MINUTES FROM FUSARIUM 'THINK TANK'

SYDNEY 5TH MAY 1999

PARTICIPANTS:

Greg Constable
Rob DeFeyter
David Jones
Helen McFadden
Stephen Allen
Joe Kochman
Jeff Ellis
Tony Pryor
Danny Llewellyn
Evan Cleland
Subbu Putcha
Bruce Lyon
Ralph Schulze
Jo Lane

MINUTES OF FUSARIUM THINK TANK MEETING – SYDNEY 5TH MAY 1999

Participants:

Greg Constable	Cotton CRC Narrabri
Rob DeFeyter	CSIRO Plant Industry Canberra
David Jones	ANU Canberra
Helen McFadden	CSIRO Plant Industry Canberra
Stephen Allen	ACRI Narrabri
Joe Kochman	DPIQ Toowoomba
Jeff Ellis	CSIRO Plant Industry Canberra
Tony Pryor	CSIRO Plant Industry Canberra
Danny Llewellyn	CSIRO Plant Industry Canberra
Evan Cleland	CRDC Director, Goondiwindi
Subbu Putcha	CAMBIA Canberra
Bruce Lyon	Sydney Uni
Ralph Schulze	Executive Director CRDC Narrabri
Jo Lane	CRDC Narrabri

The meeting was held at the Qantas club at Sydney airport, and was chaired by Greg Constable. The objective of the day was to:

> *Update Canberra biotechnology group on the ecology and severity of fusarium wilt of cotton with a view to deciding whether novel genes being researched may be used in cotton.*

PRESENTATIONS
Joe Kochman

FUSARIUM WILT OF COTTON

Living with fusarium wilt

Dr Joe Kochman
Principal Plant Pathologist
Farming Systems Institute, DPI

FUSARIUM WILT OF COTTON
Symptoms:
- wilting
- dark brown discolouration of vascular system
- leaf and stem death, often appearing to be from top down

FUSARIUM WILT OF COTTON
In the field, symptoms may appear as:
- seedlings dying in patches
- a few wilted or dead plants in a patch
- large areas with wilted and dead plants

- large dead areas along rows, usually starting from the tail drain

FUSARIUM WILT OF COTTON

Is caused by a fungus:

Fusarium oxysporum f.sp. *vasinfectum*

lives in soil

two strains in Australia which appear to have developed locally

FUSARIUM WILT OF COTTON

Factors affecting incidence

- Presence of the pathogenic strain
- cotton varieties
- climatic conditions
- soil types
- interaction with soil-borne organisms
- fertilisers
- alternative hosts - weeds, crops

FUSARIUM WILT OF COTTON

Disease development factors

temperature: cold soils appear to induce seedling infection, symptoms severe in adult plants in hot dry conditions

soil type: usually worse on heavy clays

FUSARIUM WILT OF COTTON

Disease development factors

varieties: a range of susceptibility in varieties

fertiliser: high N low K tends to increase wilt

weeds: some species are symptomless hosts

FUSARIUM WILT OF COTTON

How to slow down the spread of fusarium wilt

- minimise spread- best practice
- less susceptible cotton varieties

FUSARIUM WILT OF COTTON

Can we live with fusarium wilt?

Yes

- *but we need to continue to improve our management strategies, particularly the resistance in cotton varieties.*

FUSARIUM WILT OF COTTON

The research team

Dr Joe Kochman, Dr Natalie Moore,

Mr Neale Obst, Mr Wayne O'Neill

Collaborators
Dr Suzy Bentley, Dr Stephen Allen
Dr Greg Constable, Dr Peter Reid
Dr Michele Dale, Mr Bo Wang
Mr Greg Salmond

Presentation: Steve Allen

Limiting the spread of Fusarium

Currently monitoring 4 properties and the incidence of the disease has worsened in the past season on all 4 properties – in all cases the disease is worsening and spreading.

Fusarium is thought to be transported by:
- irrigation water
- flood water
- soil and trash attached to vehicles and machinery
- earthmoving equipment
- seed – not in all seed by it can occur – seed hulls are more a problem than kernels

How to stop the spread:
- ensure seed production in clean areas only
- cannot stop floodwater
- minimise dribbling on highways i.e the cotton on the sides of the road – this is usually confined within valleys (in some cases modules are transported inter valley and this can cause problems)
- fuzzy seed transported to crushing plant at Narrabri and this could potentially bring infected seed in from other valleys
- need a come clean / leave clean policy for all contractors on farms

Steve – don't think that reducing the spread will stop it – it is only buying time.

Presentation: Subbu Putcha

Biocontrol of Fusarium

- There is no major control measure for fusarium
- Have looked at using bacteria to reduce the disease intensity and reduce yield losses
- Work has been going for 3 years
- 90% of plants treated with biocontrol had some mild form of the disease

- the biocontrol agents reduce yield loss by fusarium
- the treatment is applied to the seed
- biocontrol agents performed consistently well in the field – there is an improvement in crop establishment
- Endophytic bacteria are more effective biocontrol agents – want the bacteria to go up the stem to induce systemic resistance as opposed to just forming resistance in the root system
- When the mechanics are well understood the traits can potentially be engineered into the plant
- Fusarium damages the plant over a range of temperatures
- The crop needs season long protection
- Pathogen isolates vary genetically – some biocontrol agents don't work on some pathogen strains

Presentation: Helen McFadden

(see notes attached)

Presentation: David Jones
(see notes attached)

Presentation: Bruce Lyon
(see notes attached)

After hearing the above presentations the group discussion focused on what future direction biocontrol / genetic research may take in helping solve the fusarium problem. There was a general consensus that the status quo will only delay the onset of the seriousness and further spread of the disease.

Tony Pryor identified three main areas that need further investigation and collaborative discussions (particularly within CSIRO Plant Industry):
1. Genes (I_2 and I_3)
2. Major genes – wild relatives
3. G.E.A.R

Tony – would like to confer more with the Canberra people, and come back to CRDC with an outline of the possible areas of work that may be undertaken.

Brief discussion about a fusarium working group meeting that is being held in Toowoomba on 24th May and being organised by Greg Salmond. Maybe an opportunity to broaden this meeting to take a more holistic view of the disease.

Actions arising from the meeting:

1. Greg Constable to contact Greg Salmond and look into the fusarium working group meeting agenda and possibility of inclusion

2. Canberra people (Llewellyn / McFadden / DeFeyter / Pryor / Ellis / Jones) to arrange to meet and speak collaboratively about possible areas of research and put together a brief proposal to CRDC

3. Jo Lane to complete minutes and distribute to participants.

NAME	FUSARIUM "THINK TANK" MEETING PARTICIPANTS ORGANISATION	ADDRESS		STATE	POSTCODE	PHONE	FAX	EMAIL
Greg Constable	Cotton CRC	Locked Bag 59	Narrabri	NSW	2390	02 - 6799 1500	02 - 6793 1105	gregc@mv.pi.csiro.au
Rob DeFeyter	CSIRO Plant Industry	GPO Box 1600	Canberra	ACT	2601	02 - 6246 5527	02 - 6246 5000	robd@pican.pi.csiro.au
David Jones	RSBN, ANU	PO Box 475	Canberra	ACT	2601	02 - 6249 4192	02 - 6249 4331	jones@rsbn.anu.edu.au
Helen McFadden	CSIRO Plant Industry	GPO Box 1600	Canberra	ACT	2601	02 - 6246 5377	02 - 6246 5000	h.mcfadden@pi.csiro.au
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Tony Pryor	CSIRO Plant Industry	GPO Box 1600	Canberra	ACT	2601	02 - 6246 5494	02 - 6246 5000	tony.pryor@pi.csiro.au
Danny Llewellyn	CSIRO Plant Industry	GPO Box 1600	Canberra	ACT	2601	02 - 6246 5470	02 - 6246 5000	D.Llewellyn@pi.csiro.au
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Jo Lane	CRDC	PO Box 282	Narrabri	NSW	2390	02 - 6792 4088	02 - 9792 4400	lane@mpx.com.au

NOTES FROM JOE KOCKMAN

FUSARIUM WILT OF COTTON

- Living with fusarium wilt

- Dr Joe Kochman
- Principal Plant Pathologist
- Farming Systems Institute, DPI

Cooperative Research Centre for Sustainable Cotton Production

C.R.D.C.

FARMING SYSTEMS INSTITUTE

Queensland Government

DPI Queensland Department of Primary Industries

FUSARIUM WILT OF COTTON

Symptoms:

- wilting
- dark brown discolouration of vascular system
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FUSARIUM WILT OF COTTON

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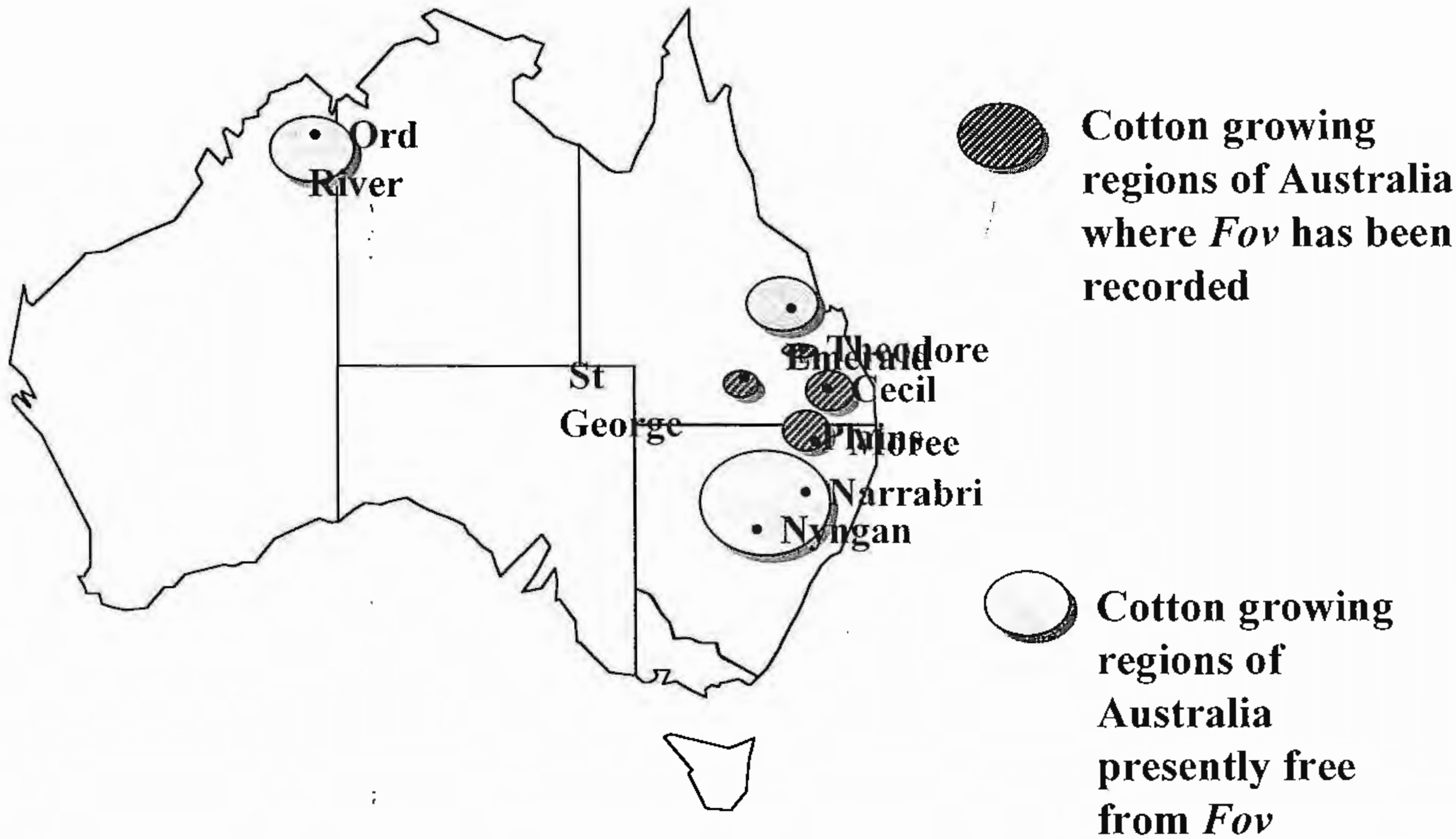
FUSARIUM WILT OF COTTON

Is caused by a fungus:

- *Fusarium oxysporum* f.sp. *vasinfectum*
- *lives in soil*
- *two strains in Australia which appear to have developed locally*



COOPERATIVE RESEARCH CENTRE FOR SUSTAINABLE COTTON PRODUCTION

C.R.D.C.

FARMING SYSTEMS INSTITUTE

QUEENSLAND GOVERNMENT DPI

FUSARIUM WILT OF COTTON

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- Presence of the pathogenic strain
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Disease development factors

- varieties: a range of susceptibility in varieties
- fertiliser: high N low K tends to increase wilt
- weeds: some species are symptomless hosts

FUSARIUM WILT OF COTTON

How to slow down the spread of fusarium wilt

- **minimise spread- best practice**
- **less susceptible cotton varieties**

FUSARIUM WILT OF COTTON

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- Yes

  -but we need to continue to improve our management strategies, particularly the resistance in cotton varieties.

FUSARIUM WILT OF COTTON

- The research team
- Dr Joe Kochman, Dr Natalie Moore,
- Mr Neale Obst, Mr Wayne O’ Neill
- Collaborators
- Dr Suzy Bentley, Dr Stephen Allen
- Dr Greg Constable, Dr Peter Reid
- Dr Michele Dale, Mr Bo Wang
- Mr Greg Salmond

COOPERATIVE RESEARCH CENTRE FOR SUSTAINABLE COTTON PRODUCTION

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FARMING SYSTEMS INSTITUTE

QUEENSLAND GOVERNMENT DPI QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES

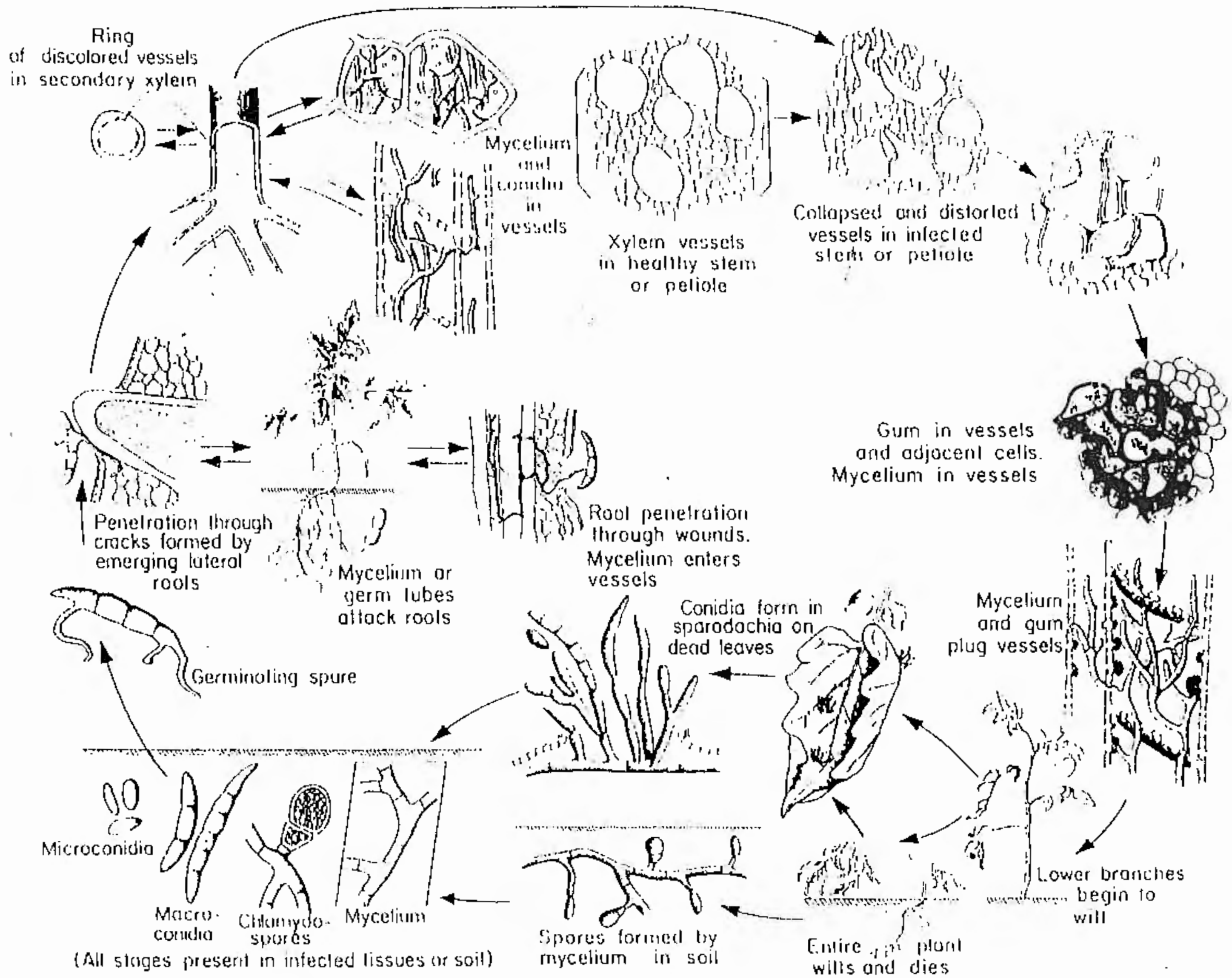


Figure 3. Stylised lifecycle of *Fusarium oxysporum* (Agrios, 1988)

NOTES FROM HELEN McFADDEN

FUSARIUM WILT MANAGEMENT OPTIONS

Germplasm selection and breeding

Chemical control

Biologicals

Anti-fungal proteins

Alternative hosts/rotations

Conclusions so far:-

Where the *avr* gene is expressed in transgenic cotton:

- Plant survival is in accordance with expected *avr/R* specificities
- In the absence of a particular *R* gene, one *avr*-expressing line shows apparent interaction with an undefined *R* gene homologue.

In this line we observe

- a stunted phenotype
- elevated chitinase enzyme activity
- increased resistance to virulent *Xanthomonas* measured as degree of watersoaking after inoculation
- no increased resistance to *Verticillium* measured as leaf symptom score

We have established that avr/R gene interactions driven by bacterial *avr* genes can occur in cotton.

More work is needed to characterise the resulting resistance phenotypes against the pathogens of interest, particularly Fusarium.

Future goals

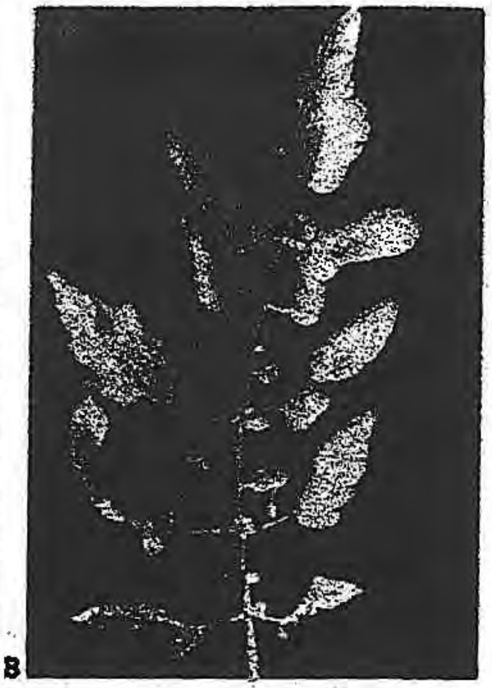
- further characterisation of line 1 in terms of resistance and gene expression

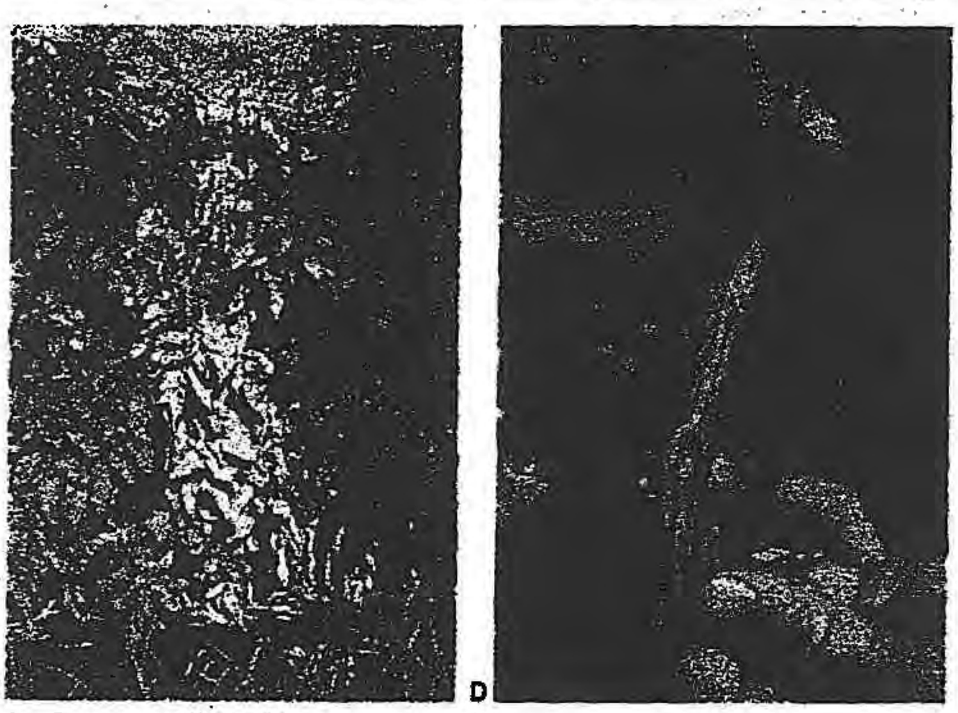
Different promoters

- lower level constitutive expression of *avr* genes in the presence of corresponding R genes for possible plant survival
- expression of *avr* genes in plant sectors, driven by the transposon-tagging expression system of GEAR
- pathogen-inducible of *avr* genes

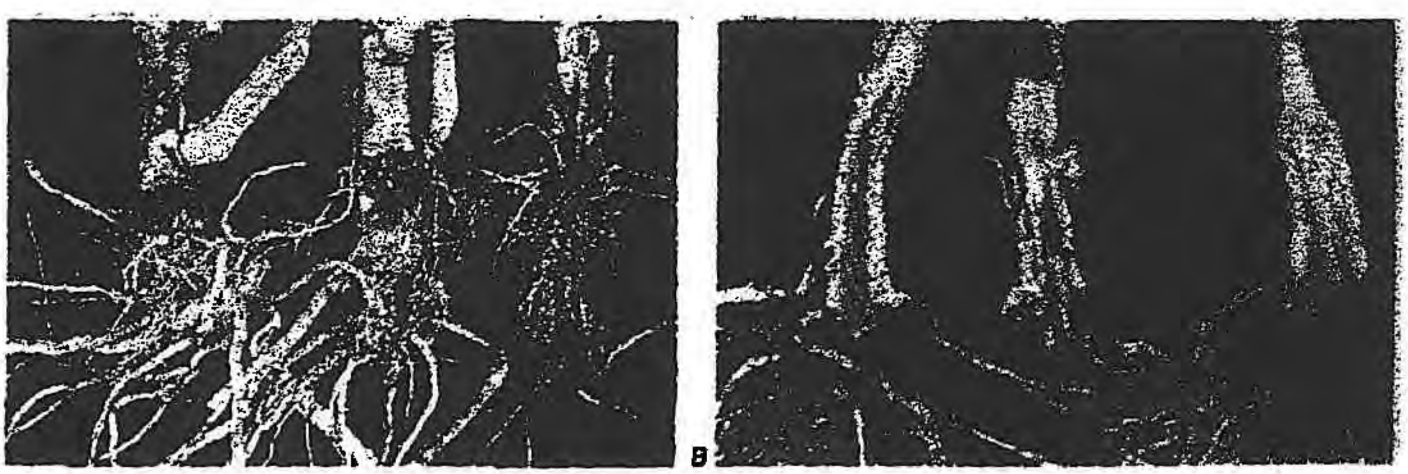
NOTES FROM DAVID JONES

Fusarium oxysporum in tomato

A  B 

C D 

17. Fusarium wilt. A, Field with high incidence of Fusarium wilt. B, Detached leaf showing unilateral yellowing. C, Mature plant showing golden yellowing. D, Longitudinal section of stem with browning of the vascular system.

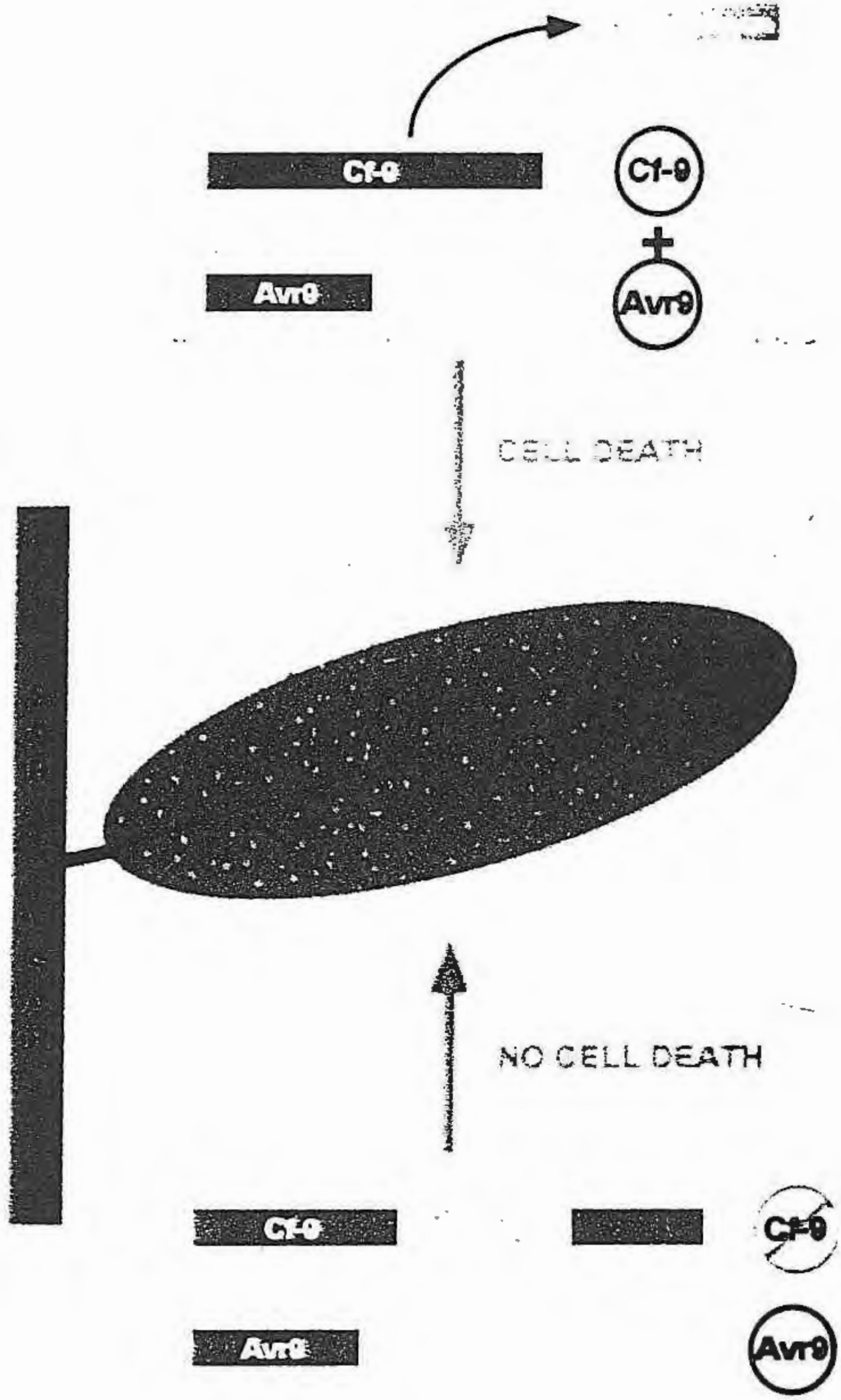
A B 

18. Fusarium crown and root rot. A, External symptoms. B, Internal discoloration. (Courtesy J. P. Jones)

TABLE 1. COMPARISON OF TOMATO FRUIT YIELD AND GROWTH OF GEAR AND NON-GEAR LINES

Genotype	Total fruit yield/ plant (g)	Total No fruits/plant	Mean fruit weight (g)	Stem height (cm) at	
				Truss 1	Truss 5
Cf0	1862 # 112a	25.8 # 1.2a	72.1 # 3.9a	41.2 # 1.9ab	136.0 # 4.7a
non GEAR M18	1888 # 91b	26.8 # 1.3b	70.4 # 2.5a	43.7 # 3.2a	136.2 # 4.2a
GEAR M18	724 # 273a	10.7 # 4.0a	68.8 # 4.9a	33.7 # 4.2c	121.8 # 4.1b
GEAR M31	1856 # 63a	26.5 # 1.3a	70.1 # 3.3a	36.8 # 1.6bc	111.3 # 7.2c
GEAR M50	1872 # 120a	26.5 # 1.9a	70.7 # 2.2a	39.0 # 2.6b	113.3 # 8.6bc

= standard deviation. Different letters within a column indicate statistically significant differences ($P<0.05$). Student-Newman-Keuls test for variability.


Cf-9
Cf-9
Avr9
Avr9
CELL DEATH
NO CELL DEATH
Cf-9
Cf-9
Avr9
Avr9

Strategies for using tomato R genes to provide Fusarium resistance in transgenic cotton

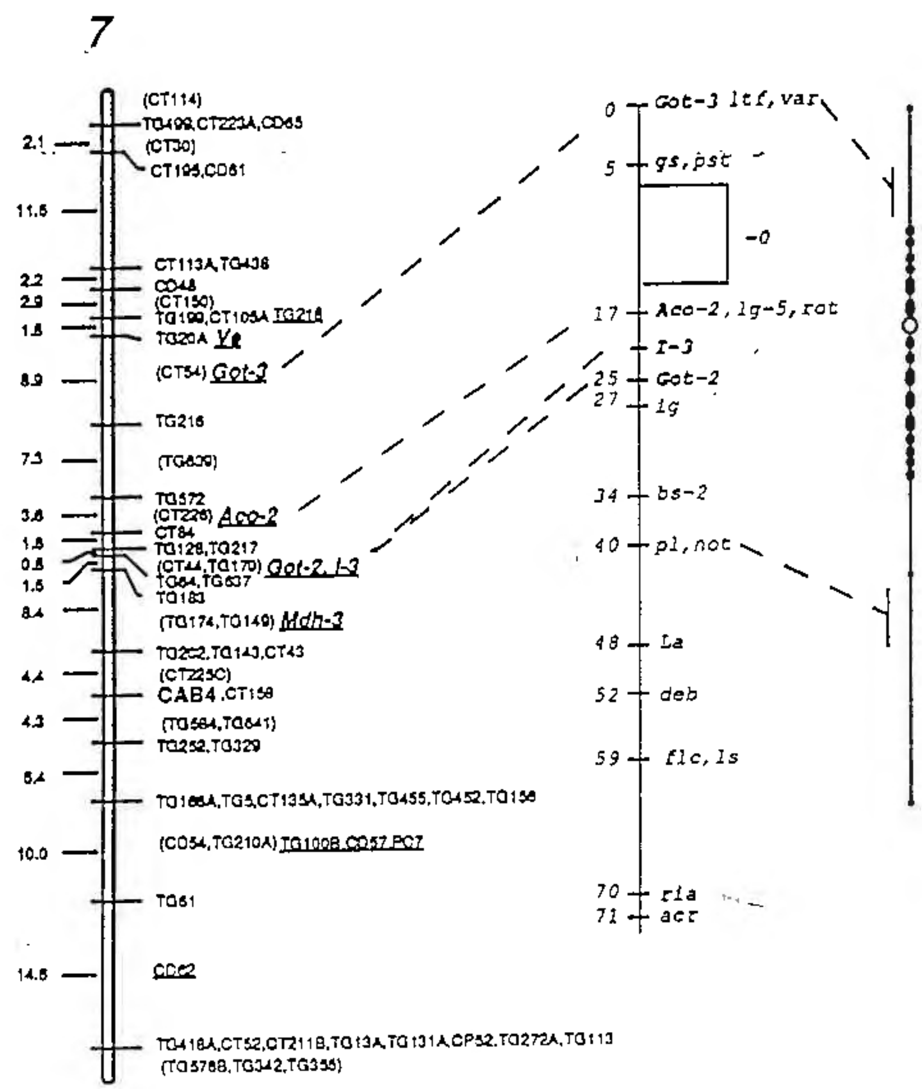
Transform cotton with *Cf-9* and *Avr9* with *Avr9* under the control of a transposable element. Somatic excision restores function and triggers HR leading to SAR (GEAR)

Different IP and licencing considerations

Strategies for using tomato R genes to provide Fusarium resistance in transgenic cotton

Transform cotton with *Cf-9* and *Avr9* with one or both under the control of a pathogen-inducible promoter to trigger an incompatible interaction at the site of pathogen challenge.

Different IP and licencing considerations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(CT114)
TG499,CT223A,CD65
(CT30)
CT195,CD61
CT113A,TG438
CD48
(CT150)
TG199,CT105A TG216
TG20A Ve
(CT54) Got-3
TG216
(TG639)
TG572
(CT226) Aco-2
CT84
TG128,TG217
(CT44,TG170) Got-2, I-3
TG64,TG637
TG183
(TG174,TG149) Mdh-3
TG262,TG143,CT43
(CT225C)
CAB4,CT159
(TG584,TG641)
TG252,TG329
TG166A,TG5,CT135A,TG331,TG455,TG452,TG156
(CD54,TG210A) TG100B,CD57,PG7
TG61
CDc2
TG418A,CT52,CT211B,TG13A,TG131A,CP52,TG272A,TG113
(TG576B,TG342,TG355)
2.1
11.5
2.2
2.9
1.6
8.9
7.3
3.6
1.6
0.8
1.5
8.4
4.4
4.3
6.4
10.0
14.6
0 Got-3 ltf, var
5 gs, pst
-0
17 Aco-2, lg-5, rot
I-3
25 Got-2
27 lg
34 bs-2
40 pl, not
48 La
52 deb
59 flc, ls
70 ria
71 acr

Strategies for using tomato R genes to provide Fusarium resistance in transgenic cotton

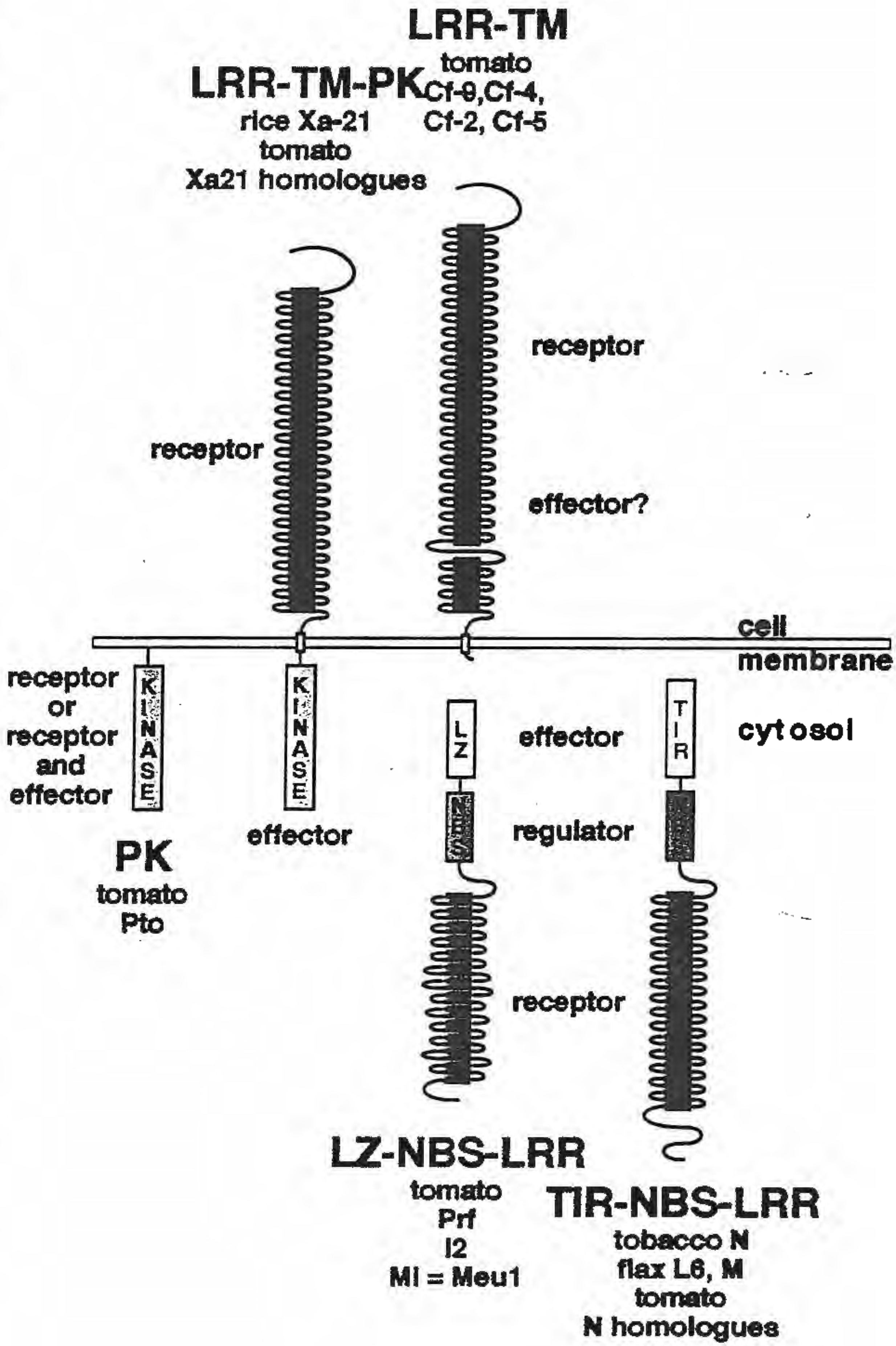
Isolate and transform cotton with *I-3*

- *I-3* can be used in the same way as proposed for *I*-2
    - different specificity
    - discovered in Australia and IP would be Australian owned

Strategies for using tomato R genes to provide Fusarium resistance in transgenic cotton

Transform cotton with *I-2* or other members of the *I-2* gene family

- *I-2* may confer resistance to *F.o.v.* if *F.o.v* has the corresponding *Avr* gene
- Overexpression of *I-2* may confer resistance in the absence of a corresponding *Avr* gene
    - constitutive overexpression
    - pathogen-inducible overexpression
- IP and licencing considerations



LRR-TM
tomato
Cf-9,Cf-4,
Cf-2, Cf-5
LRR-TM-PK
rice Xa-21
tomato
Xa21 homologues
receptor
receptor
effector?
cell
membrane
receptor
or
receptor
and
effector
KINASE
KINASE
effector
LZ
effector
TIR
cytosol
regulator
PK
tomato
Pto
receptor
LZ-NBS-LRR
tomato
Prf
I2
MI = Meu1
TIR-NBS-LRR
tobacco N
flax L6, M
tomato
N homologues

Predicted I-2 protein
1266 amino acids

Leucine Zipper/Coiled Coil Domain

```
MEIG AVGGAF SSA NV FDR APNGDL NM RKHKDHV
KL KK KMT RG QIV SD ENKQASNPSVRDWLNELRDA
VDSAENLIEEVNYEALRLKVEGQHQNFSETSNQQVSDDFF
LNIKDKLEDTIETLKDLQEQIGLLGLKEYFDSTKLETRRP
STSVDDES
```

Nucleotide Binding Site Domain

```
DIFGRQSEIEDLIDRLLSEGASGKKLTVVPIVGMGGQGKT
TLAKAVYNDERVKNHFDLKAWYCVSEGFDALRITKELLQE
IGKFDSKDVHNNLNQLQVKLKESLKGKKFLIVLDDVWNEN
YNEWNDLRNIFAQGDIGSKIIVTTRKDSVALMMGNEQIRM
GNLSTEASWSLFQRHAFENMDPMGHPELEEVGRQIAAKCK
GLPLALKTLAGMLRSKSEVEEWKRILRSEIWELPHNDILP
ALMLSYNDLPAHLKRCFSFCAIFPKDYPFRKEQVIHLWIA
NGLVPVKDEINQDLGNQYFLELRSRSLFEKVPNPSKRNIE
ELFLMHDLVNDLAQLASSKLCIRLEESQGSHMLEQCRHLS
YSIGFNGEFKKLTPLYK
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Leucine-rich Repeat Domain

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 EQ  RT   LP R EFRLHNLSKRVLHNI
 PT  RS   RA S SQYK    KE   NDL
 TK  KL   RF D SR W    TK  DS
 CG  YN   ET L SS AD   EE   LQ
 EK  IN   RH D SN RR   K    LH
 SR  KS   Q  L GPKFFVDGWRMEDLGE
 QN  HGS  SV K ENVVDRREAVKAK
 REKNH    EQ S EWSESSIADNSQTESDILDE
 CPHKN    KK E SGYRGTNFPNWVADPL
     LK   VN S RN KD CYS    A
 GQ  PC   KF S KGMHG   RV  TEEFYGRLSSKKP
     NS   EK E EDMTEWKQWHALG
 GE  PT   EN S KN PE   SLE
 IQ  SS   KR E SD PV   FDDAQLFRSQ
 EA  KQ   EE D CD NS   TS    FS
 ILPTT    KR Q SR PK   KLE
 VGEMF    EY R ND GC   DD   SP
 EF  PT   RQ S EN QN   TR  FL
   IPTA  TET R SN EN   EK   SVA
CGG  AQ   TS N WG KK   KC    E
   L PS   KE R SD PE   EGEL
     FN   EI R IY KK   VNGRK
EWH  QR   TE W DHDGSDEDIEHWE
   LPCS   QR T KNLKT   SSQH
 KS  TS   QY C EGYLSQ QSQGQLSS
 SH  TS   QT Q WNFLN   QS   AE
SALPSS    SH E DD PN   QS   FE
SALPSS    SQ F QD PN   QS    F
KGMPSS    SK S FN PL   TP   E
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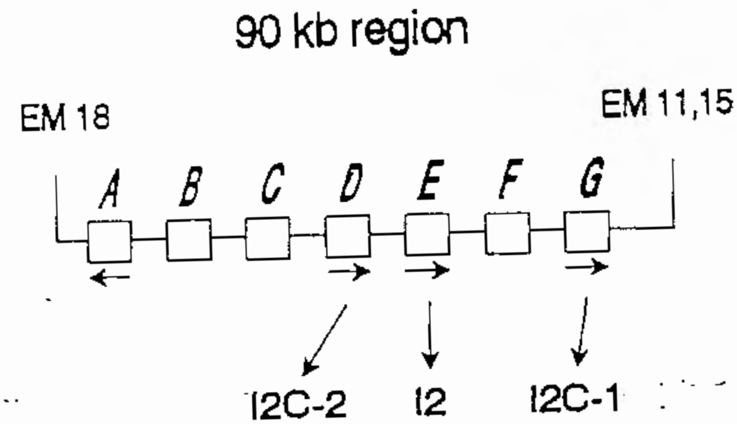

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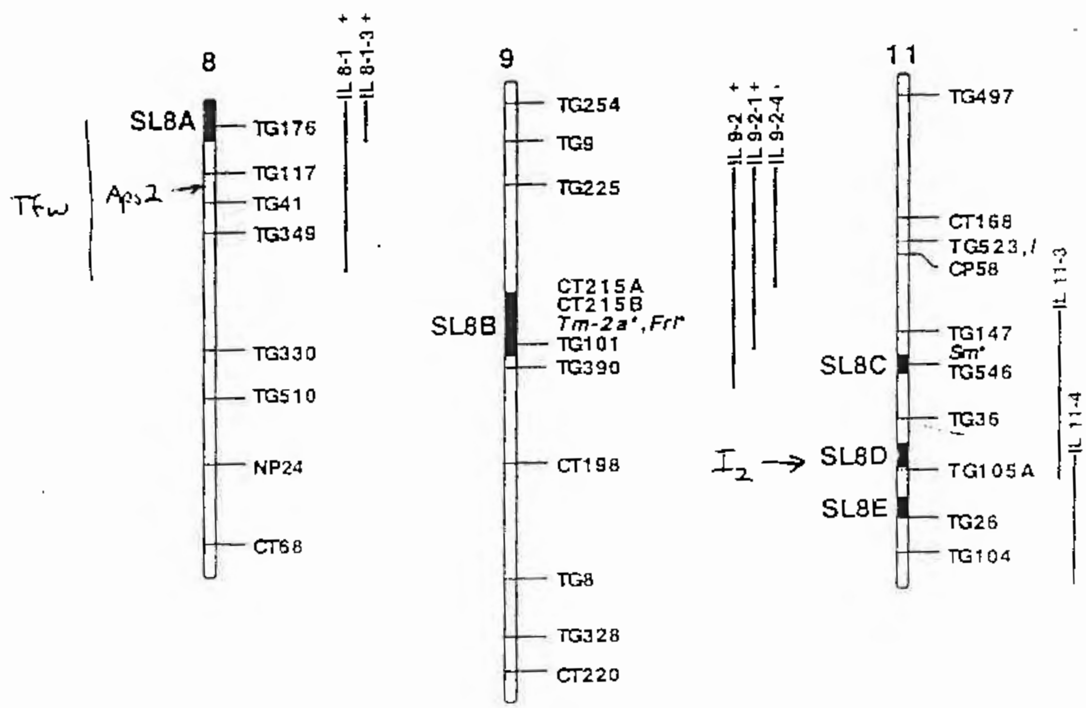

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CT68
Tfw
Aps2
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IL 8-1-3
9
TG254
TG9
TG225
CT215A
CT215B
SL8B
TG101
TG390
CT198
TG8
TG328
CT220
IL 9-2
IL 9-2-1
IL 9-2-4
11
TG497
CT168
TG523,I
CP58
TG147
SL8C
TG546
TG36
SL8D
TG105A
SL8E
TG26
TG104
IL 11-3
IL 11-4
I2

Tomato genes for resistance to Fusarium oxysporum

R gene	Source	Location	Resistance
I-1	*L. pimp.*	11 S	race 1 of *F.o.l.*
I-2	*L. pimp.*	11 L	race 2 of *F.o.l.* and some isolates of race 1
I-3	*L.penn.*	7 L	races 1, 2 and 3 of *F.o.l.*
Tfw	*L. penn.*	8	tolerance to race 3 of *F.o.l.*
Frl	*L. peru.*	9	resistance to *F.o.r.l.*

NOTES FROM BRUCE LYON

Identification of Genes for Resistance to Fungal Wilt Diseases in Cotton

Dr. Bruce R. Lyon
The University of Sydney

Fungal Wilt Diseases of Cotton

- Verticillium wilt disease
  - *Verticillium dahliae* Kleb.
- Fusarium wilt disease
  - *Fusarium oxysporum* f.sp. *vasinfectum*

Fungal Wilt Diseases of Cotton

- Molecular plant breeding:
  - Assays for fungal wilt disease in cotton
    - Relative disease tolerance of cultivars
  - DNA markers for disease tolerance
    - Enhanced breeding for disease tolerance

Fungal Wilt Diseases of Cotton

- Assays for plant disease tolerance:
  - Assay of plant disease symptoms
    - Leaf necrosis
    - Vascular discolouration
    - Plant stunting
  - Assay for fungi in plant tissues
    - PCR of fungal DNA from stems

Molecular Genetic Markers

- Molecular genetic marker technologies:
  - Restriction Fragment Length Polymorphism
  - Random Amplification Polymorphic DNA
  - Simple Sequence Repeat (microsatellite)
  - Amplified Fragment Length Polymorphism

Molecular Genetic Markers

- Fingerprinting of cotton varieties
  - Plant identification & PVR
- Linkage of important agronomic genes
  - Molecular plant breeding
- Mapping of important agronomic genes
  - Gene isolation & cloning