Molecular mechanism of powdery mildew resistance in tobacco

Masao ARAI
Tomoyuki TAJIMA
Seiki SATO
Tomoyuki KOMATSU
Tomoya FUJIMURA

JAPAN TOBACCO INC.

Powdery mildew

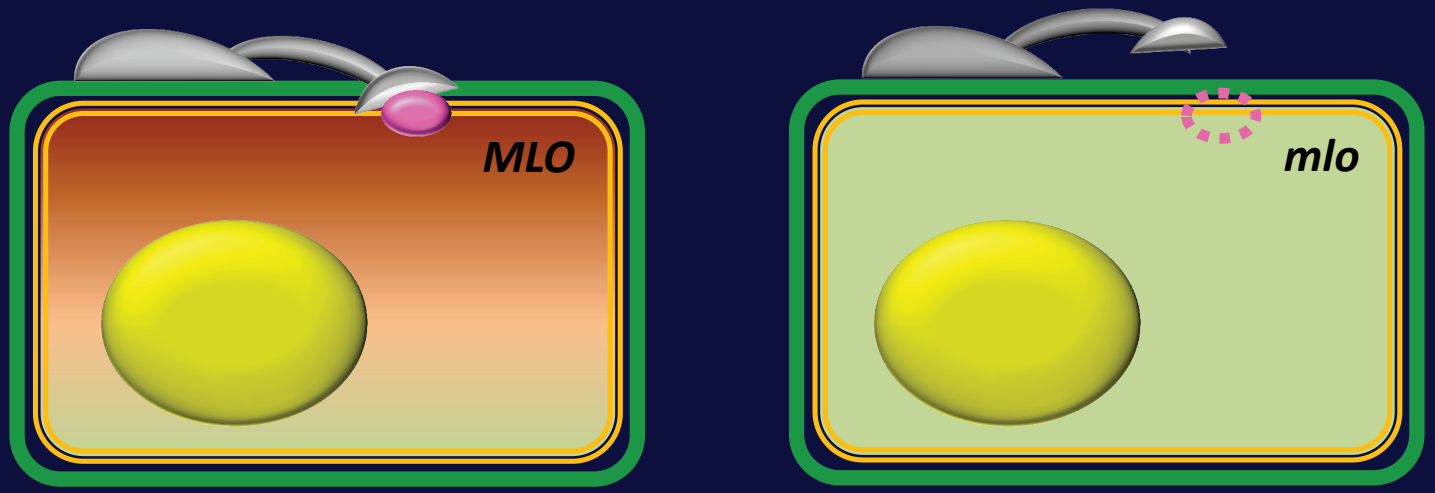
- ✓ Pathogen: *Golovinomyces cichoracearum* var. *cichoracearum* (syn. *Erysiphe cichoracearum*)
- ✓ One of the major diseases of tobacco
- ✓ Symptom occurs under damp, shady or nitrogen rich condition
- ✓ Severe infection leads to leaf chlorosis and premature senescence

Powdery mildew resistance in tobacco

- **Wild tobacco (*N. debneyi*, *N. glutinosa*, *N. tomentosiformis*)**
  - ✓ Single dominant locus
- **Japanese domestic cultivar Kokubu (syn. Kou-fan)**
  - ✓ Two recessive loci (*pm1*, *pm2*)
  - ✓ Introduced into some cultivars (Tsukuba 1 etc.)
  - ✓ Responsible genes have not been identified

Powdery mildew resistance in other plants

- *Mildew resistance locus O (MLO)*
  - ✓ Loss-of-function mutants confer broad-spectrum resistance to powdery mildew .
    (barley, tomato, pea etc.)
  - ✓ Gene encodes plant specific seven transmembrane domain protein.
  - ✓ Fungi can not enter *mlo* mutant host cells.



Objective

To elucidate the molecular mechanism of powdery mildew resistance in Kokubu by analyzing tobacco *MLO* orthologs

Hypothesis

Powdery mildew resistance of cultivar Kokubu is due to the loss-of-function mutations of tobacco *MLO* orthologs.

Approach

1. Isolation of tobacco *MLO* orthologous transcripts
2. Comparison of *MLO* transcript sequence between powdery mildew susceptible and resistant cultivars
3. Comparison of MLO genome sequence between powdery mildew susceptible and resistant cultivars
4. Complementation test (transgenic plants)
5. DNA marker development

- Susceptible cultivars : Bright Yellow 4, Burley 21, K326, Xanthi
- Resistant cultivars : Kokubu, Kutsaga E1, Tsukuba 1
  (containing resistance alleles from Kokubu)

Alignment of tobacco and tomato MLO protein

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NtMLO1   MAKERSMEATPTWAVAAVCFILLAISIFIEQIIHHLGEWLLKKHKKPLYEALEKIKAELMLLGFISLLLTVVQSPVSNLC  80
NtMLO2   MAKERSMEATPTWAVAVVCFILLAISIFIEQIIHHLGEWLLKKHKKPLYEALEKIKAELMLLGFISLLLTVVQTPVSNLC  80
S1M1o1   MAKERSMEATPTWAIAVVCFILLAISIFIEQIIHHIGEWLLEKRKKSLYEALEKIKAELMLLGFLSLLLTVLQDPVSNLC  80

NtMLO1   VPKSVGYSWHPCKSDEAAKNKYDDPCLPKGKVQFASSYAIHQLHIFIFVLAVAHVLYSIATFALGRLKMRKWRAWEEETK 160
NtMLO2   VPKSVGYSWHPCKADEDAKSKYDDPCLPKGKVQFASSYAIHQLHIFIFVLAVAHVLYSIATFALGRLKMRKWRAWEDETK 160
S1M1o1   VPKSVGYSWHPCMAKEDAKSEYDDPCLPKGKVQFASSYAIHQLHIFIFVLAVAHVLYCIATFALGRLKMRKWRAWEDETK 160

NtMLO1   TIEYQFYNDPERFRFARETSFGRRHLHYWSKSPVLLWIVCFFRQFFSSVAKVDYLTLRHGFMMAHLTPQNQENFDFQIYI 240
NtMLO2   TIEYQFYNDPERFRFARETSFGRRHLHYWSKSPVLLWIVCFFRQFFSSVAKVDYLTLRHGFMIAHLTPQNQENFDFQIYI 240
S1M1o1   TMEYQFYNDPERFRFARETSFGRRHLHFWSKSPVLLSIVCFFRQFFSSVAKVDYLTLRHGFMMAHLTPQNQNNFDFQLYI 240

NtMLO1   NRAVEKDFKFVVEISPALWLFTVLYFLTTTNGLYSYLWVPFIPLVIILLVGTKLEMIIAEMGVRISKRGDIVRGVPVVET 320
NtMLO2   NRAVEKDFKFVVEISPALWLFTVLYFLTTTNGLYSYLWVPFIPLVIILLVGTKLEMIIAEMGVRISKRGDIVRGVPVVET 320
S1M1o1   NRAVDKDFKVVVGISPALWLFTVLYFLTTTDRLYSYLWVPFIPLVIILLVGTKLQMIITEMGVRISERGDIVKGVPVVET 320

NtMLO1   GDHLFWFNRPGFVLFLINFVLFQNAFQVAFFVWSWWKFSYPSCFHQNAADIAIRLTMGVIIQVHCSYVTLPLYALVTQMG 400
NtMLO2   GDHLFWFNRPAFVLFLINFVLFQNAFQVAFFVWSWWKFGYPSCFHQNAADIAIRLTMGVIIQVHCSYVTLPLYALVTQMG 400
S1M1o1   GDHLFWFNRPALVLFLINFVLFQNAFQVAFFFWSWWKFGFPSCFHKNAADLAIRLTMGVIIQVHCSYVTLPLYALVTQMG 400

NtMLO1   TSMKPIIFGDNVATALRSWHNTAKKRVKHGRLSENTTPVSSRPATPLHGTSPVHLLRSYPQYSNE----ESRTSNAENEG 476
NtMLO2   TSMKPIIFGDNVATALRSWHNTAKKRVKHGRLSGNNTPVSSRPATPLHGTSPVHLLRSYPQYSNE----ESRTSNAENEG 476
S1M1o1   SSMKPIIFGDNVATALRSWHHTAKKRVKHG-LSGHTTPANSRPTTPLRGTSPVHLLRGYPQYNEDSVQASPRTSNVENEG 479

NtMLO1   WANEIPTSPRRQIENIKDDDHQEGEIHASSSV-------HQVEIAMSEFTFGNKMS 525
NtMLO2   WANEISTSPRRQIENTKDDDHQEGEIHASSSMQRPHPDQHQVEVTLSEFTFGNKTS 532
S1M1o1   WANEN----------------QEGEILQHASPDHN----KQIEITMSDFTFGNK   513
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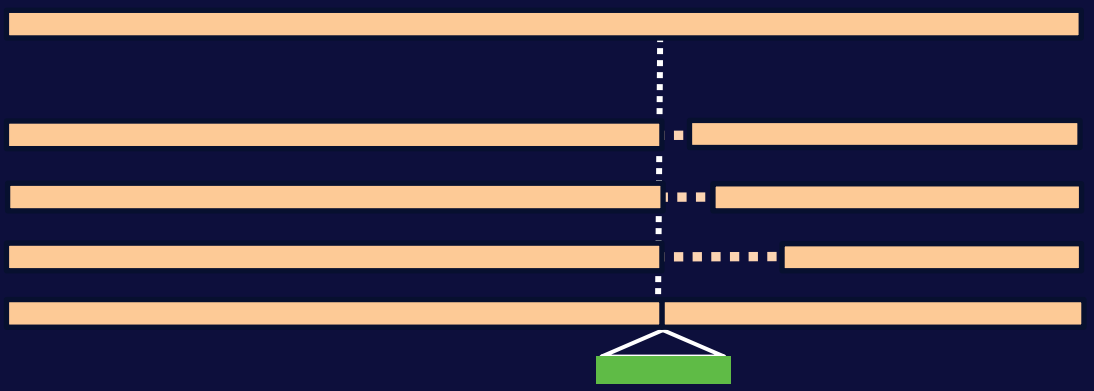
- **Two MLOs (NtMLO1 & NtMLO2) are expressed in tobacco leaves.**

Aberrant *MLO* transcripts in resistant cultivar

NtMLO1 transcripts

Susceptible cultivar :

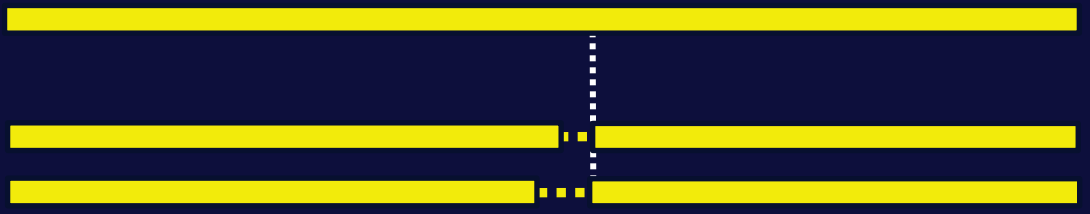
Resistant cultivar :
(1-95 nt deletion or
153 nt insertion)



NtMLO2 transcripts

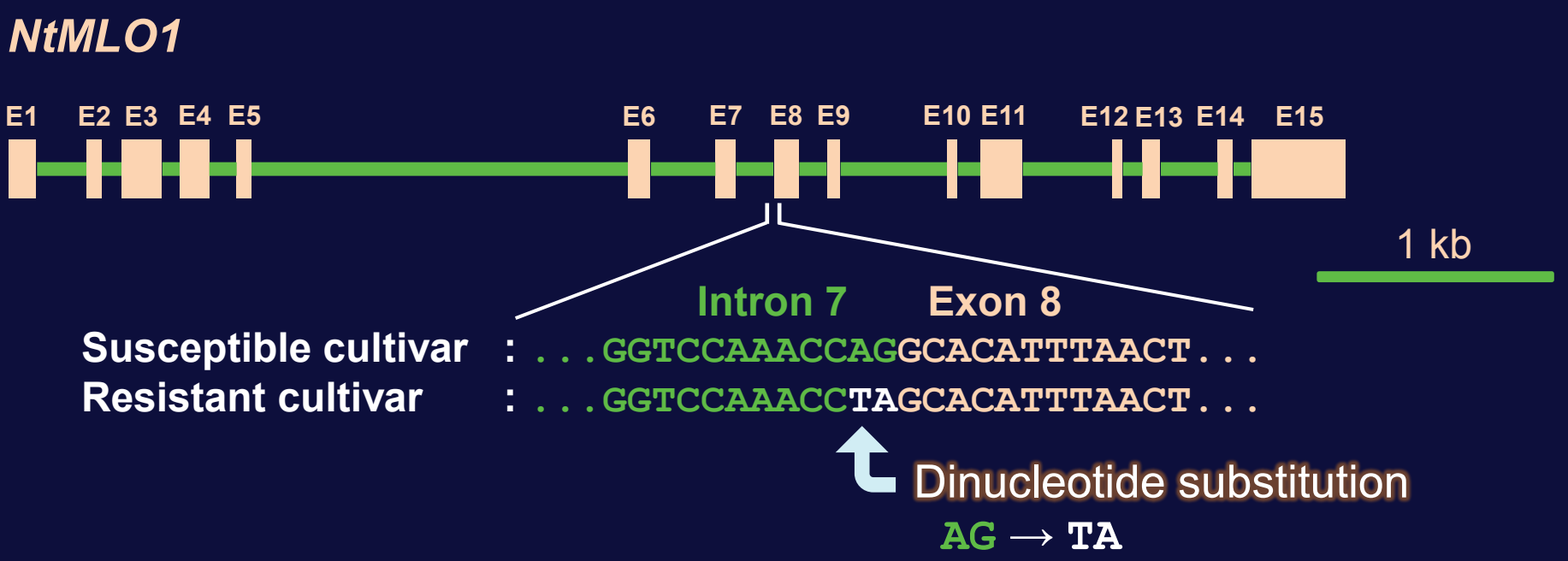
Susceptible cultivar :

Resistant cultivar :
(4 or 15 nt deletion)

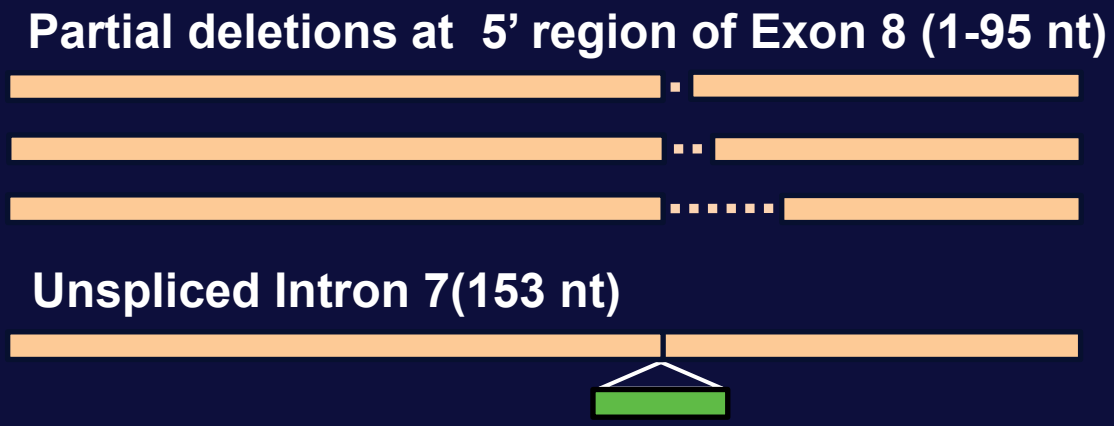


- Various aberrant *NtMLO* transcripts (deletion or insertion) were isolated from the powdery mildew resistant cultivar.

Splice site mutation in *NtMLO1* of resistant cultivars



NtMLO1 transcripts of resistant cultivar



Splice site mutation in *NtMLO2* of resistant cultivars

NtMLO2

NtMLO2 transcripts of resistant cultivar

Partial deletions at 3' region of Exon 6 (4 or 15 nt)

Transgenic Kokubu expressing functional MLO protein

Non-transformant

Transformants

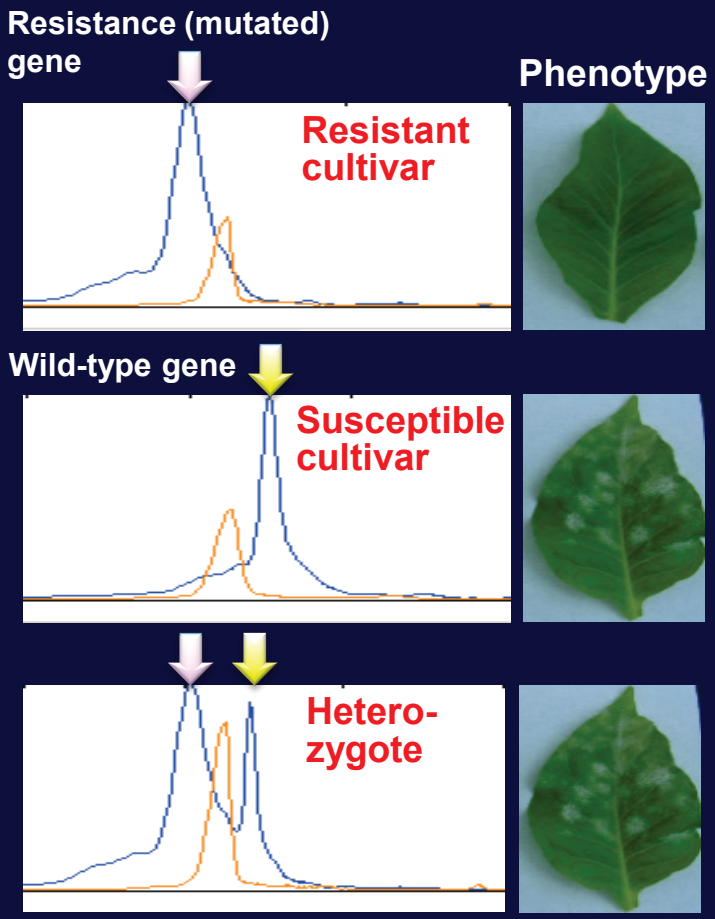
p35S::NtMLO1

p35S::NtMLO2

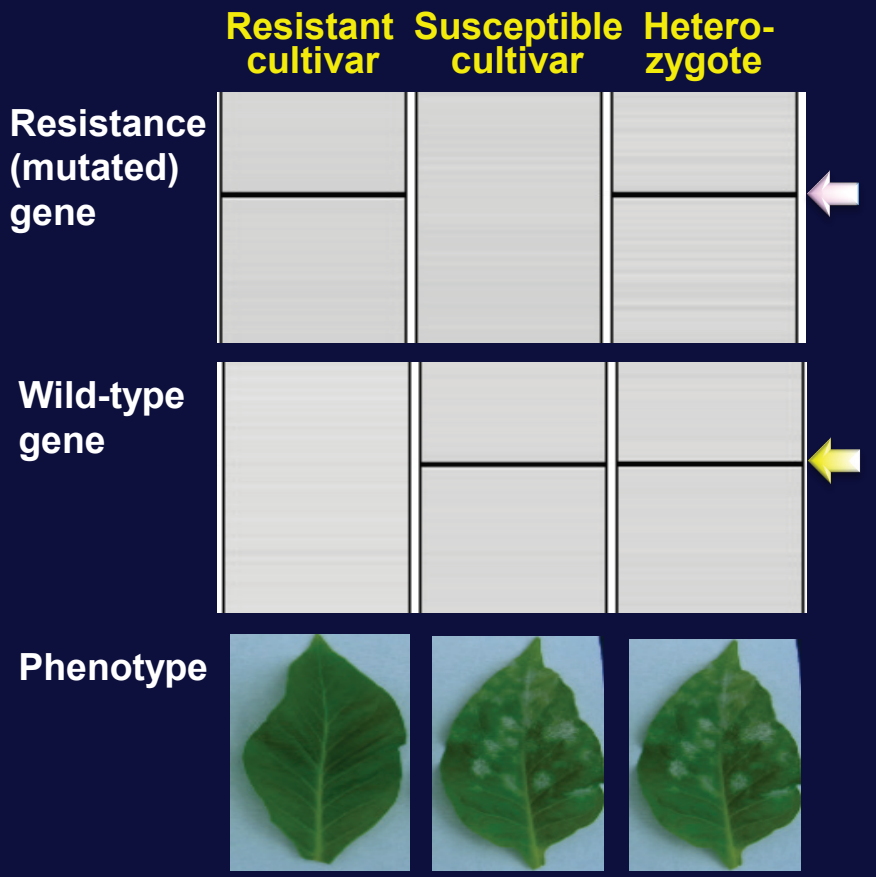
- Transformants expressing wild-type MLO protein exhibited severe disease symptom like susceptible cultivars.
- NtMLO1 and NtMLO2 are functionally redundant.

DNA marker development

SSCP
(Single Strand Conformation Polymorphism)



AS-PCR
(Allele Specific Polymerase Chain Reaction)



- These markers can detect heterozygotes.
- These methods obviate the test crosses for genotype determination and improve efficiency of breeding

Summary

- Tobacco expresses two *MLO* genes related to powdery mildew susceptibility.
- Powdery mildew resistance in Kokubu is caused by mutations in the intron region of two *NtMLO* genes.
- These mutations inhibit functional MLO protein synthesis.
- DNA markers for the breeding of powdery mildew resistance were developed.