A light-dependent selection marker system in plants

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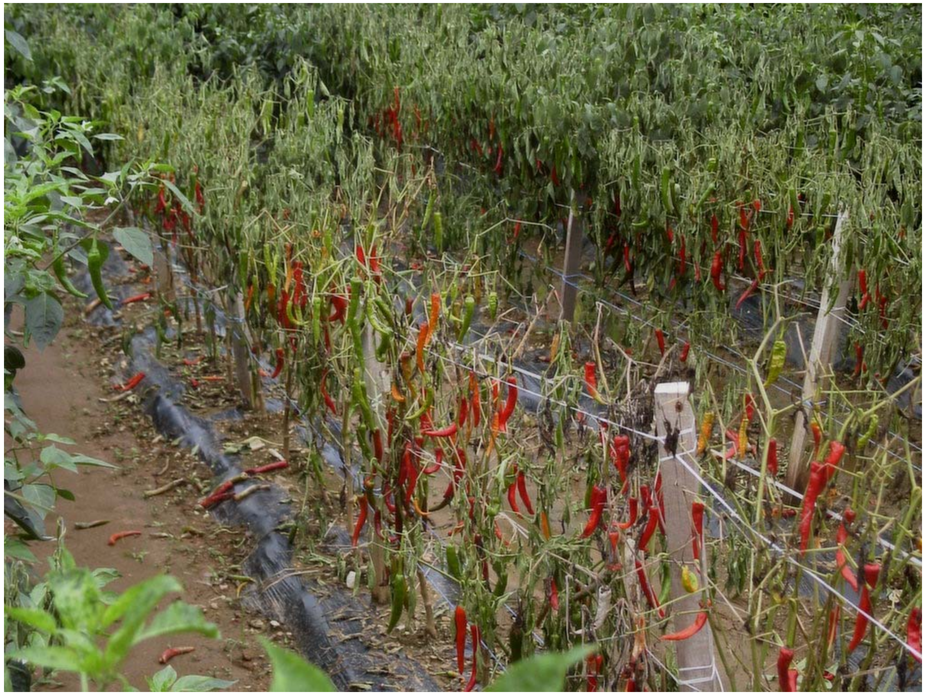
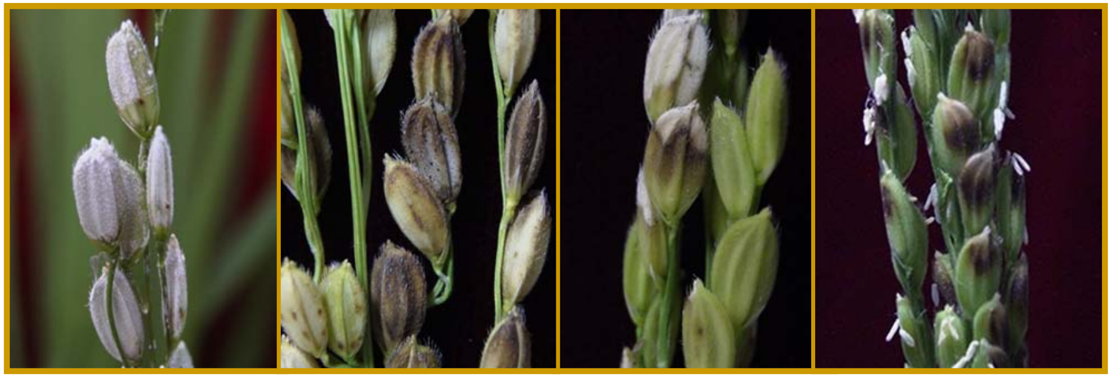
***Burkholderia glumae*:**

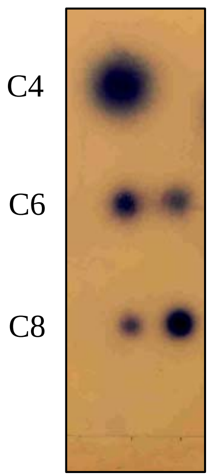
The causal agent of rice panicle blight (bacterial rice grain rot) **and bacterial wilt**

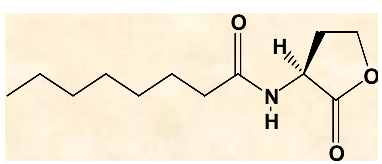




Burkholderia glumae

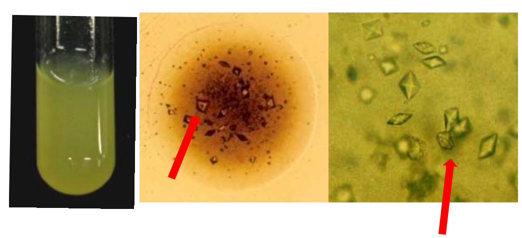
- Infects roots, stems, and flowers.
- Produces toxoflavin and *N*-octanoyl-L-homoserine lactone.
- Optimum growth temperature is 37^{o}C.
- Possesses 2-4 polar flagella.

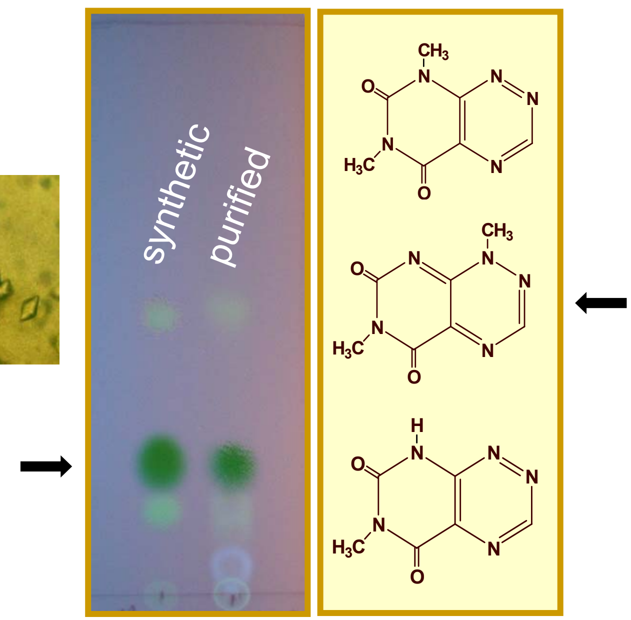




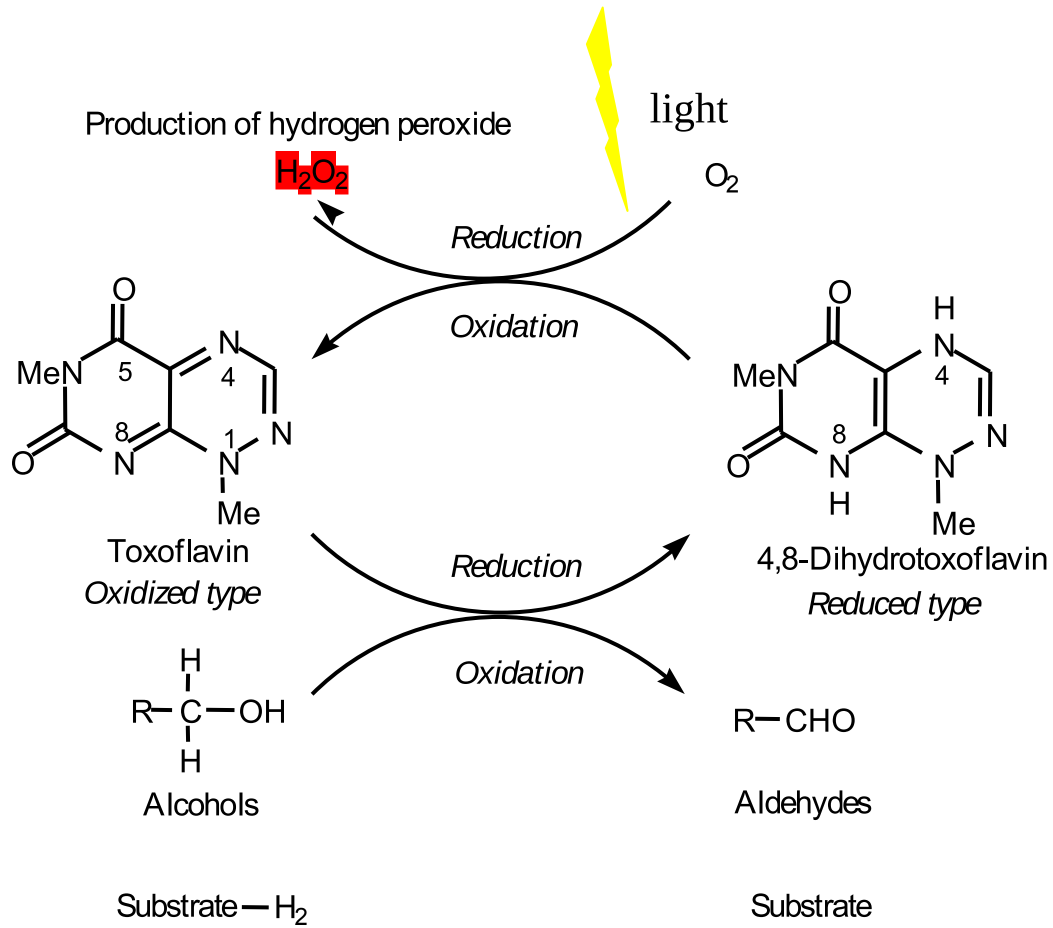
N-octanoyl-L-homoserine lactone (C8-HSL)

Toxoflavin





Toxoflavin is a photosensitizer.



T. Nagamatsu, Y. Hashiguchi, Y. Sakuma, and F. Yoneda, *Chem. Lett.,* **1982**, 1309.

Toxoflavin is a key pathogenicity factor.

Disease severity

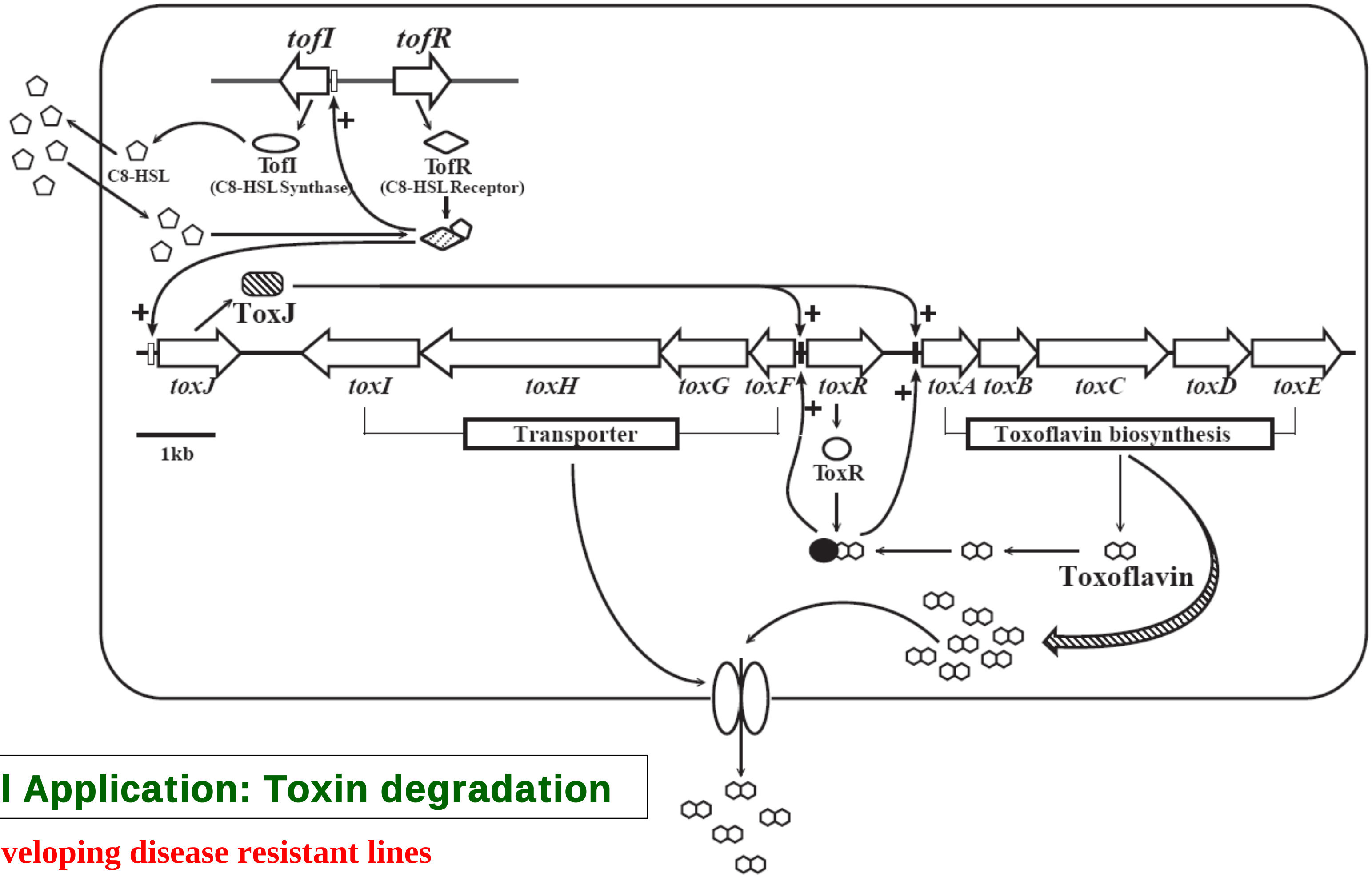
5 5 3.5 2 0.6 0

40 μg/ml 20 μg/ml 10 μg/ml 5 μg/ml 2.5 μg/ml SDW

Milyang 23

10 days after treatment

Regulation of toxoflavin biosynthesis by quorum sensing

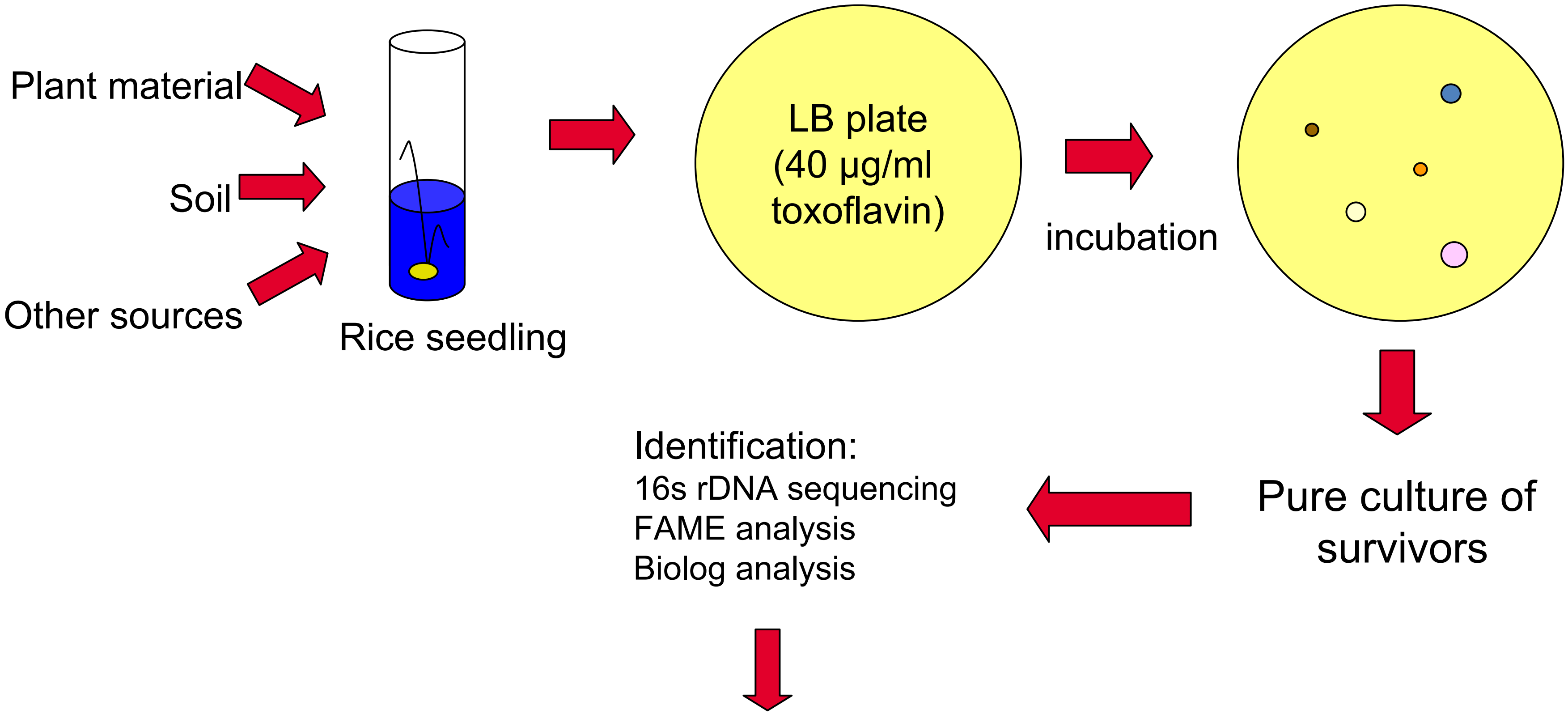


Practical Application: Toxin degradation

1. Developing disease resistant lines
2. A novel selection marker for plant transformation

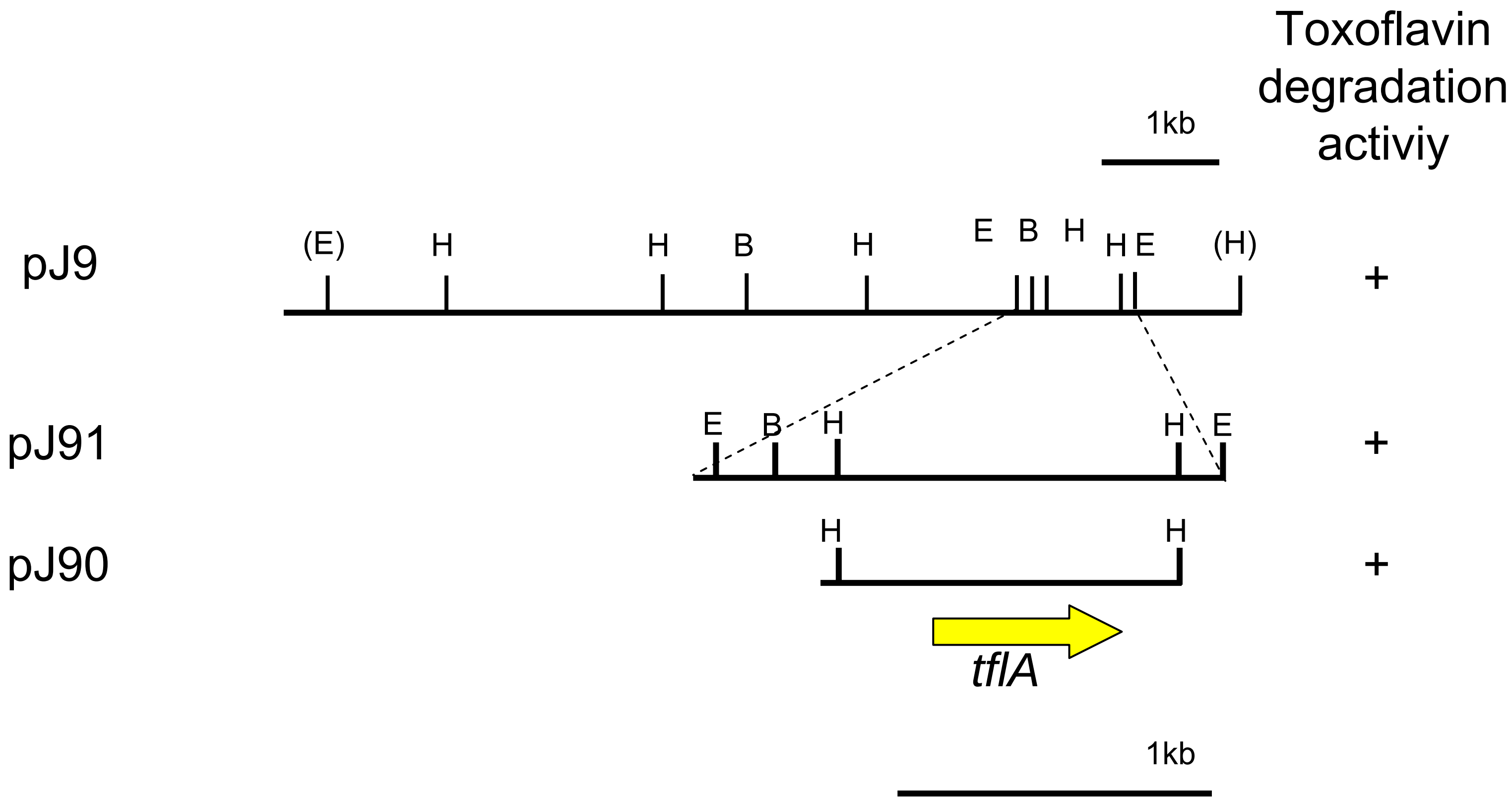
Kim J. *et. al*. Mol. Microbiol. 54: 921-934 (2004)

Isolation and identification of toxoflavin-degrading bacteria from natural environment

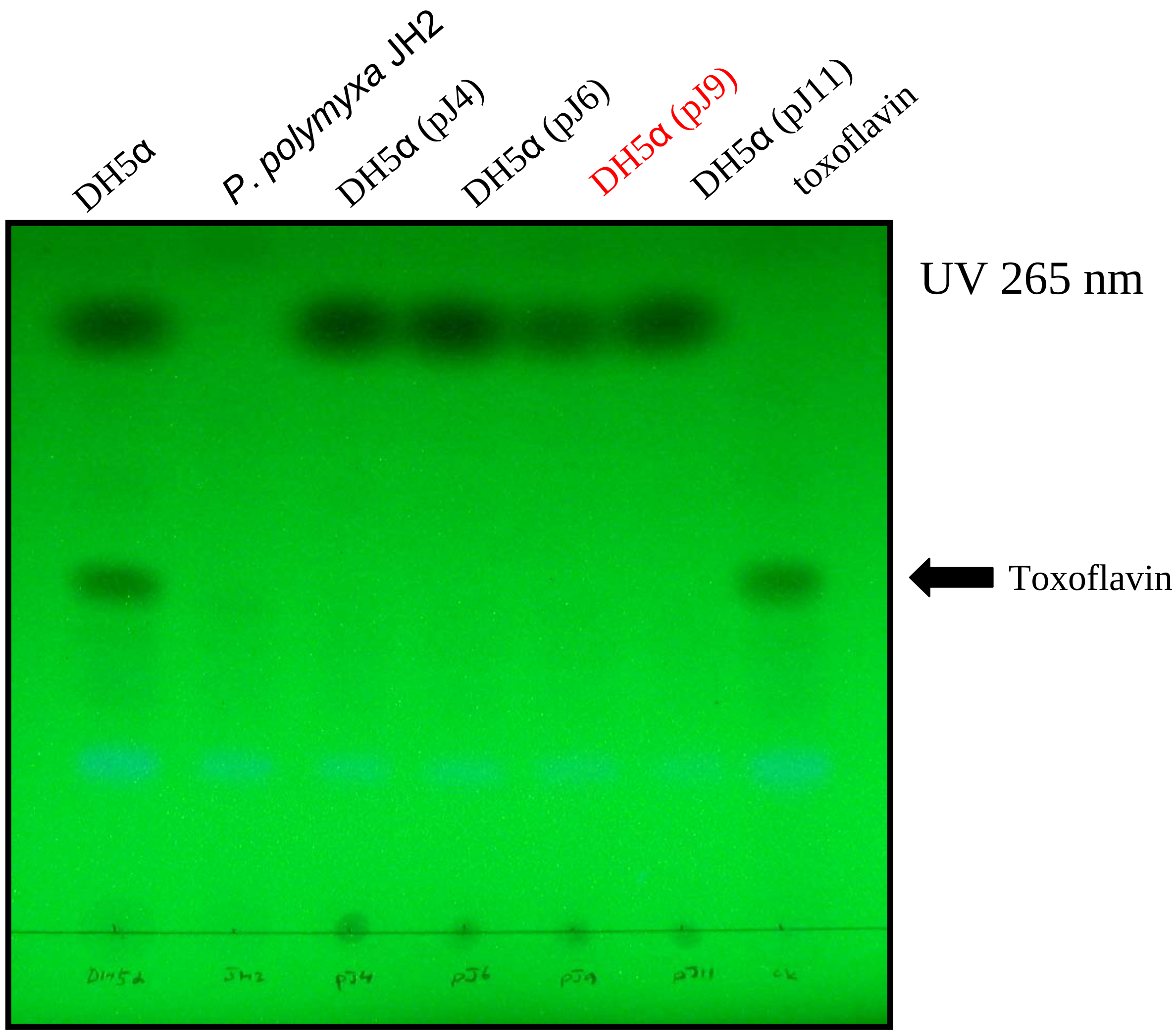


Isolation of *Paenibacillus polymyxa* strain JH2 from rice seeds

Deletion analysis of pJ9



E. coli DH5α carrying pJ9 degrades toxoflavin.



After 16 hr in LB broth (Toxoflavin 40 μg/ml)

TflA is similar to ring-cleavage extradiol dioxygenases.

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                               :                                      . .:
1 -----MTSIKQLTLYTAELDRMLAFYTNMLGAQHVHEQADAFTIQLG---VSQIQFRAAADGTKPFYHIAINIAANHFQEGKAWLSGFGELLTE--NDEDQAYFPFFNAY
2 ------MRITAATLWTNDVKPMQDFYTETLGFPLVEETATAFTVQIG---TSQLRFELDTTQQPKQYHFAFNVPGDAFRLAKDWLRHCVPLLNE--DGEDEIFFENINAH
3 ------MEFEAVTFATKKLDSVKSFYKETLGLRVTHETTSQVTILVG---STLVTFEQFEDDGEPFYHYAINIPSNKLQEAKMWLEKRVTLLQH--EGKNVVPFPRWNAE
4 -----------------------------MGLRVTHETTSQVTILVG---STLVTFEQFEDDGEPFYHYAINIPSNKLQEAKMWLEKRVTLLQH--EGKNVVPFPRWNAE
5 ----MILRLGHAELFVTDLERAREFYVHILGFREND-SDKEHIYLRGVDEFDAHTLTLTKNSYAGLGHFALRVSEHEDLERLADQHKKLGIPCQ--LVPAGVEPGQGMA-
6 MSHMGIFKFANGTIRVKDLEKSIEFYRDVIGMHELA-REKGVVYFG--CGADETFDIAIKEGGTGVEQFALQVADEEDIQYYEKKLTNLGISTQ--RMSN-PEPGKKVA-
7 MDKASVTELGYIGISVSDAAAWRDYACCIVGMELVDDGEEDRFYLR--MDGWHHRIVVHTNGGDDLEYLGWRVDGPNELDAIAAQLDAAGYSYR--VASAEEAAERRVLG
8 ---MSIRSLGYMGFAVSDVAAWRSFLTQKLGLMEAG-TTDNGDLFR--IDSRAWRIAVQQGEVDDLAFAGYEVADAAGLAQMADKLKQAGIAVT--TGDASLARRRGVTG
9 ------MHLRHLVLPCLDPGLTLRFYRDVLQLPVHG-----NTVRIG----WSTLECVQAQHPVGSAHLAFNVAPSRFQAAAQWIGARATLLSDPHGRKHFALDGAWQSH
  1.......10........20........30........40........50........60........70........80........90.......100.......110

     .  .     *                                    .                                                 .
1 SCYVEDPSGNIIELISR-QQAAP---------VLDKPFSADQLLSIGEINITTSDVEQAATRLKQAELPVKLDQ-------------IEPAGLNFIGDQD--LFLLLGPP
2 SVYFYDTDENVVELIAR-HDVNPDK-------SLEK-FTVRDILDIGEMNVTTPDVASVGAQFAELGVFHRYHQP------------INVDFLNFLGASEDGTHLLLGRE
3 ALYFEDPAGNIVEWIAR-HNLNQT--------TSEPFVPSEQLLYVSEVGIVTAELGEQTKLFESLGYPVWDQG---------------TEHFRAIGDEHG--LFITVTK
4 ALYFEDPAGNIVEWIAR-HNLNQT--------TSEPFVPSEQLLYVSEVGIVTAELGEQTKLFESLGYPVWDQG---------------TEHFRAIGDEHG--LFITVTK
5 -LRVIEPNGHPIEFYHEMQQIDVYNENGRLKRLPMRSTHTDKGIPPLRIDHVNLRVANVDYALRYWRDELNFSVSEYVERD----GETFAAWTRRFPGTHDVALV-KANG
6 -LRFAAPSGHQLELALMEDRPHYLH--------PVAGHKGSRGIGILDADHITLHTKDVKGLAEFLQEALDFRVADVFEPAP---GVWGAAWTHASDYHHDVALIGTSED
7 LLKLEDPGGNPTEIFWGPEQTLRRP------FHPGRPMHGRFLTGSQGLGHCLLRQDDDEAAYRFYR-TLGFKGGVEYHLPLPDGATAKPTFMHCNDRQHAVAFGLGPMP
8 LITFADPFGLPLEIYYGASEVFEKP------FLPGAAVSG-FLTGEQGLGHFVRCVPDSDKALAFYTDVLGFQLSDVIDMKMGPDVTVPAYFLHCNERHHTLAIAAFPLP
9 SVYFGGPDGAVLELIARNALQDAAAG--------EGQFRGEELLCLSEIGLPSHNVEVVTRSLAHHFGLRPFAPP--------------LEGIAALGDDHG--LLIVVDR
  .......120.......130.......140.......150.......160.......170.......180.......190.......200.......210.......220

1 GRRWLFSERVAVIYPLQMELDNGVSLAITETGELVI-------------------------------------------------------------------------
2 DRTWLFSPKPAITSPLVLELDGNLHVRLDADGMLHHEK-----------------------------------------------------------------------
3 DRQWFMSERVSDFFPLEVMTKSNGKLYFY--------------------------------------------------------------------------------
4 DRQWFMSERVSDFFPLEVMTKSNGKL-----------------------------------------------------------------------------------
5 PSLHHIAYTVQGPAEIIRTADLLADAGYQDAIEFGPGRHGLSNAFFLYIRDPDGNRMEIYTGDYLRDLDMPPIKWTWEEYDQKGRLWWGPSYPERFLETSPVNQNWISK
6 TTLHHFAFLVSGFEDMKRACDLLAQAGYK--IETGPGRHGVGGNLFTYFLDPSGNRIELSAEMPRADRSVP--HKVWPDFPP-AFSTWGALPPESFAKGS---------
7 KRINHLMIEYTELDDLGIAHDMIRERQIP--VAMQLGKHSNDQALTFYHASPSGWLWELGWGGVKSS-----DQLVYYKADLFGHGIEASGFGLDVELGDDG-------
8 KRIHHFMLEVASLDDVGFAFDRVDADGL---ITSTLGRHTNDHMVSFYASTPSGVEVEYGWSARTVD-----RSWVVVRHDSPSMWGHKSVRDKAAARNKA--------
9 RRRWFPQQRQLPWADGLRISVDAPEPGLRLRDAQGWELLAA--------------------------------------------------------------------
  .......230.......240.......250.......260.......270.......280.......290.......300.......310.......320.........
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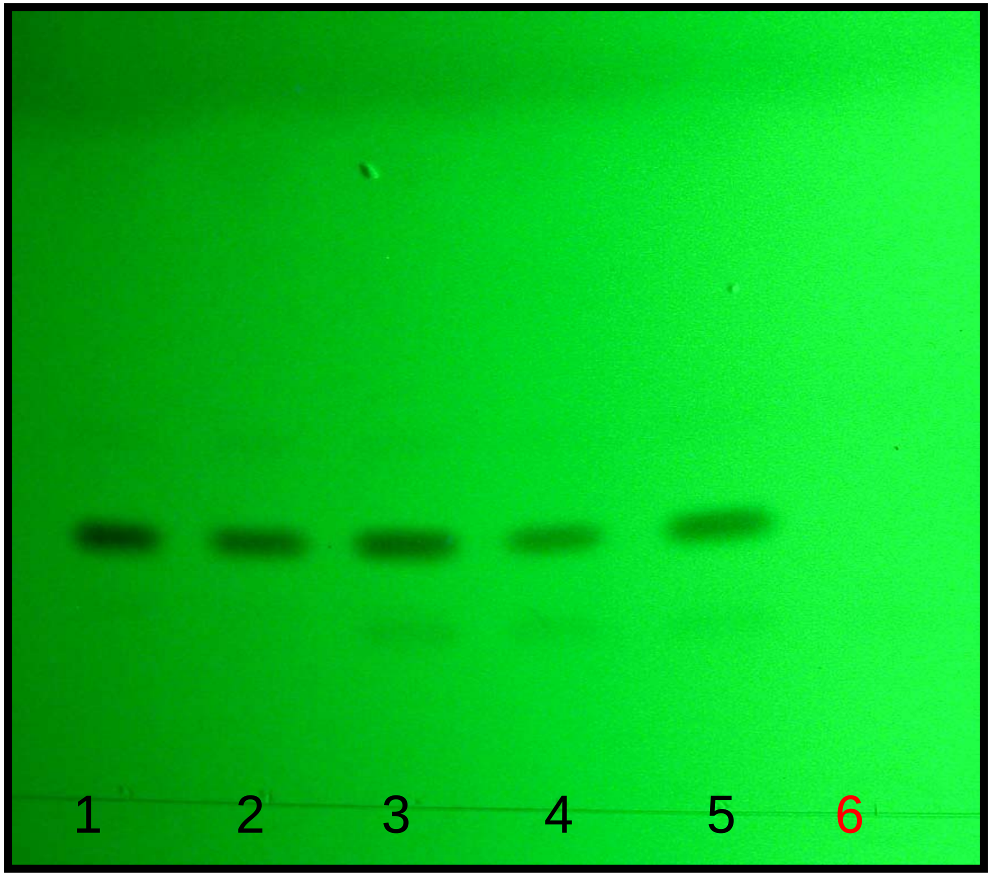
Degradation of toxoflavin by TflA depends on Mn^{++} and DTT.



100μM toxoflavin plus

1: CK (10μM $MnCl_2$)
2: Purified His-TflA (10μM $MnCl_2$)
3: CK (5mM DTT)
4: Purified His-TflA (5mM DTT)
5: CK (5mM DTT/10μM $MnCl_2$)
6: Purified His-TflA (5mM DTT/10μM $MnCl_2$)

Comparison of TflA with other extradiol dioxygenases

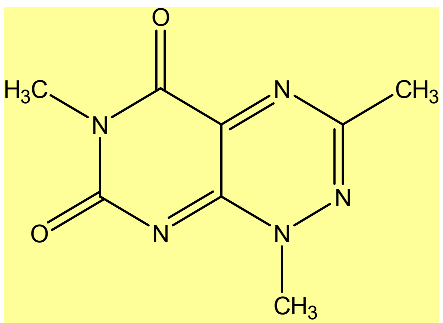
	Specific activity (units/mg)	Metal	Temperature	pH	K_M (µM)	Substrate
TflA	0.04	$MnCl_2$	25°C	6.5	69.72	Toxoflavin
NahC	1.5	$MnCl_2$	25°C	7.5	400	2,3-dihydroxybiphenyl
NahH	7	$FeCl_2$	25°C	7.5	0.25	2,3-dihydroxybiphenyl
ThnC	2	$FeCl_2$	30°C	6.8	18.6	1,2-dihydroxybiphenyl
BphC	0.65	$MnCl_2$	25°C	8.0	7	2,3-dihydroxybiphenyl

Purified TflA degrades toxoflavin derivatives.

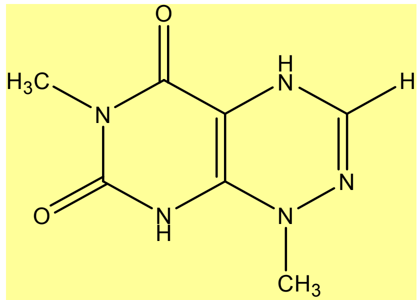
Toxoflavin	+++	Reumycin	+
3-Methyl toxoflavin	+++	3-Methyl reumycin	+++
3-Phenyl toxoflavin	–	3-Phenyl reumycin	–
4,8-Dihydro toxoflavin	+++	Fervenulin	+
3-Methyl 4,8-Dihydro toxoflavin	++	5-Deazaflavin	–

Degradation activity:-0~20% degradation; +,20~50%; ++, 50~80%; +++, 80~100%

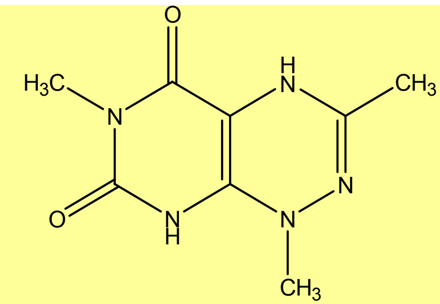
Toxoflavin derivatives with phenyl group on 3 carbon were not degraded by TflA.



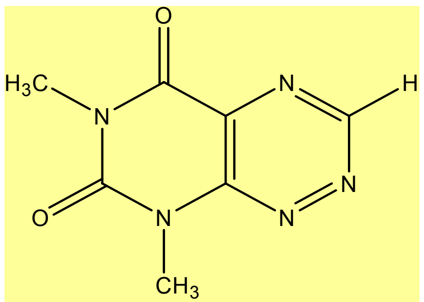
3-Methyltoxoflavin



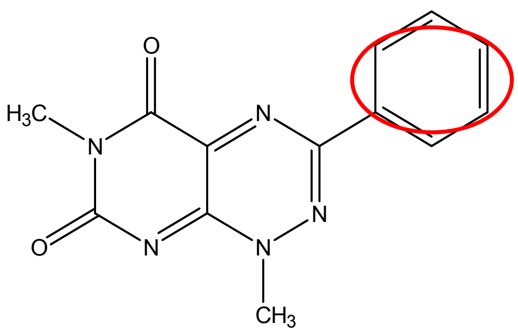
4,8-Dihydrotoxoflavin



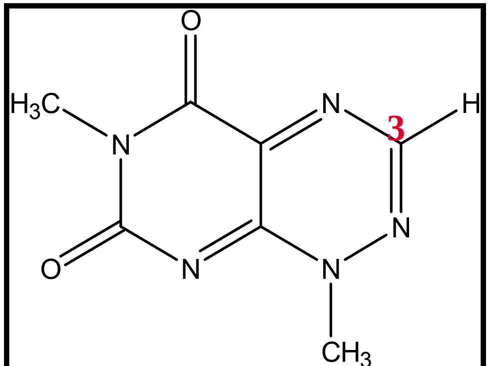
3'-Methyl 4,8-Dihydrotoxoflavin



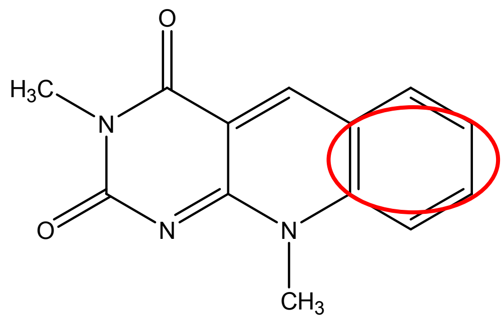
Fervenulin



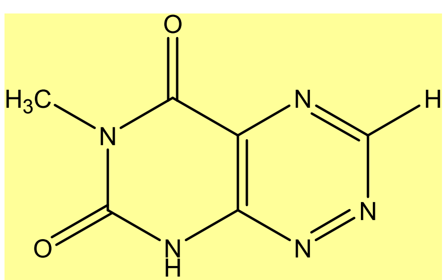
3-Phenyltoxoflavin



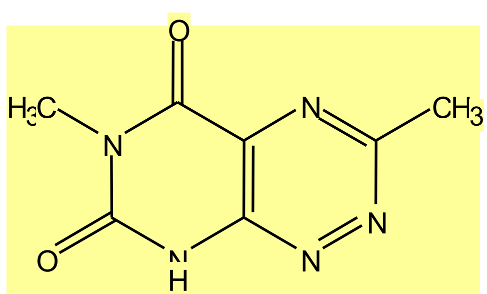
Toxoflavin



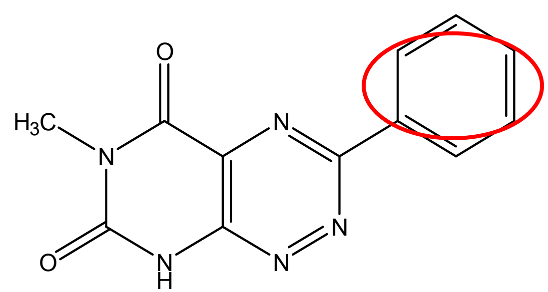
5-Deazaflavin



Reumycin



3-Methylreumycin



3-Phenylreumycin

Extradiol dioxygenases

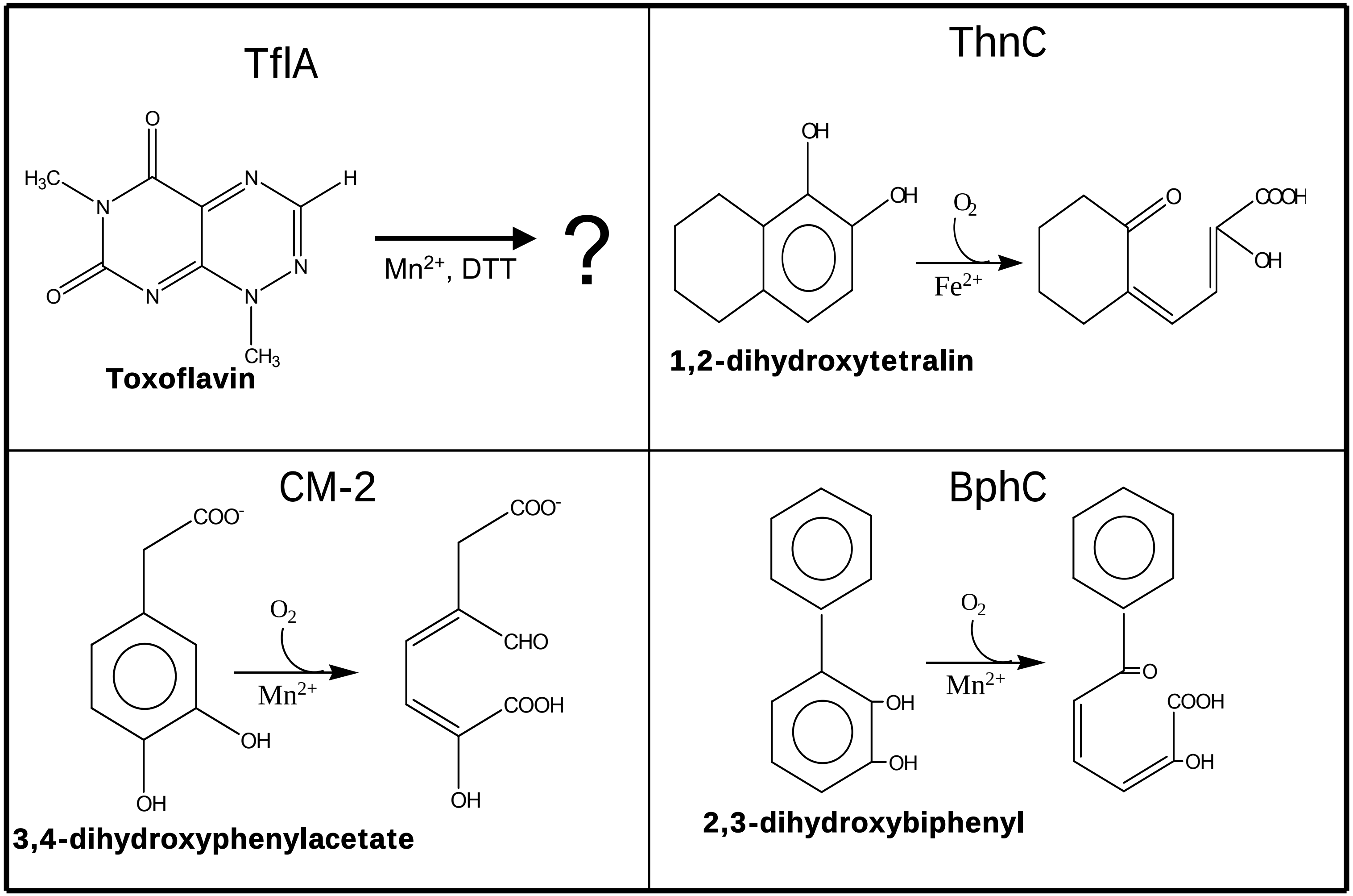


Table 1. Selectable markers currently used in transgenic plants confer resistance to antibiotics, herbicides or metabolic inhibitors

Gene	Gene product	Selection agent(s)	Gene source	Ref.
nptII/neo[P]	Neomycin phosphotransferase II	Kanamycin, neomycin, geneticin (G418), paromomycin, amikacin	*Escherichia coli*, transposon Tn*5*	45
bar[P]	Phosphinothricin acetyltransferase	Glufosinate, L-phosphinothricin, bialaphos	*Streptomyces hygroscopicus*	45
pat[P]	Phosphinothricin acetyltransferase	Glufosinate, L-phosphinothricin, bialaphos	*Streptomyces viridochromogenes*	29,45
bla	β-Lactamase	Penicillin, ampicillin	*Escherichia coli*	45
aadA[P]	Aminoglycoside-3′-adenyltransferase	Streptomycin, spectinomycin	*Shigella flexneri*	45
hpt	Hygromycin phosphotransferase	Hygromycin B	*Escherichia coli*	45
nptIII	Neomycin phosphotrasferase III	Amikacin, kanamycin, neomycin, geneticin (G418), paromomycin	*Streptococcus faecalis R* plasmid	45
epsps/aroA[P]	5-Enoylpyruvate shikimate-3-phosphate	Glyphosate	*Agrobacterium CP4*, *Zea mays*, *Petunia hybrida*	45
gox	Glyphosate oxidoreductase	Glyphosate	*Achromobacter LBAA*	45
bxn	Bromoxynil nitrilase	Bromoxynil	*Klebsiella pneumoniae* var. *iozaenae*	45
als	Acetolactate synthase	Sulfonylureas, imidazolinones, thiazolopyrimidines	*Arabidopsis thaliana*, *Nicotiana tabacum*, *Brassica napus*	45
cat[P]	Chloramphenicol acetyltransferase	Chloramphenicol	Bacteriophage P1 Cm^R	7
TDC	Tryptophan decarboxylase	4-Methyltryptophan	*Catharanthus roseus*	46
uidA/GUS[P]	β-Glucuronidase	Cytokinin glucuronides	*Escherichia coli*	47
xylA	Xylulose isomerase	D-Xylose	*Thermoanaerobacterium thermosulfurogenes*	48
manA	Phosphomannose isomerase	Mannose-6-phosphate	*Escherichia coli*	49
BADH[P]	Betaine aldehyde dehydrogenase	Betaine aldehyde	*Spinacia oleracea*	7

[P]Shown to be functional in plastids[6].

Nature Biotechnology (2006) 20:575-580

Generation of transgenic rice plants expressing *tflA*

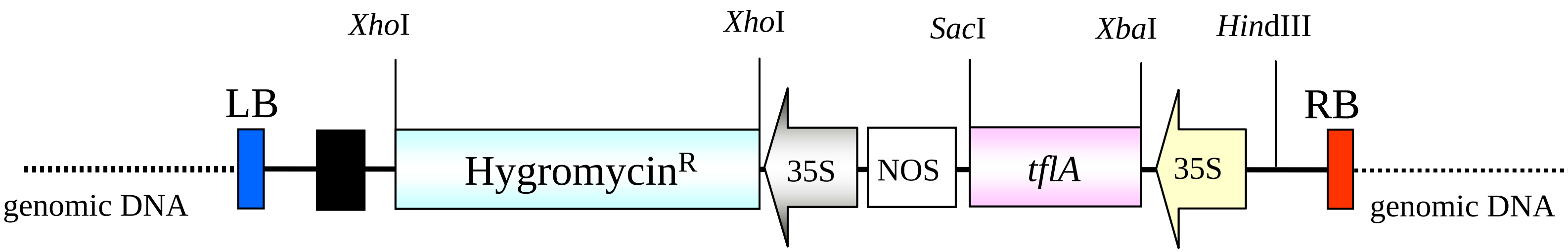
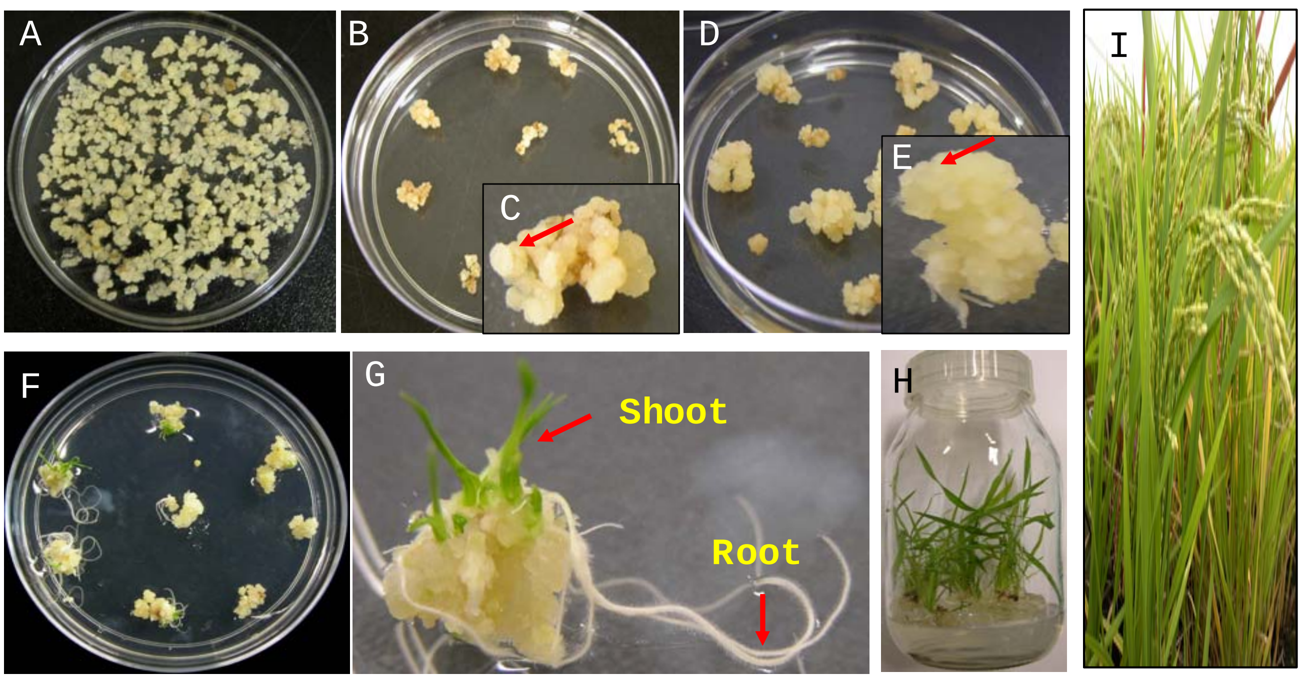
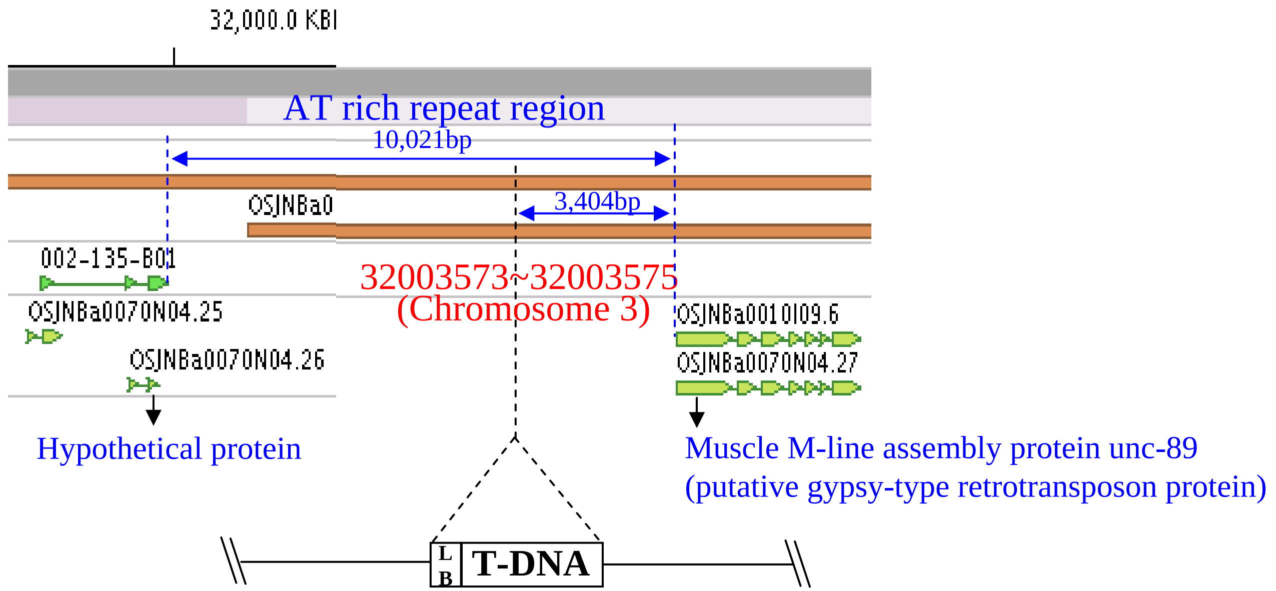


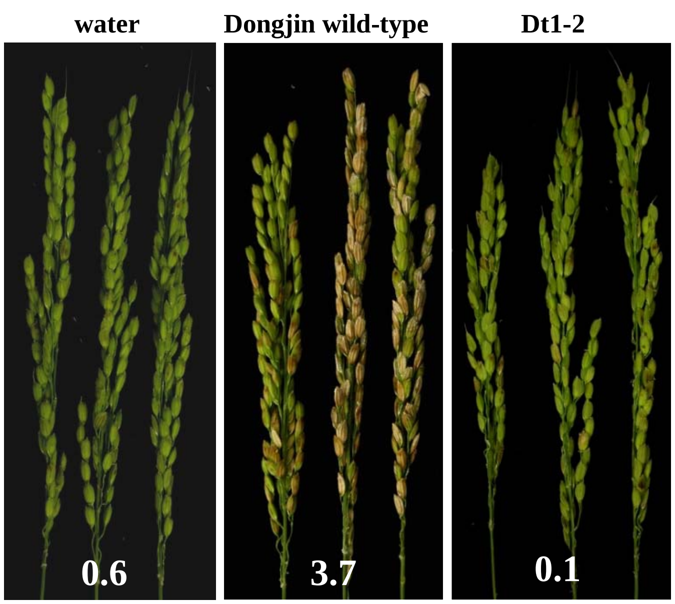
Fig. 1. Genetic construction of pJ904(pCamLA::*tflA*).



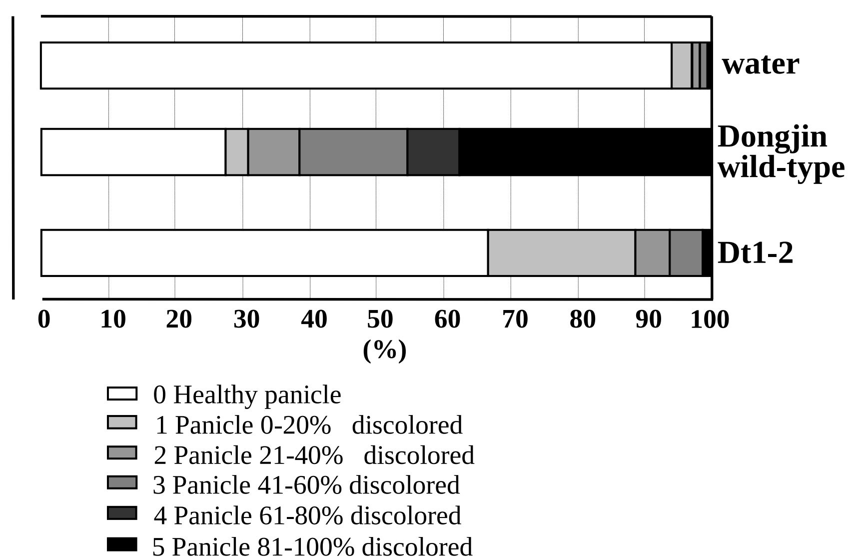
Dt 1-2

T4 plants

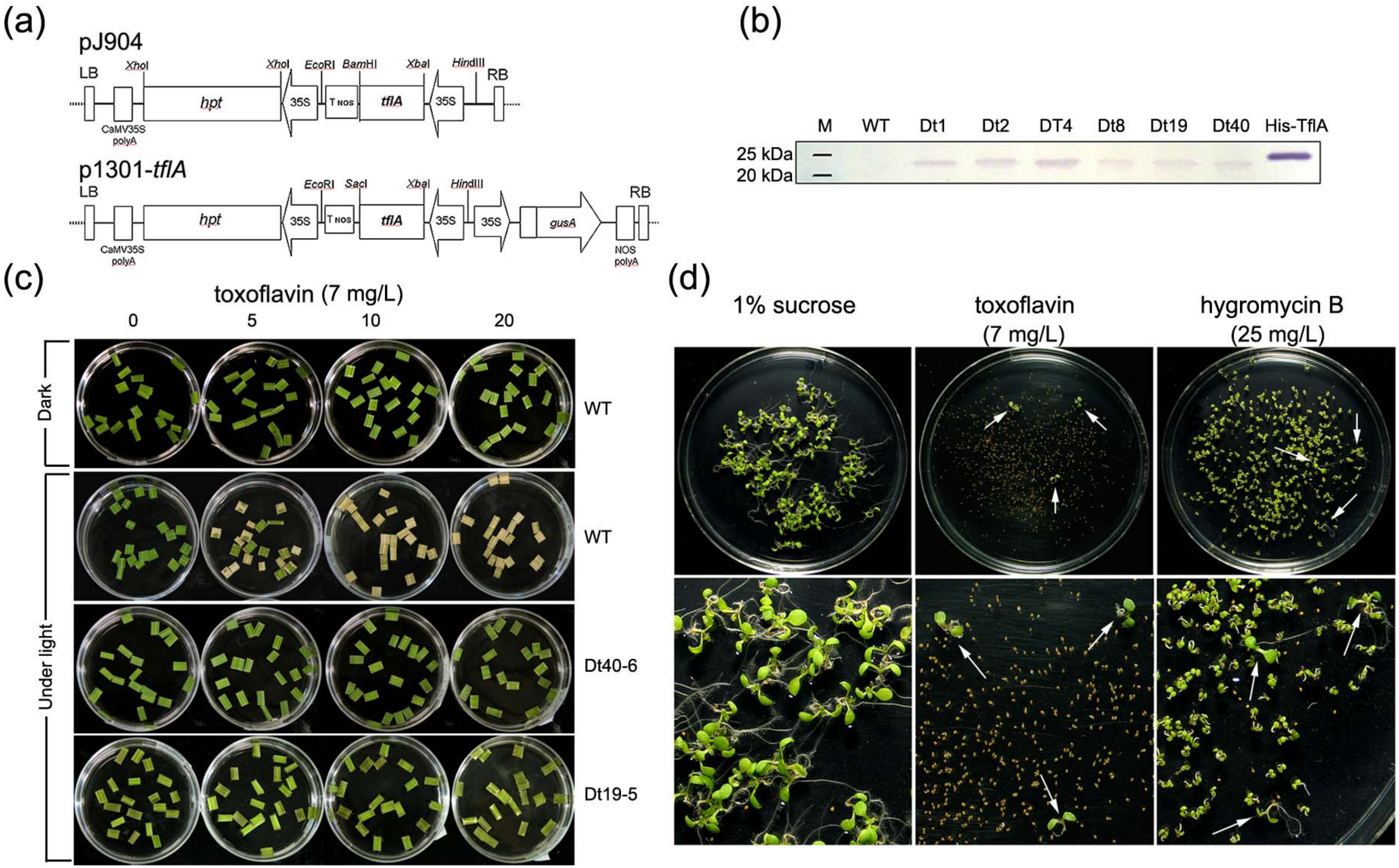




Disease degree (1~5)



Comparison of selection markers in rice and Arabidopsis



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Efficiency of rice transformation

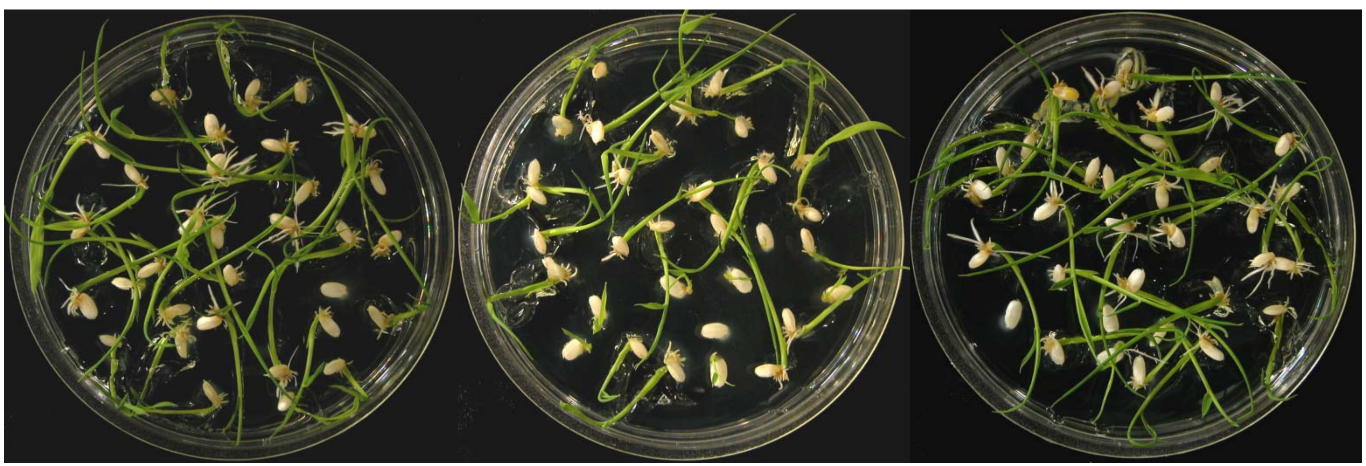
Marker	No. of calli inoculated	No. of resistant calli	No. of putative regenerated plants	PCR positive independent plants[a]	Transformation frequency (%)[b]
pCamLA (hpt)	300	80	20	16	80
	300	134	43	38	88.37
pTflA	300	84	28	25	89.28
	300	192	26	21	80.76

[a] Independent plants : plants coming from different calli

[b] Number of PCR-positive plants/number of putative regenerated plants

Seed selection with toxoflavin (7mg/L)

Dongjin wild-type (Hygromycin;30mg/L) | Dongjin wild-type | Dt7-3-5



Dt27-4-1 | Dt28-2-5 | Dt40-9-3

The photograph was taken 10 days after seeding.

Spray screening of transgenic rice plants

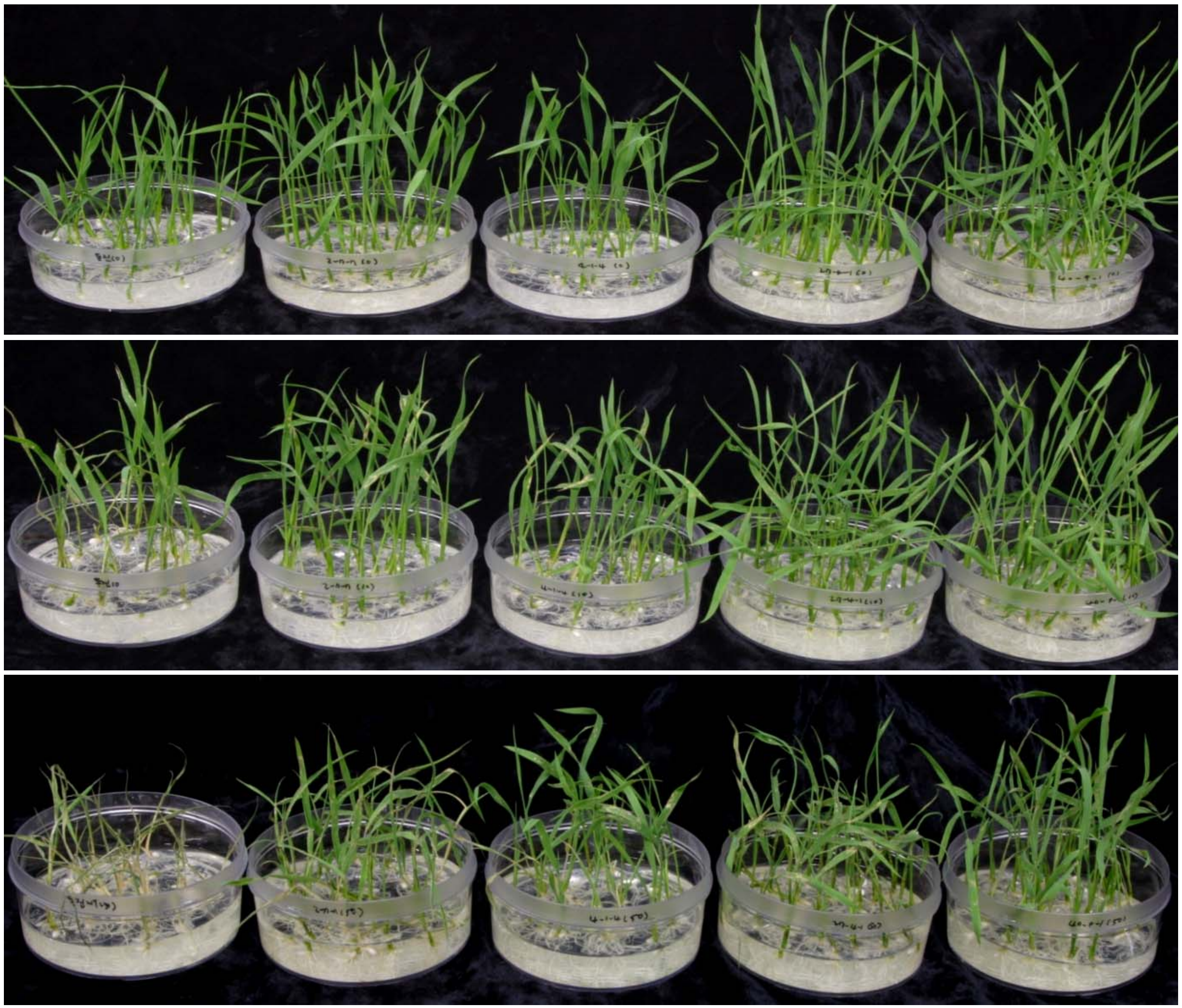
WT Dt2-7-2 Dt4-1-4 Dt27-4-1 Dt40-9-1

Toxoflavin (mg/L)

0

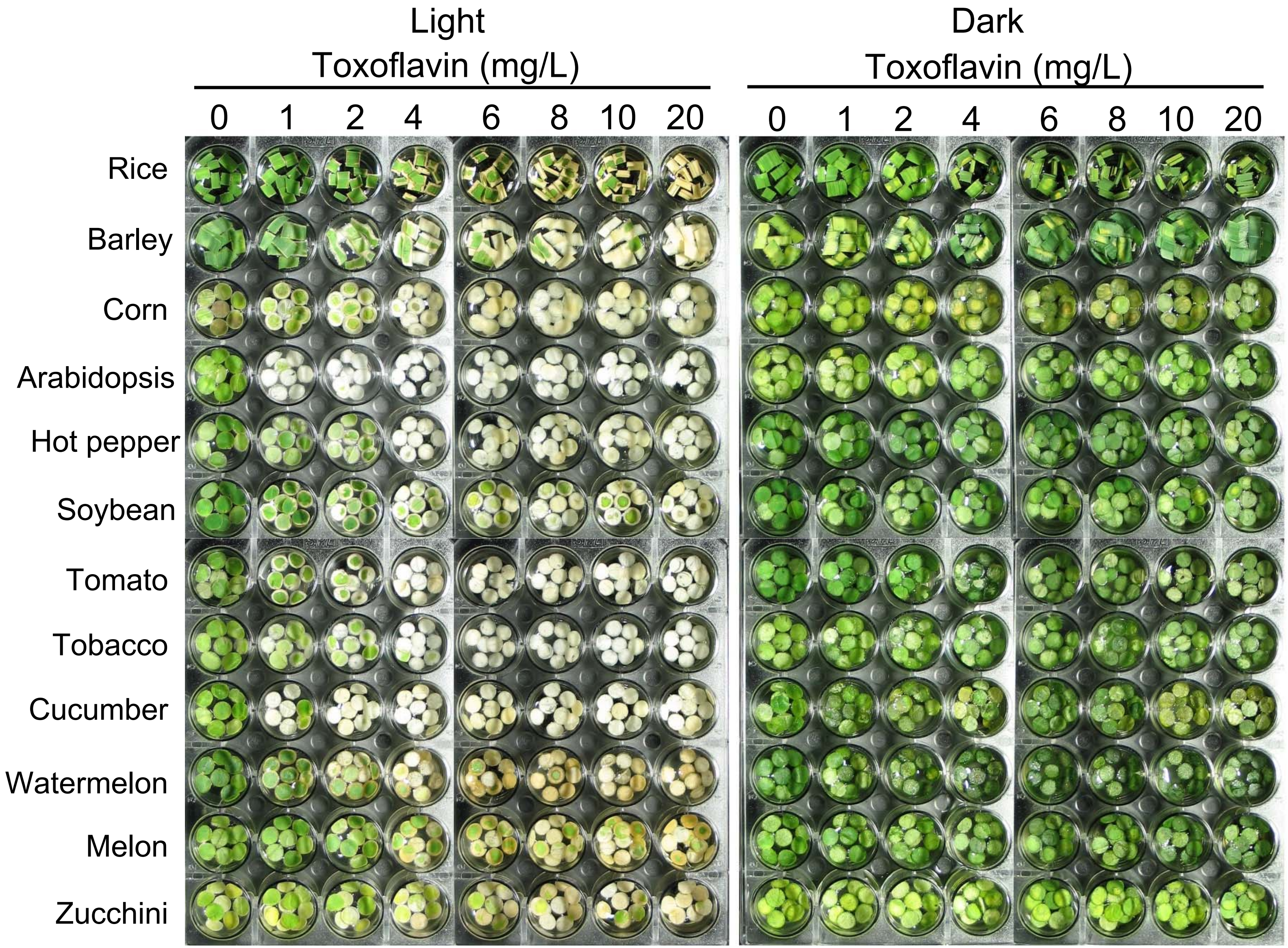
10

50



The photograph was taken after 48h after spray.

Light-dependent sensitivity of various crops to toxoflavin



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  - Jae Sun Moon, Serry Koh (KRIBB)
  - Nam-Soo Jwa (Sejong Univ.)