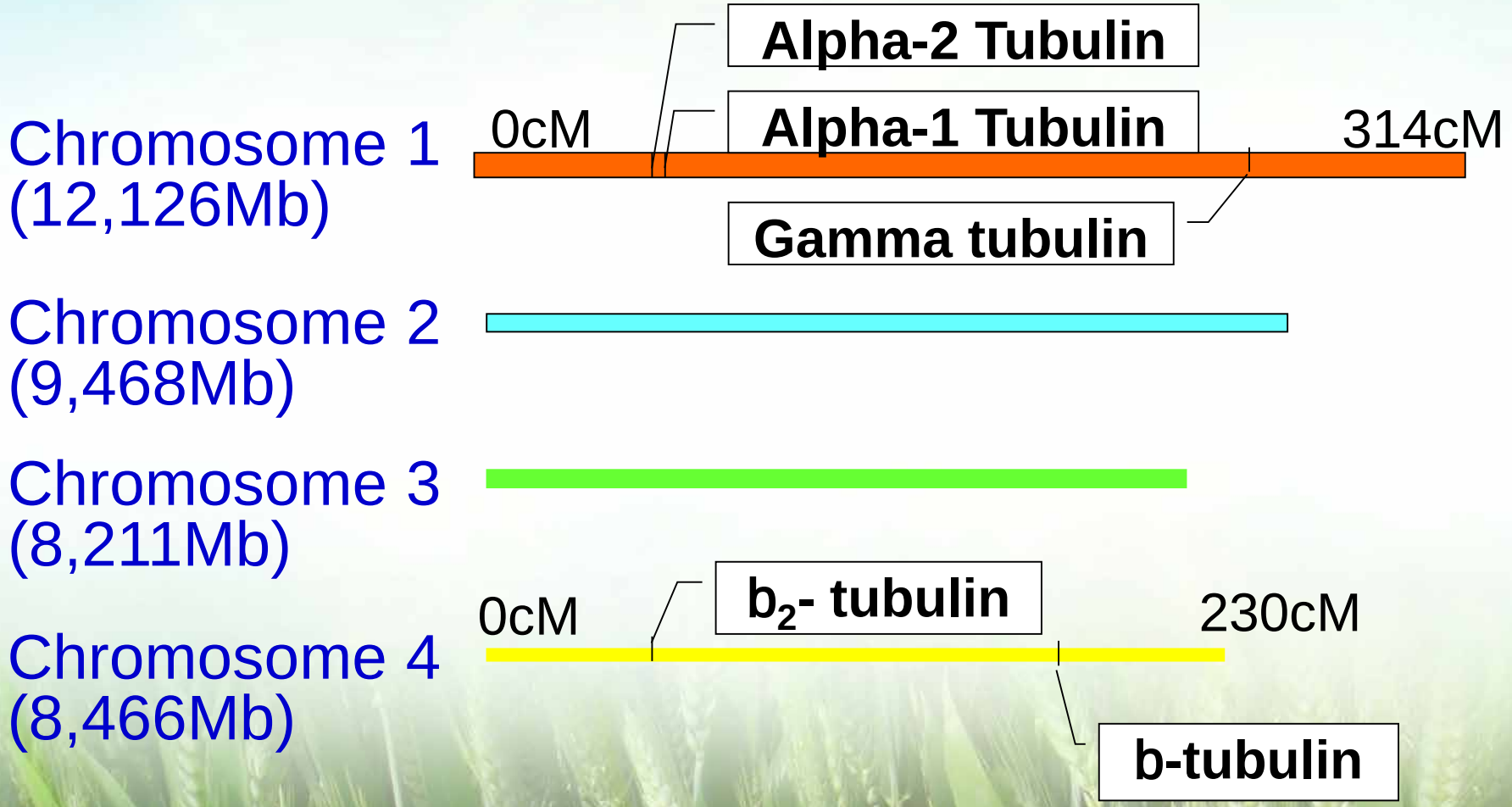
Point Mutation on *b_2-tubulin* Companied with MBC-Resistance

- **All tubulin genes involved in mitosis were analyzed between sensitive and resistant.**
- **99% of 110 moderate resistance isolates substituted TAT for TTT at codon 167 (F167Y) in *b_2-tubulin*. Others corresponded to shift at codon 17, 50 or 200**
- **Less 5% of resistance population appeared high resistance degree and responded the shift at codon 198 or 17 and 167 in the *b_2-tub***

Amino Acid Shift on b_2-tubulin

Phenotype	Number of tested strains	Codon Number						
		166	167	168	197	198	199	200
MBC^S	14	ACC T	TTT F	TCC S	GAC D	GAG E	ACC T	TTC F
MBC^{MR}	108	ACC T	T**A**T **Y**	TCC S	GAC D	GAG E	ACC T	TTC F
MBC^{MR}	1	ACC T	TTT F	TCC S	GAC D	GAG E	ACC T	T**A**C **A**
MBC^{IMR}	1	ACC T	T**A**T **Y**	TCC S	GAC D	GAG E	ACC T	TTC F
MBC^{HR}	1	ACC T	TTT F	TCC S	GAC D	**CT**G **L**	ACC T	TTC F
MBC^{HR}	4	ACC T	TTT F	TCC S	GAC D	**A**AG **K**	ACC T	TTC F

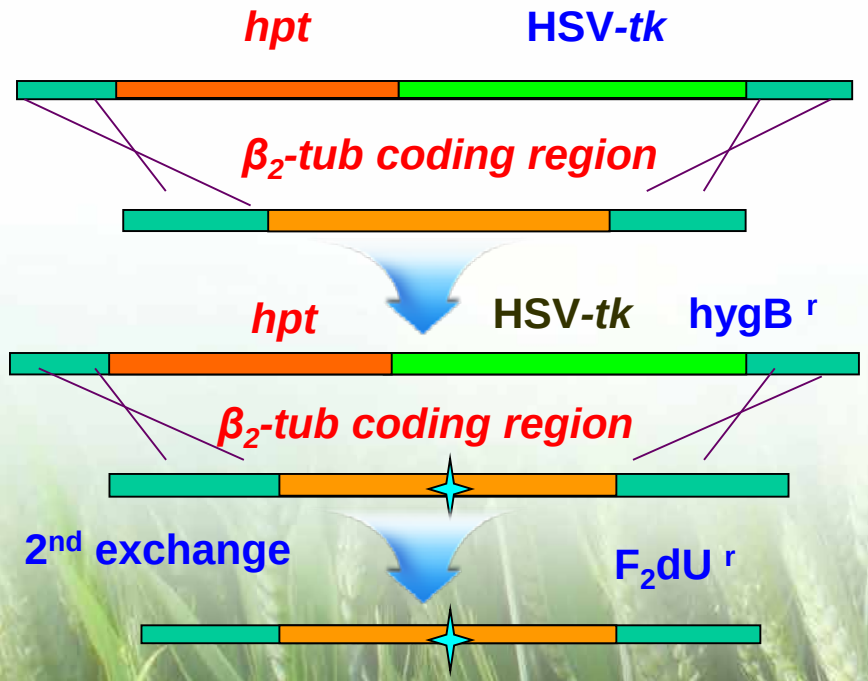
Orientation of *b_2-tubulin*

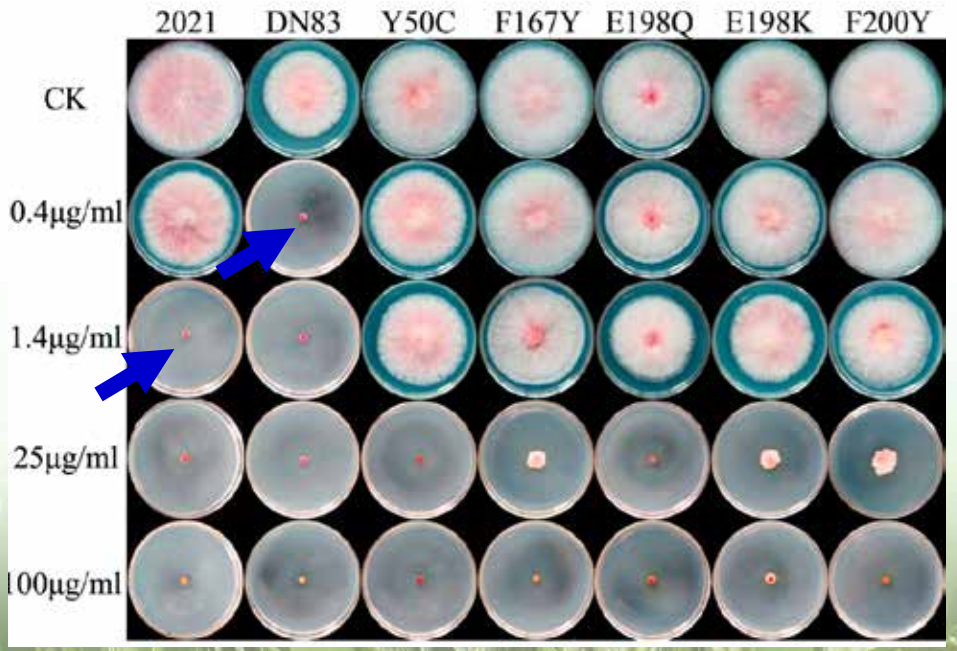


Does the Single Nucleotide Shift at Different Site Decide Phenotype of MBC-Resistance?

Point mutation at codon G50S or F167Y or E198K or F200Y

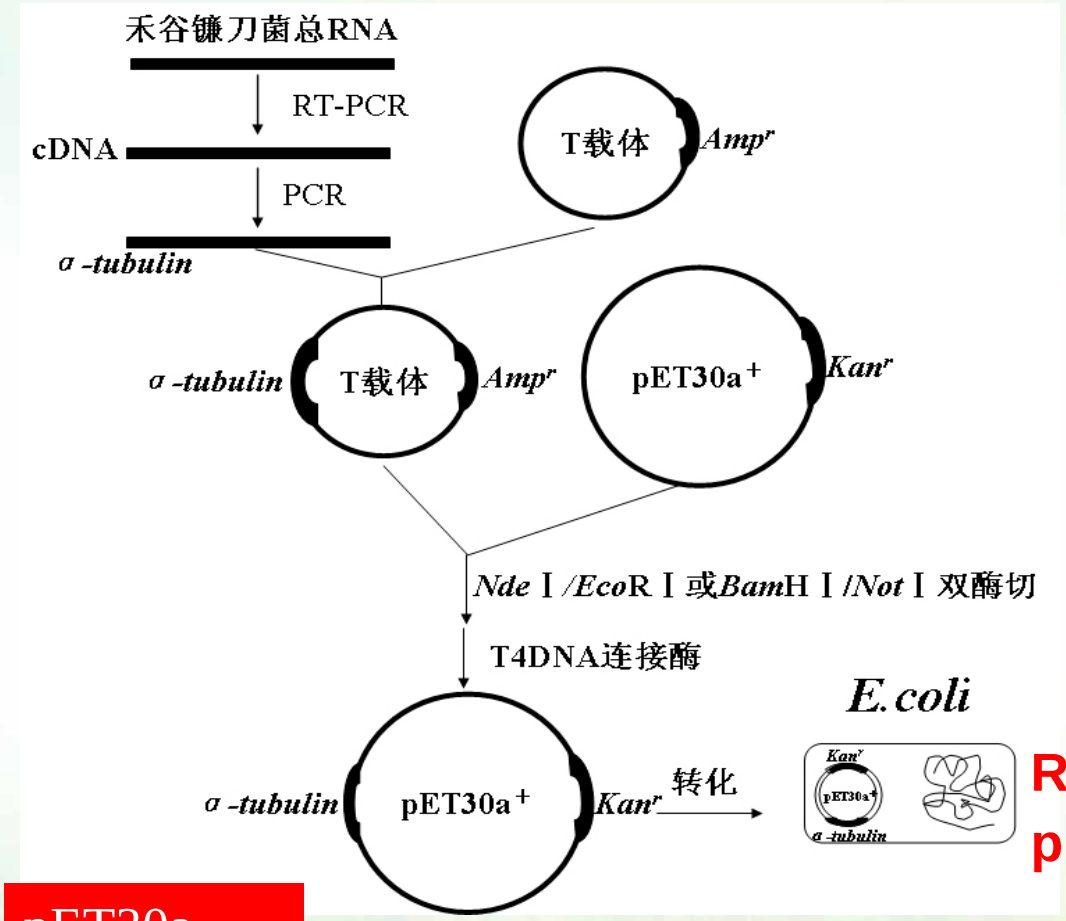
GGT→TAT、TTT→TAT、 GAG→AAG/CTG、TTC→TAC



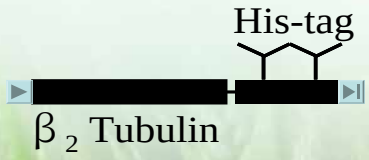


Why does a single amino acid change in b2-tubulin result in MBC-resistance?

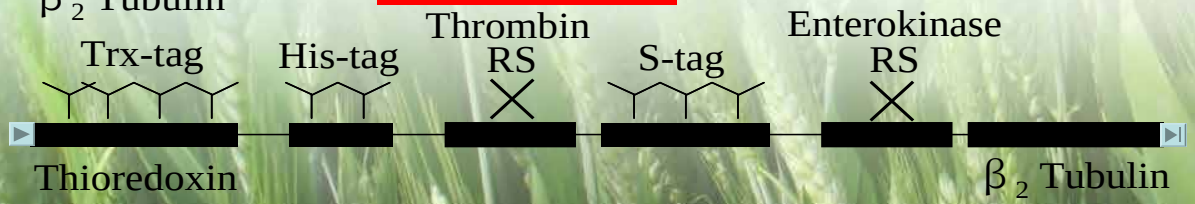
Tubulins Expressed in *E. coli*



Rossatta (DE3) pLysS

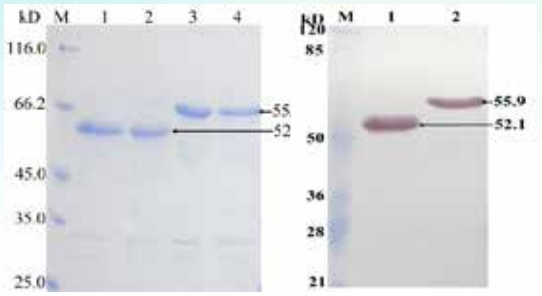


pET30a



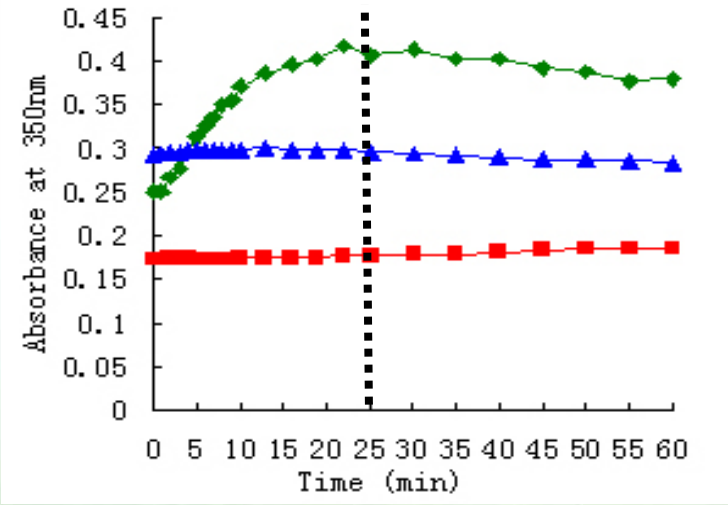
pET32a

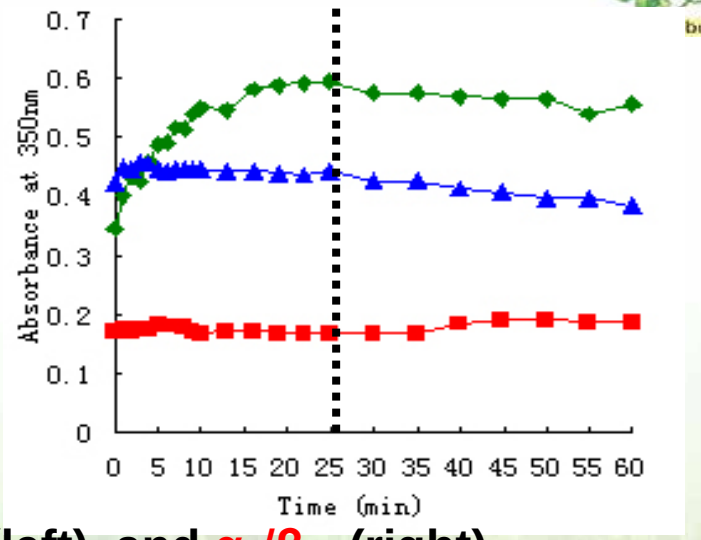
Carbendazim inhibited microtubule assembly of β_2-tubulin and α-tubulin *in vitro*



GTP GDP

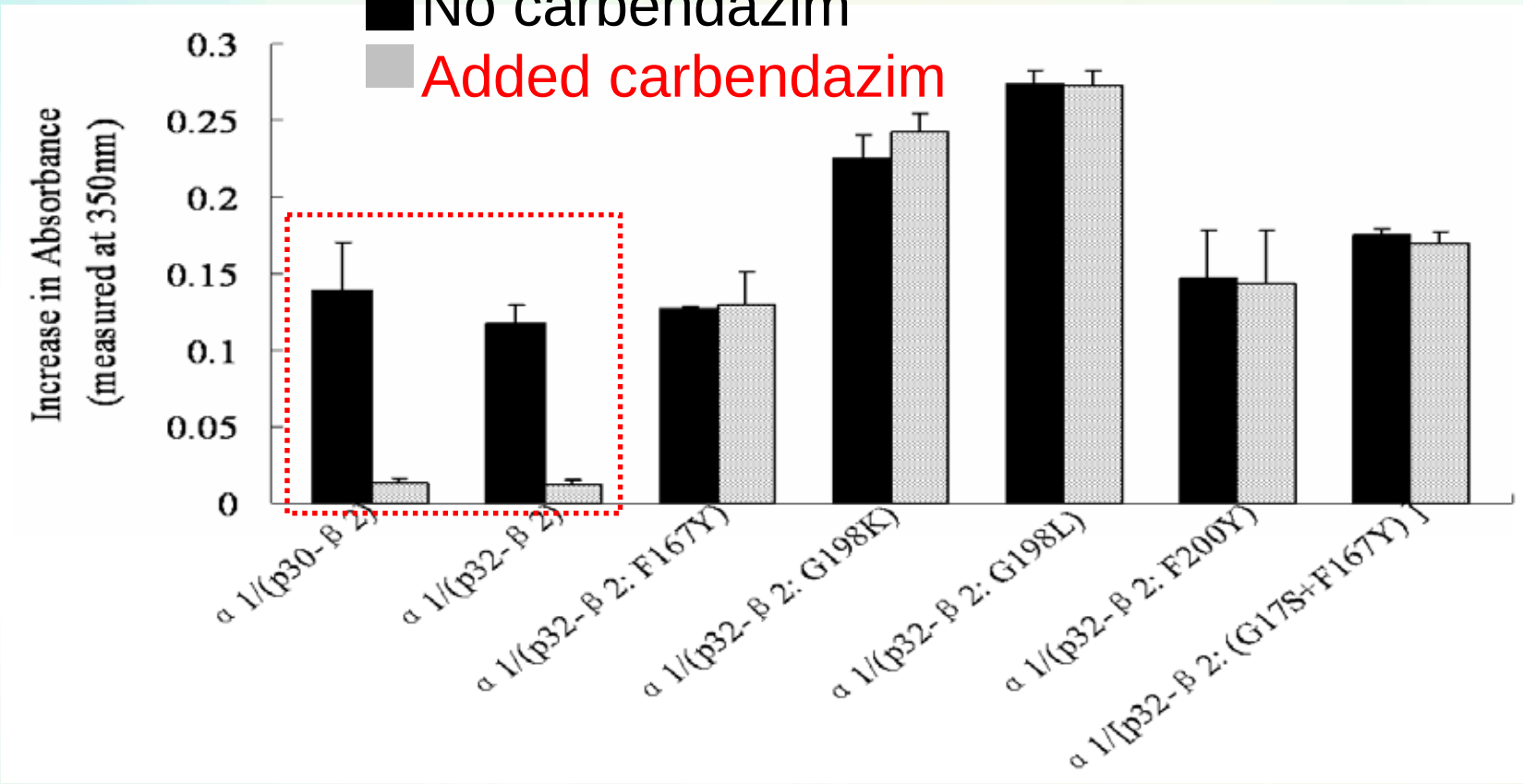
β_2-tubulin + α-tubulin





Assembly dynamic curves of α_1/β_2 (left) and α_2/β_2 (right)

u untreatment; • treated after assembly; < treated before assembly



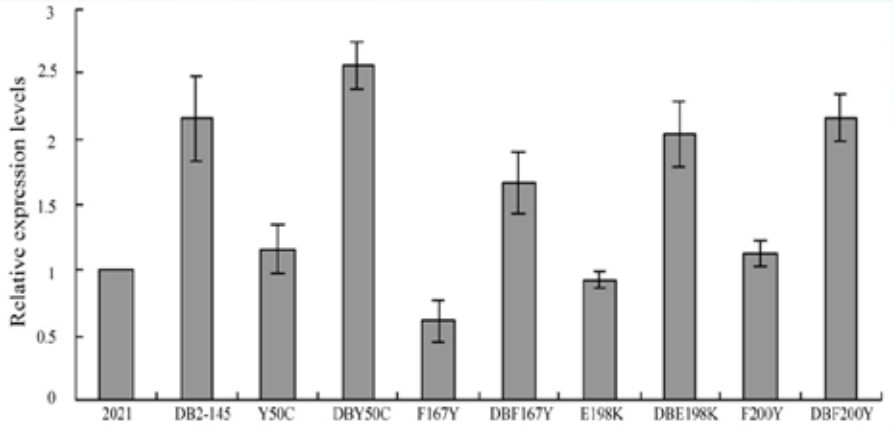
Assembly of b_2- and α_1-tubulin from wild-type was inhibited, mutant b_2-tubulines were not inhibited by carbendazim

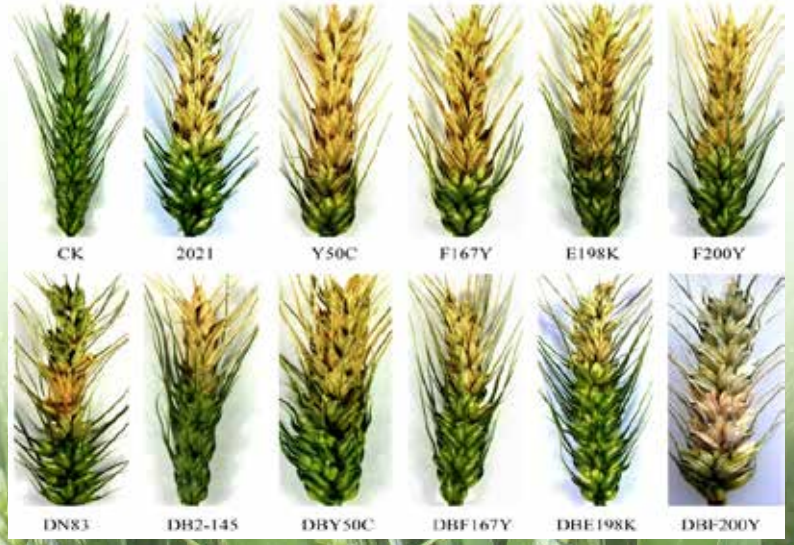
What is the relationship between b1- and b2-tubulin?

Interaction between b_1- and b_2-tubulin

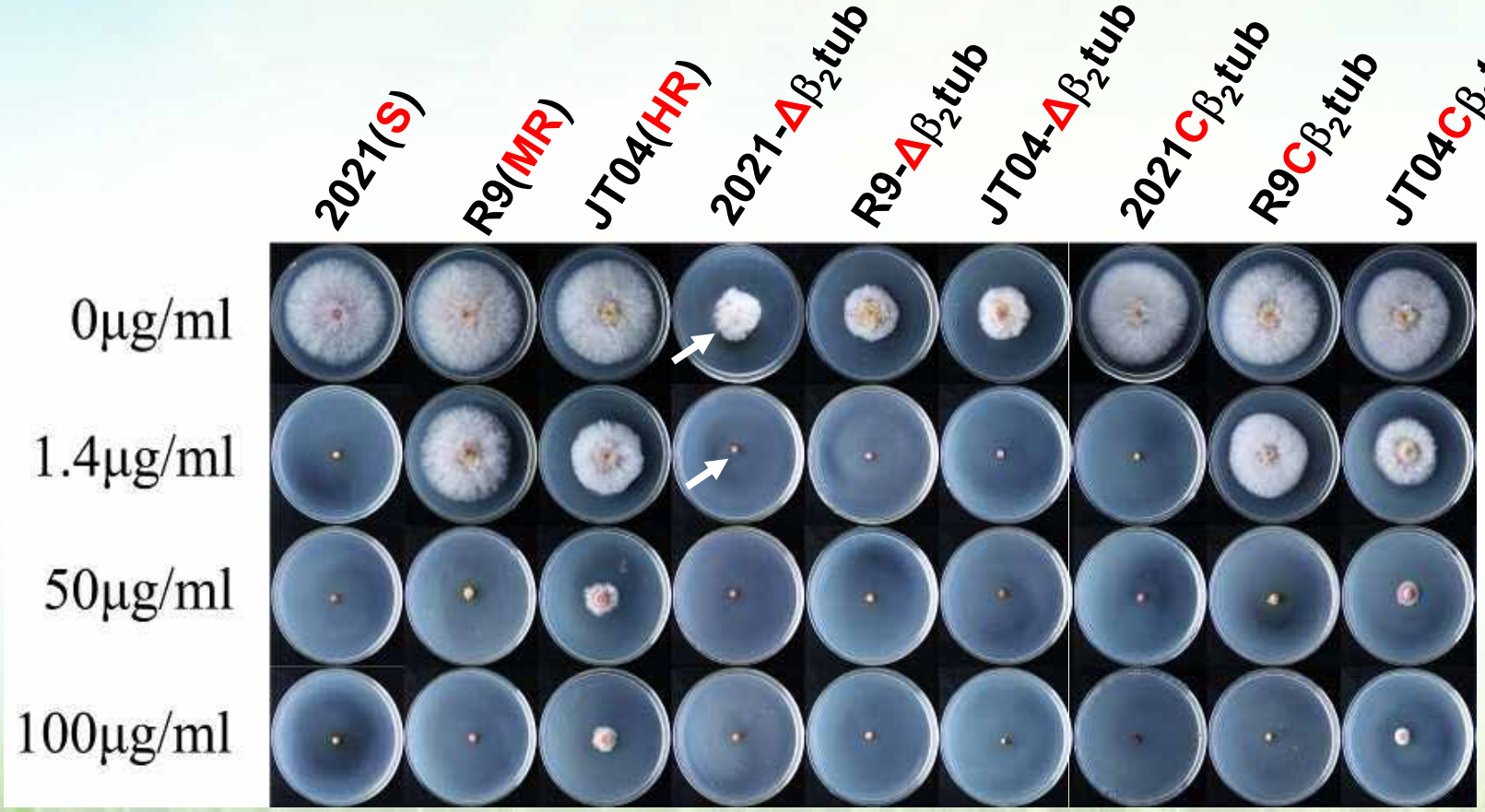
Effect of β_1-tub on MBC-Resistance

Isolate	EC_{50}(μg/mL)	MIC (μg/mL)
2021	0.60	1.4
DB2-145	0.68	1.4
Y50C	3.24	20
DBY50C	9.72	50
F167Y	10.85	50
DBF167Y	25.18	100
E198K	3.52	>100
DBE198K	10.16	>200
F200Y	10.92	50
DBF20Y	55.87	>100

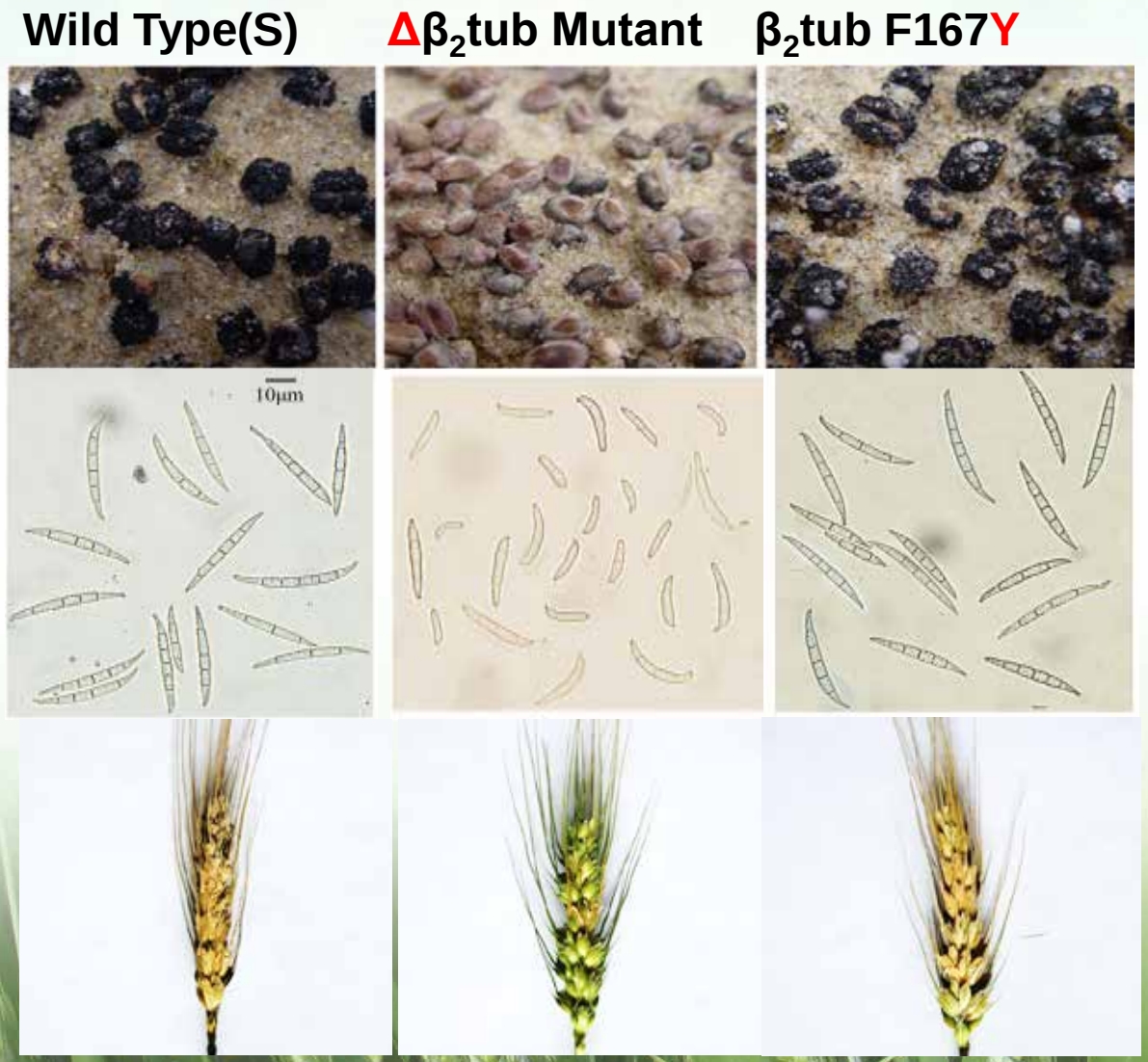




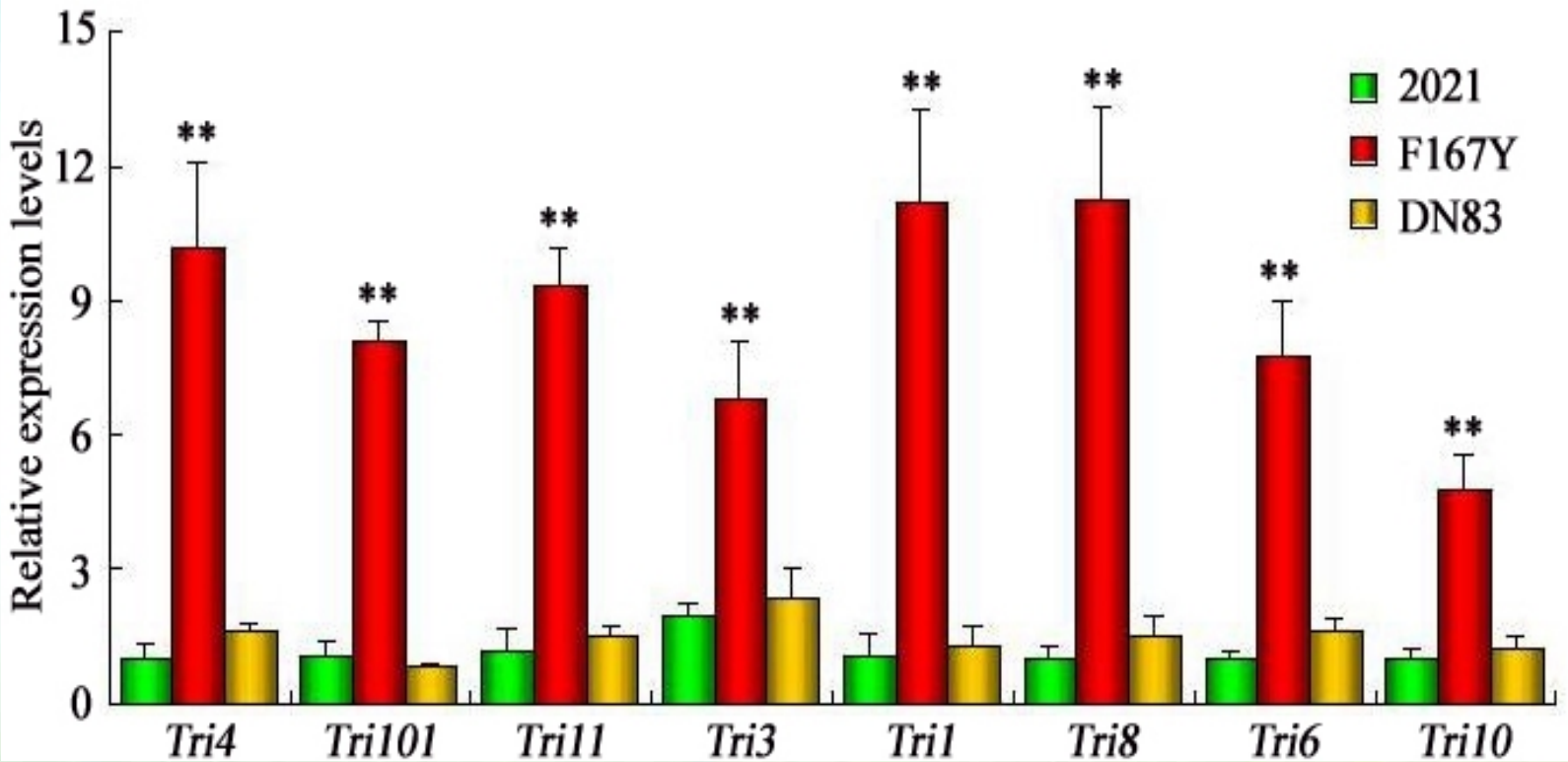
Effect of β_2-tub on MBC-Sensitivity



Difference in Bio-Property of Point Mutation and Deletion of β_2-*tub*



Difference in DON Production

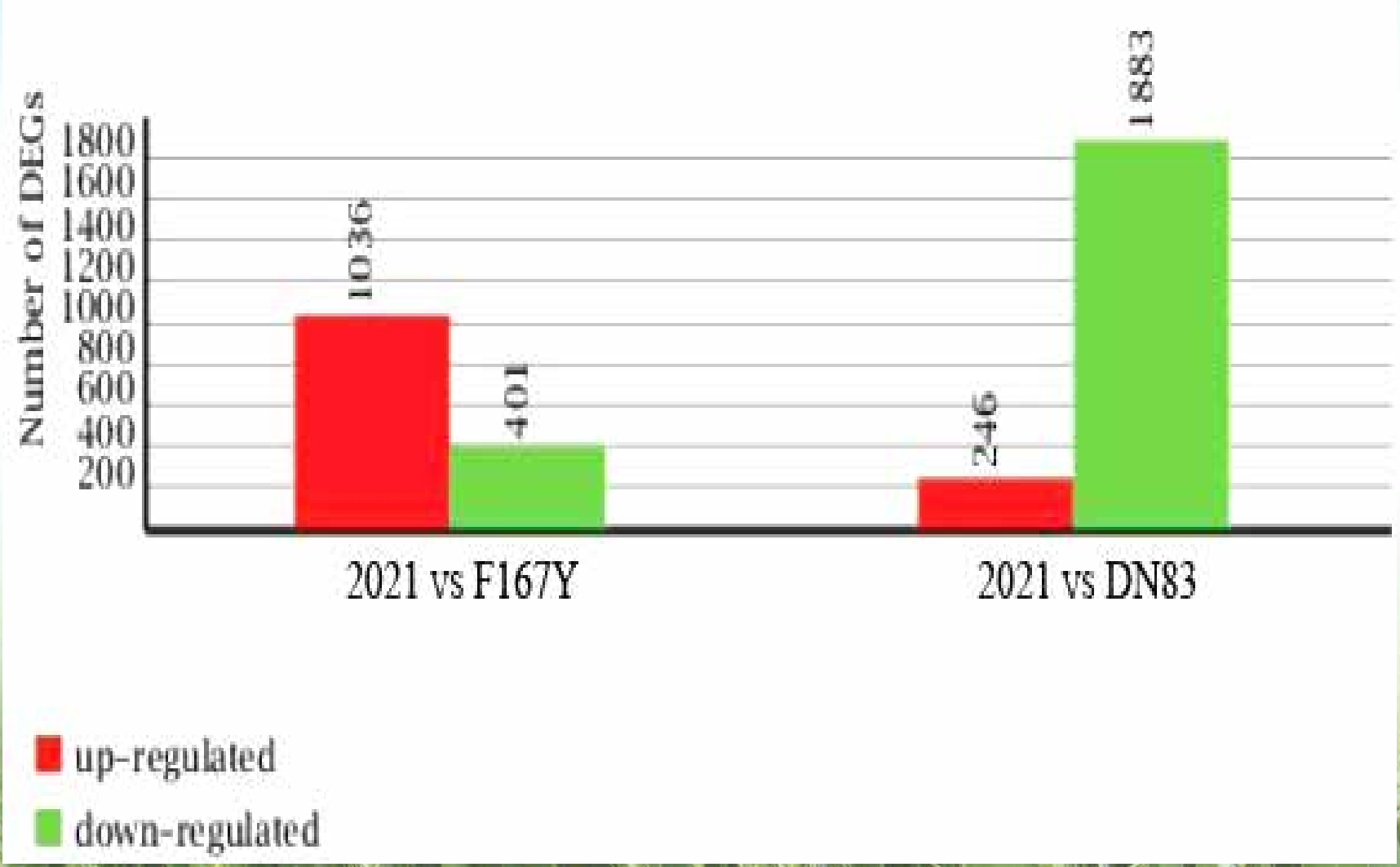


DON from resistance isolates： 2.4 times as sensitive in mycelia and 2.2 times in inoculated seed kernels

- **Why did the b2-tubulin gene deletion cause viability decrease?**

- **Why did the point mutation of the gene increase DON synthesis?**

Difference in Gene Expression



Difference in Pathway

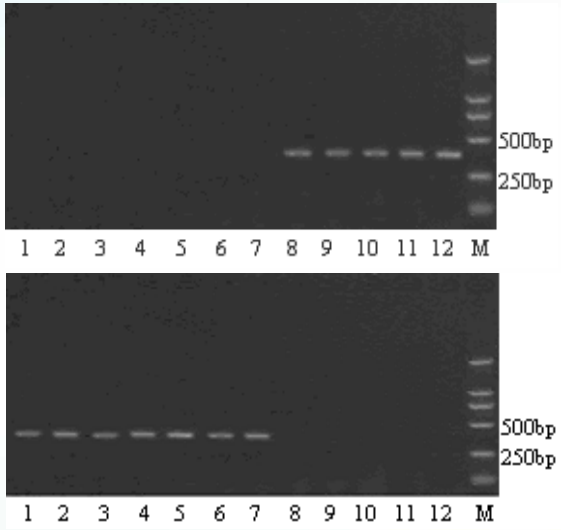
DN83 VS 2021

1	Valine, leucine and isoleucine degradation(22/5/77)
2	Ribosome biogenesis in eukaryotes(36/1/116)
3	N-Glycan biosynthesis(18/1/53)
4	beta-Alanine metabolism(16/5/63)
5	Protein processing in endoplasmic reticulum(32/8/140)
6	Cytosolic DNA-sensing pathway(8/0/18)
7	Alanine, aspartate and glutamate metabolism(17/3/64)
8	mTOR signaling pathway(6/1/16)
9	Phenylalanine, tyrosine and tryptophan biosynthesis(14/0/42)
10	Inositol phosphate metabolism(14/1/47)

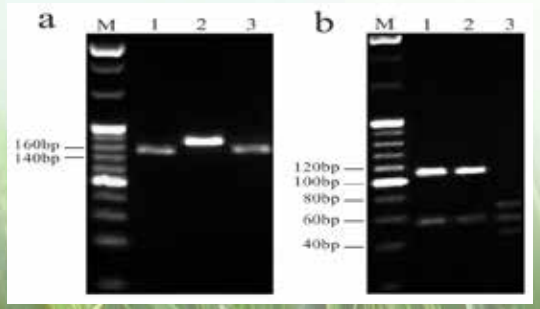
F167Y VS 2021

1	Biosynthesis of secondary metabolites(no map in kegg)
2	Fat digestion and absorption(1/3/7)
3	Pentose phosphate pathway(2/9/37)
4	Phenylalanine, tyrosine and tryptophan biosynthesis(5/7/42)
5	Oxidative phosphorylation(2/21/101)
6	Citrate cycle (TCA cycle) (3/9/45)
7	Protein processing in endoplasmic reticulum(6/23/140)
8	Aminoacyl-tRNA biosynthesis(7/7/56)
9	Vasopressin-regulated water reabsorption(2/5/21)
10	NOD-like receptor signaling pathway(0/3/5)
11	Glyoxylate and dicarboxylate metabolism(5/5/36)
12	Pyruvate metabolism(8/10/80)
13	Novobiocin biosynthesis(3/1/9)
14	Cytosolic DNA-sensing pathway(1/5/18)
15	Tropane, piperidine and pyridine alkaloid biosynthesis(4/10/59)
16	Phagosome(20/0/49)
17	Geraniol degradation(3/7/40)

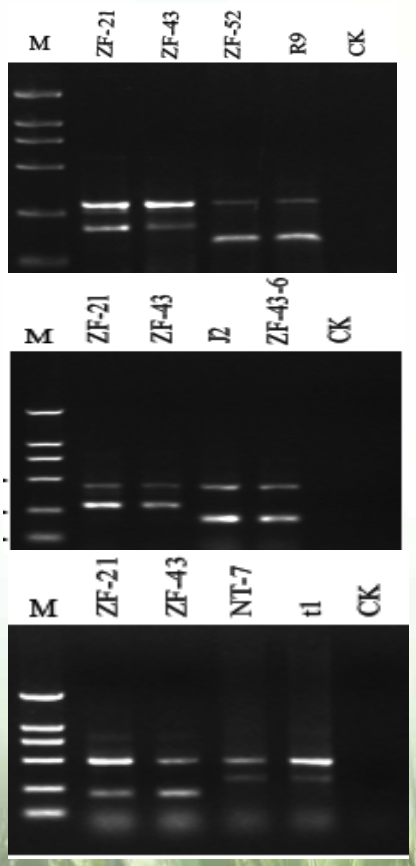
Molecular detect of resistant population



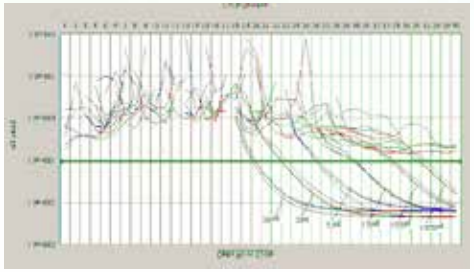
ASO-PCR

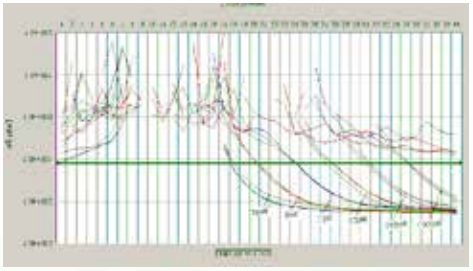


PIRA-PCR

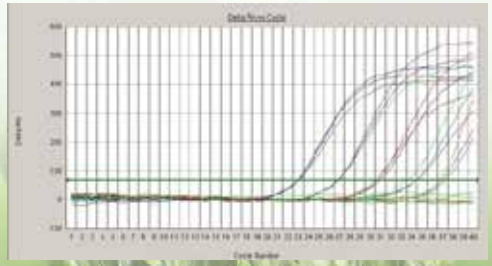


Tetra-primer ARMS PCR





Real-time PCR with mismatch primers



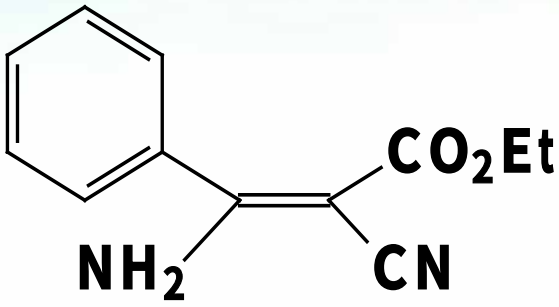
Real time PCR with Cycling Probe

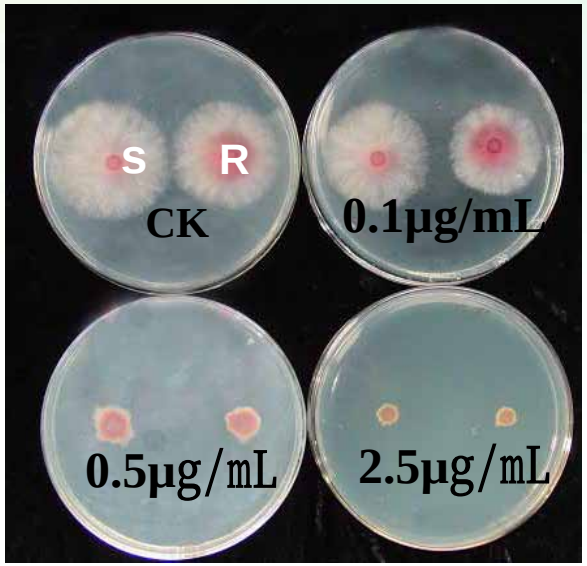
MBC-Resistance Management of *G. zeae*

- **Decreasing pressure of disease**
- **Decreasing pressure of fungicide**
- **Novel fungicide development**

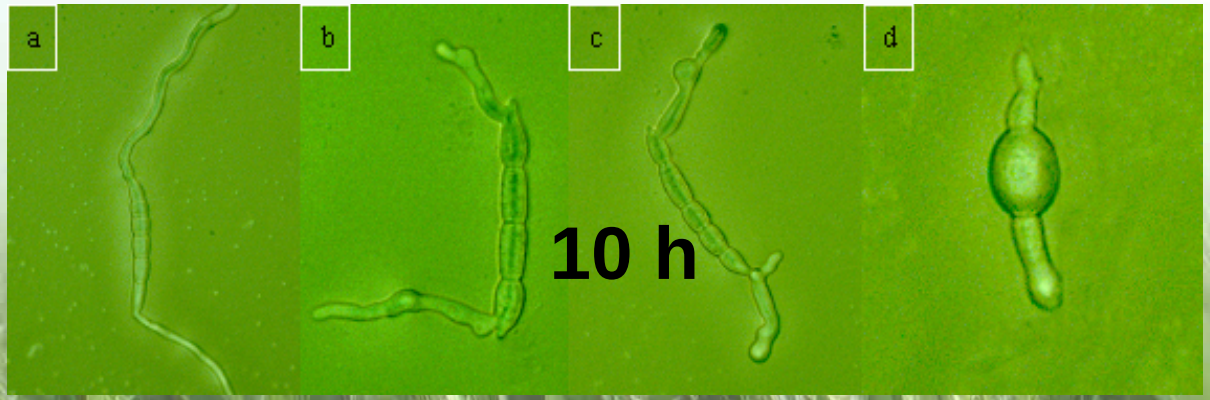
A Novel Fusarium Specific Fungicide JS399-19

JS399-19: Phenamacril

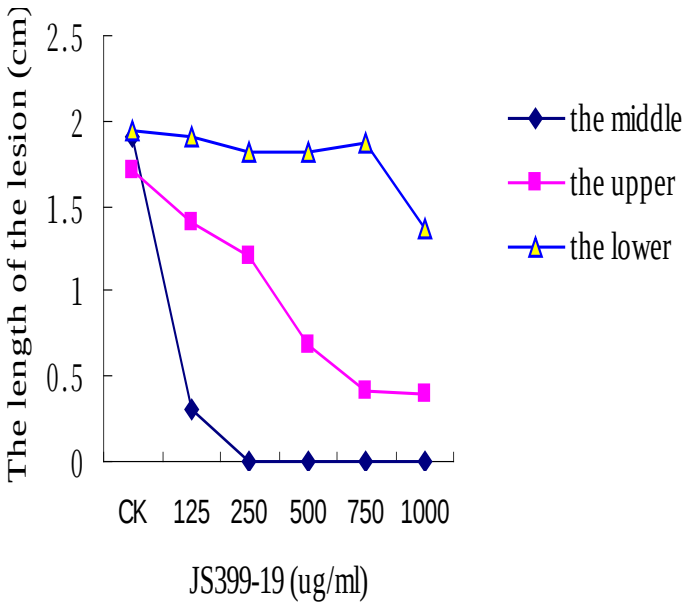




EC_{50}：0.15 μg/mL，activity is 3 times higher than that carbendazim



Systemic of JS399-19



It could be absorbed by leaves and moved upward, well-distributed

Efficacy of JS399-19 Controlling FHB on Baimahu Farm（Jun 2010）


Diseased
Panicle Rate
4.1%


Diseased
Panicle Rate
96.2%


JS399-19
100 毫升/亩
×2 次


CK
CK

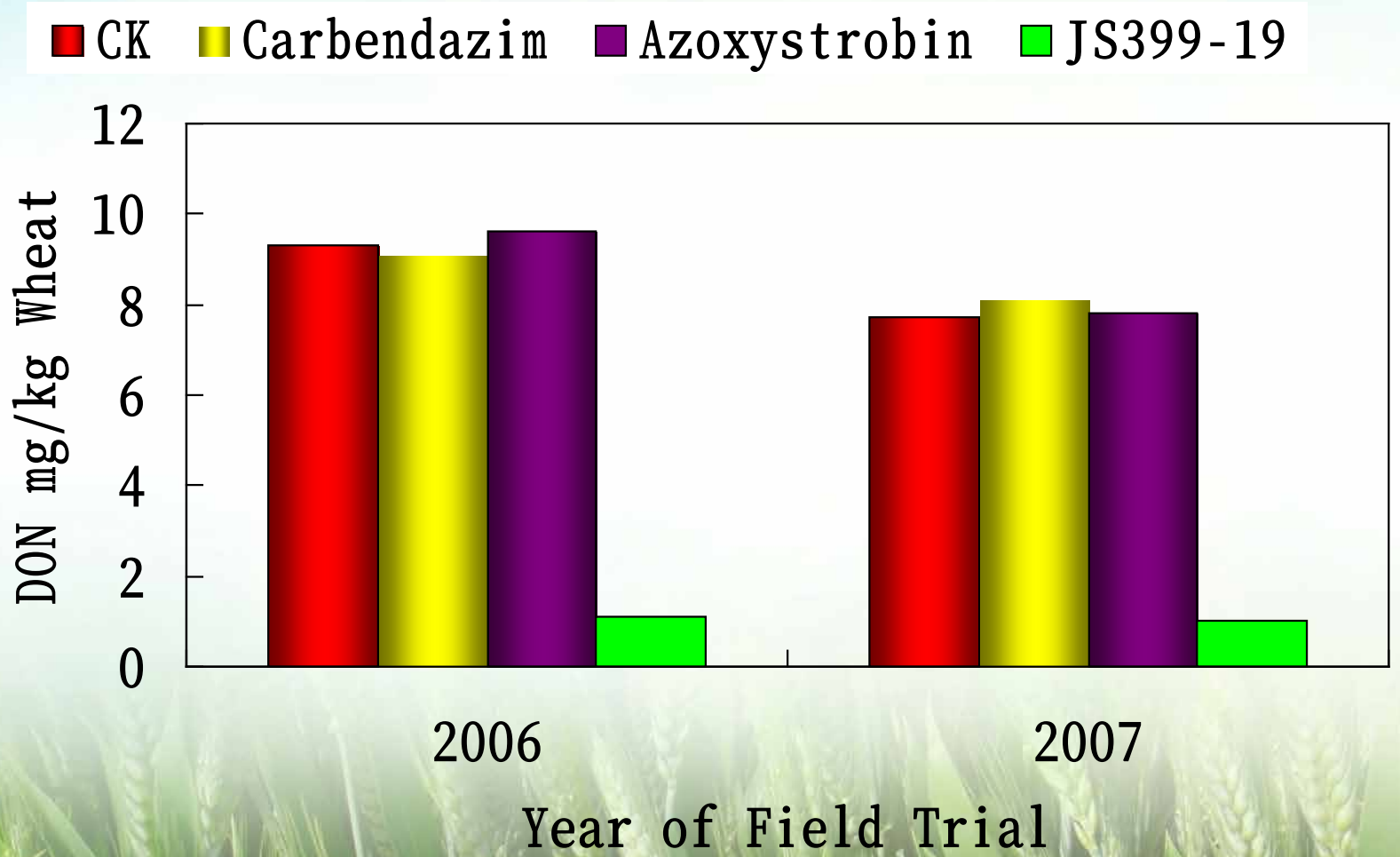
Efficacy of JS399-19 Controlling FHB in Field Trial

Treatment	Dosage (g a.i.ha^{-1})	2006	2007	2013
		Efficacy (%)	Efficacy (%)	Efficacy (%)
CK	—			
Carbendazim	375	64.8	44.6	40.8
Azoxystrobin	250	51.4	54.0	/
JS399-19	375	87.9	89.5	86.5

Effect of Fungicides on Quality and Yields

Treatment	Dosage (g a.i.ha^{-1})	2006		2007	
		DON (mg kg^{-1})	TKW (g)	DON (mg kg^{-1})	TKW (g)
CK	--	9.3 a	32.5 c	7.7 a	39.9 c
Carbendazim	375	9.1 a	38.9 bc	8.1 a	43.5 b
Azoxystrobin	250	9.6 a	39.8 b	7.8 a	44.7 ab
JS399-19	375	1.1 b	45.1 a	1.0 b	47.3 a

Effect of Fungicides on DON Production



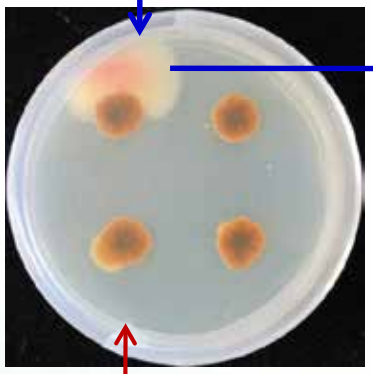
Monitoring of JS399-19 Resistance Population

Monitoring site: Jiangsu and Anhui Province

2012-2014, No resistant mutants were found.

Mechanism of JS399-19 resistance in *G. zeae*

YP-1(resistant mutant)



PH-1

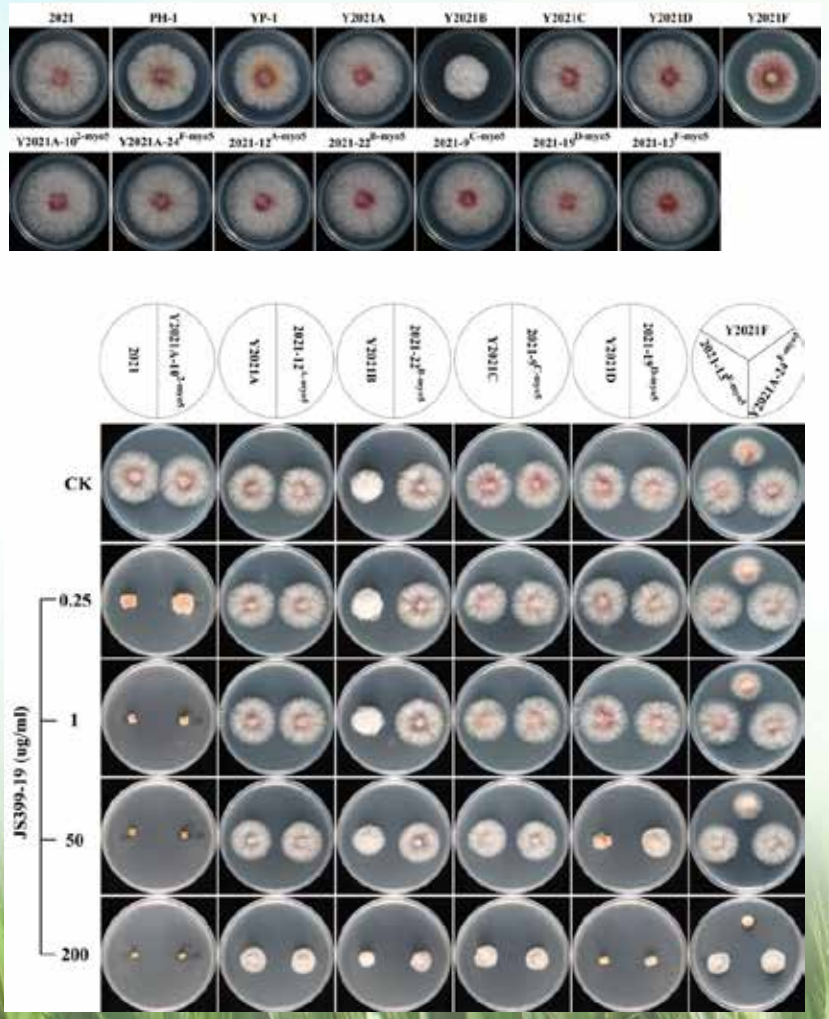
re-sequencing

Sample	YP-1
SNPs in CDS	364
Affected Genes	161
Synonymous SNPs	102
Nosynonymous SNPs	262
Genes with Amino acids change	132

Point mutation of Myosin5 (S217L, TCA→ TTA)

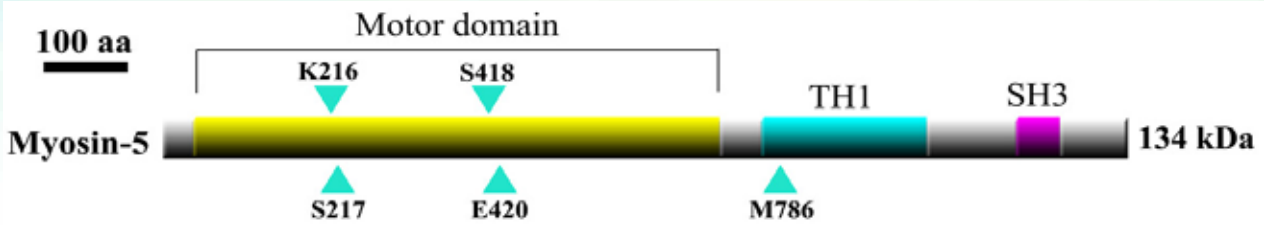
In animal, myosins comprise a family of ATP-dependent motor proteins and are best known for their role in muscle contraction. They are responsible for actin-based motility.

Point mutation of myosin5 confers resistance of *G. zeae* to JS399-19



Strain or mutant	Growth rate (cm/day)	EC_{50} (μg/ml)
2021	2.23±0.02D	0.21
PH-1	2.07±0.03E	0.26
Y2021A-10$^{2\text{-myo5}}$	2.32±0.04ABC	0.27
Y2021A-24$^{F\text{-myo5}}$	2.33±0.01AB	158
Y2021A	2.32±0.03AB	204
YP-1	2.06±0.03E	146
Y2021B	1.39±0.03G	202
Y2021C	2.30±0.05ABC	213
Y2021D	2.28±0.02BCD	42
Y2021F	1.69±0.03F	149
2021-12$^{A\text{-myo5}}$	2.35±0.02A	183
2021-22$^{B\text{-myo5}}$	2.35±0.02A	186
2021-9$^{C\text{-myo5}}$	2.28±0.03BCD	194
2021-19$^{D\text{-myo5}}$	2.26±0.03CD	65
2021-13$^{F\text{-myo5}}$	2.28±0.02BCD	151

Amino Acid Shift on *Myosin5* Conferred JS399-19 Resistance Phenotypes of *G. zeae*



Strain or mutant	Codon 216	Codon 217	Codon 418	Codon 420	Codon 786
PH-1	AAA (Lys)	TCA (Ser)	AGT (Ser)	GAA (Glu)	ATG (Met)
YP-1	AAA (Lys)	TTA (Leu)	AGT (Ser)	GAA (Glu)	ATG (Met)
2021	AAG (Lys)	TCA (Ser)	AGT (Ser)	GAA (Glu)	ATG (Met)
Y2021A	AAG (Lys)	CCA (Pro)	AGT (Ser)	GAA (Glu)	ATG (Met)
Y2021B	GAG (Glu)	TCA (Ser)	AGT (Ser)	GAA (Glu)	ATG (Met)
Y2021C	AAG (Lys)	TCA (Ser)	AGT (Ser)	GGA (Gly)	GTG (Val)
Y2021D	AAG (Lys)	TCA (Ser)	AGG (Arg)	GAA (Glu)	GTG (Val)
Y2021F	AAG (Lys)	TTA (Leu)	AGT (Ser)	GAA (Glu)	GTG (Val)
Y2021A-10$^{2\text{-myo5}}$	AAG (Lys)	TCA (Ser)	AGT (Ser)	GAA (Glu)	ATG (Met)
Y2021A-24$^{F\text{-myo5}}$	AAG (Lys)	TTA (Leu)	AGT (Ser)	GAA (Glu)	GTG (Val)
2021-12$^{A\text{-myo5}}$	AAG (Lys)	CCA (Pro)	AGT (Ser)	GAA (Glu)	ATG (Met)
2021-22$^{B\text{-myo5}}$	GAG (Glu)	TCA (Ser)	AGT (Ser)	GAA (Glu)	ATG (Met)
2021-9$^{C\text{-myo5}}$	AAG (Lys)	TCA (Ser)	AGT (Ser)	GGA (Gly)	GTG (Val)
2021-19$^{D\text{-myo5}}$	AAG (Lys)	TCA (Ser)	AGG (Arg)	GAA (Glu)	GTG (Val)
2021-13$^{F\text{-myo5}}$	AAG (Lys)	TTA (Leu)	AGT (Ser)	GAA (Glu)	GTG (Val)

Summary

- **Point mutation at *b_2-tub* confers carbendazim resistance, and *b_2-tub* is a target of the fungicide.**
- ***b_1-tub* negatively regulates MBC-resistance degree.**
- **Bio-property of *b_2-tub* point mutation and gene deletion is different in the MBC-sensitivity, mycelium growth, reproduction, virulence and DON biosynthesis.**
- **Point mutation at *myosin5* confers JS399-19(Phenamacril) resistance, and *myosin5* is a target of the fungicide in lab.**
- **JS399-19(Phenamacril) is an excellent fungicide for controlling wheat scab.**

Thanks

- Sponsors
- Work by students

Thanks for your attention!