Isolation of a TIR-NBS-like gene promoter from triploid white poplar and its characterization in transgenic tobacco plants

Ph.D. Student Huiquan Zheng & Prof.

Zhiyi Zhang

Email: zhenghuiquan2005@sina.com

Outline

- Background
- Our study
- Future work

Plant improvement for disease resistance

A major goal in plant science is the production of commercial plants with increased and durable resistance to a spectrum of diseases. In the past, two general approaches, including the conventional breeding and chemical treatment methods, have been sought. However, they are present to have several problems.

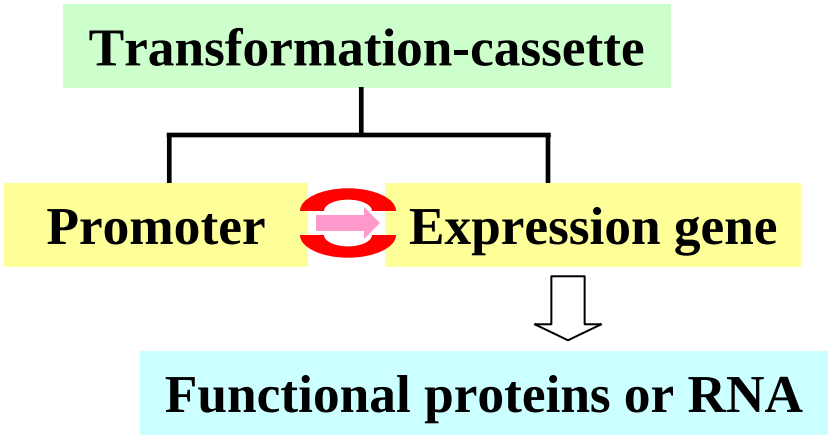
- **Conventional breeding: Laborious, time-consuming (especially for the long-lived woody species, e.g. poplars)**
- **Chemical treatment: Expensive, hazardous to the environment**

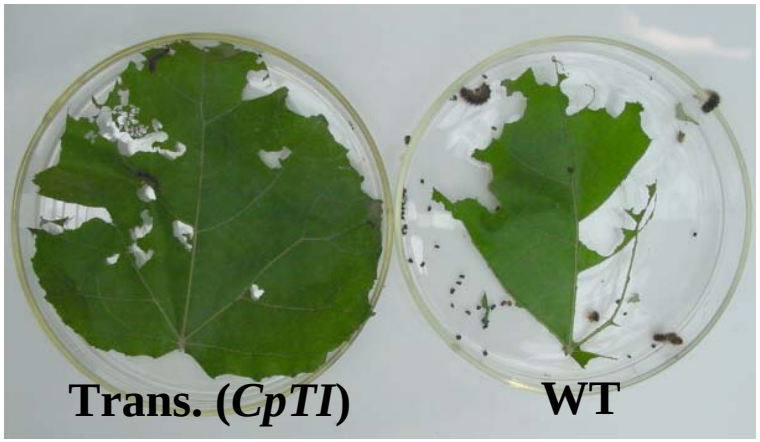
The plant genetic engineering

Genetic engineering has been used to introduce a set of valuable traits, such as pest resistance and herbicide tolerance, into a variety of commercial plants (e.g. Triploid white poplar).

The genetic engineering technology: offering an alternative avenue for the plant improvement with increased disease resistance.

The core genetic element : transformation-cassette





Zhang et al. *Silvae Genetica*, 2005.

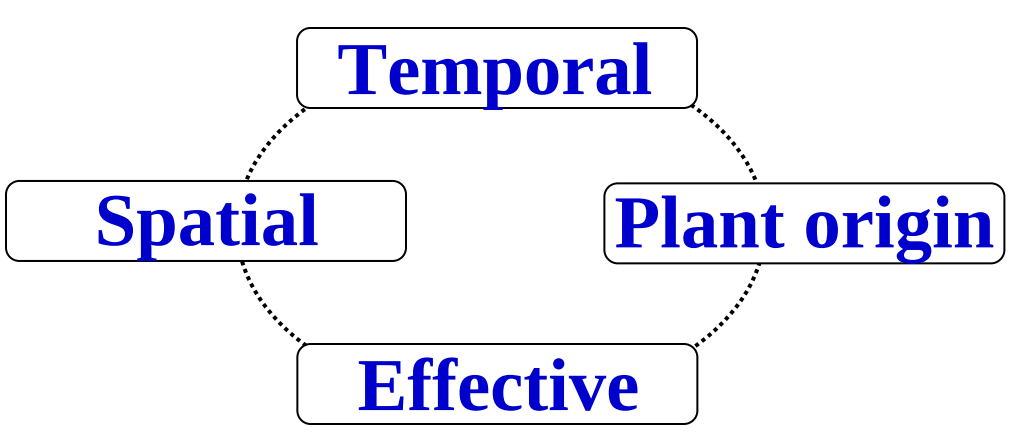
One of the pivotal problems for genetic engineering:

How to express the transgenes in the host plants?

- The high, constitutive promoters (e.g. CaMV 35S) ?
- The tissue/cell/organelle-specific promoters ?
- The inducible promoters ?

Overexpression of the defense component in transgenic plants may resulted in a set of problems, such as homology dependent gene silencing, unexpected disease symptoms, altered morphology and reduced size (dwarfish/stunted), especially for the perennial Poplar trees.

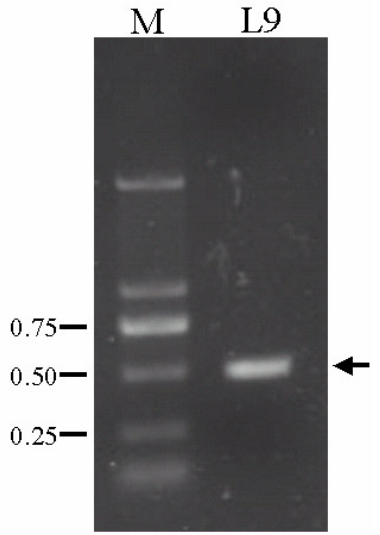
The poetic promoter

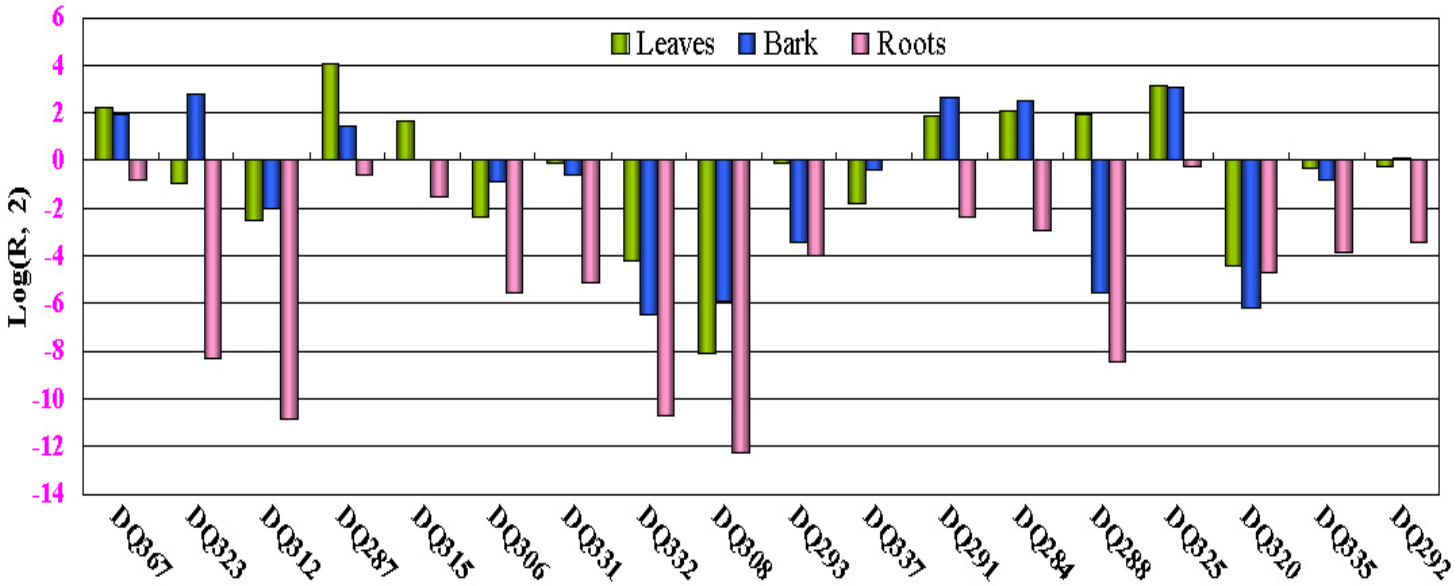


Our work

Focusing on the ‘Cloning and testing of the pathogen and/or defense signals inducible, tissue-specific promoters’, which may be promising for the Poplar genetic engineering in disease resistance.

The NBS-type resistance gene analogs (RGA) in triploid white poplar clone ‘ L9’



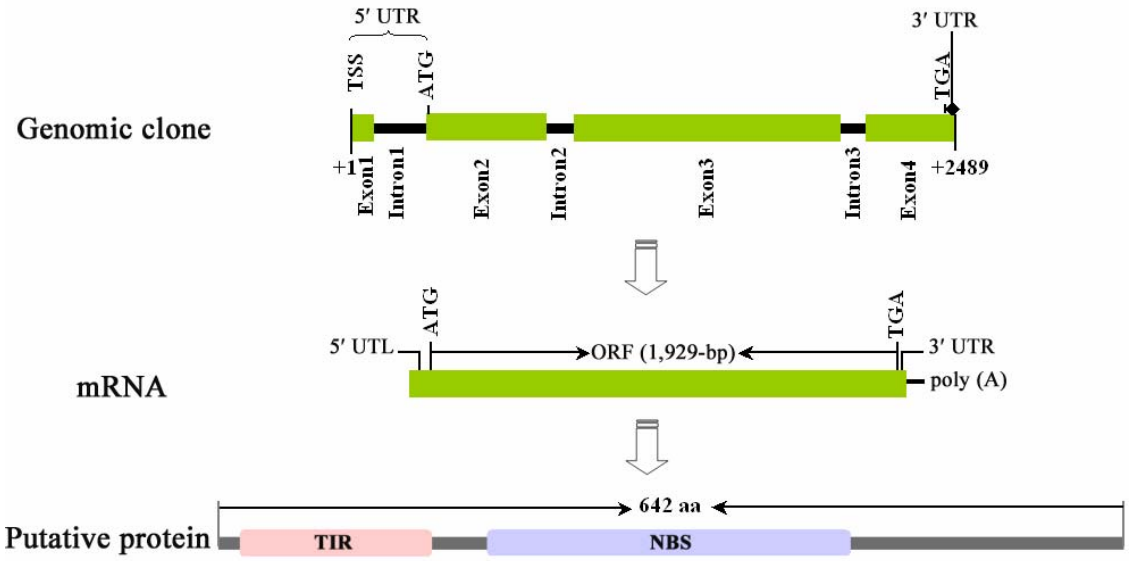


Zhang et al. *Plant Biology*, 2008

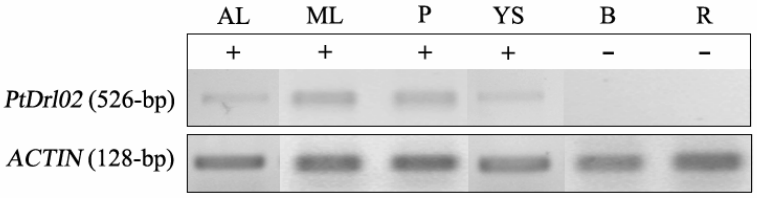
Characterization of the *PtDrl02* gene

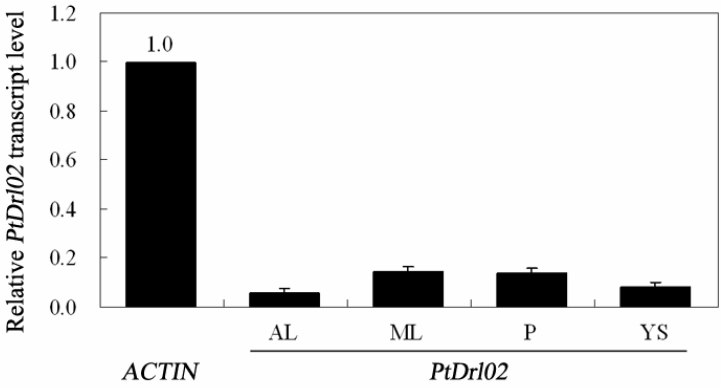
The RGA DQ324288

A TIR-NBS-like gene (*PtDrl02*)



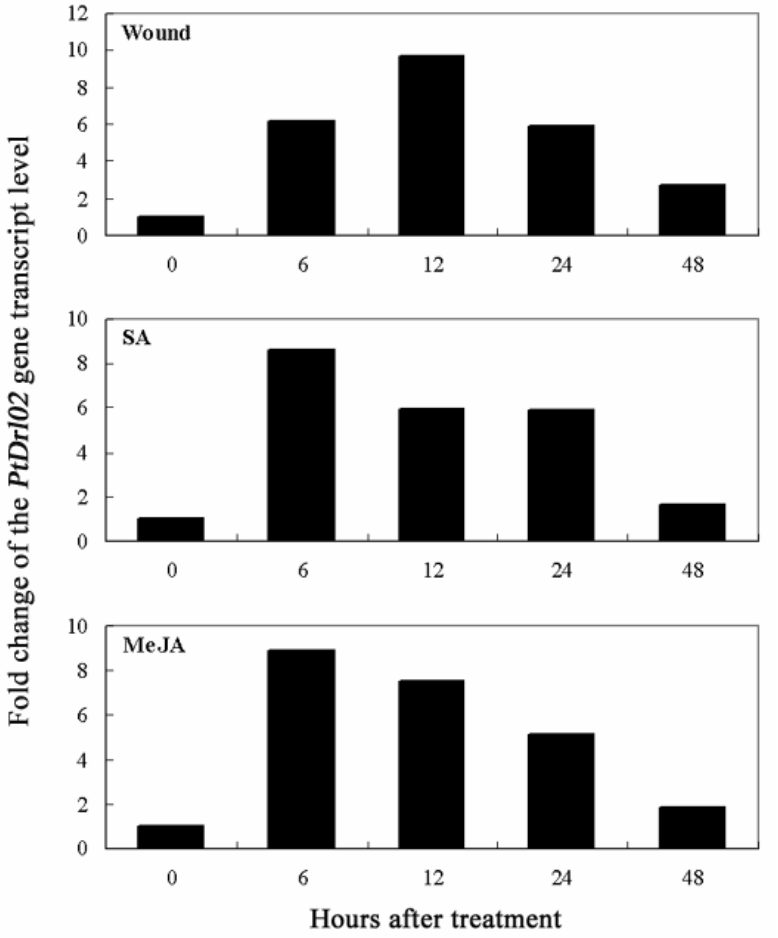
Basal expression pattern of the *PtDrl02* gene in 18-month-old triploid white poplar





Inducible expression pattern of the *PtDrl02* gene

4-month-old seedlings

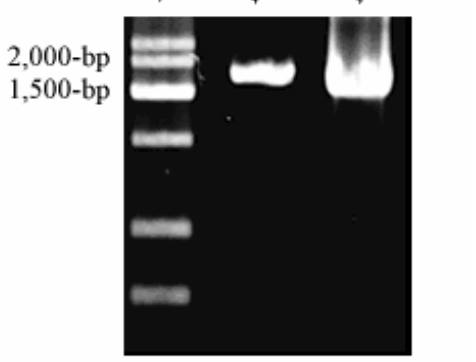


Time-course expression of the *PtDrl02* gene in response to defense-related signals

➢ Isolation and computer analysis of the *PtDrl02* gene promoter

Genome walking

5′ part of the *PtDrl02* gene



Sequencing of the primary PCR product

```
-985  CTAATACCTTCCCTTTACGCAACCAGTCTCCGTACCCGATCTCTAAAGACCAGTTAAGTTTCCTAGTAACTAGAAAACTA
-905  GGTGGCGACTCCCAGTCCATATTTTCACTTGTATAGACAAAAATTCCTTGTCTCTCCCTATTTGCCAGGAATAAATATTT
                HVAls
-825  CTGATTTTTCCTTGAGGGTGGACGATCGCCGCGCCGCCGCACACGTGCGACACCTAAAATTATTCAAAATATAGTATTAG
                                GC-rich   [box1/HVAls/G-box/ABRE-motif]
-745  AATTACATAATGCTACGTTTTAATATATAATTAAAGAGTCTCTACTGAACCAAATAATAAAATATTGTATAACTCTCTTT
                                                              A/T-rich
-665  TGGATGAATTGTTAACGGAGGAGGGACTGTTTTGGGTCCCTGTCTTGGCTGATTTTATTTGAATATATTATTTGATTAGT
                                              I-box
-585  CCATTTATAGGTTGACTCTACTATTCCTGCCTTGCATTGACAGTAAATTTCACTAGAAATTTCAAGTAATTTACACAGGA
                W-box                 W-box
-505  AATTTCCTGGCGATAAAGCTGGAATATGATTTTCAACTTTCGTAGGCGAGCAATAAAGTCCCAAACTTCAAGAATTTCTC
                I-box
-425  TTCTTTTCCTTTTGTATTTTCTCATTTACTTGGCATGCGCGCAGCTGCTTCATGAAAAAAACGTTTCAGATGCAGAAAAT
                P-box                                   GT-1 motif
-345  ATAGATAAATGTGGAAAGAGCGGGAAAAAATCCCAAATAAAGATATATTTTCGTCCCTTGGTATCGTGTGGATAATATTT
                I-box          GT-1 motif      3-AP1 binding site              I-box
-265  TAGAAAATCAATTCAACCGTACTCCAAAAACTCTCATACTCCACCACGAAAGTTACCAATAAGAATATGTTTGATACTCT
                                                              CAAT-box
-185  AAATTCCAGCTCAATAATTTATTCTTTGACAATTATTGCATTGCTAATAATTTGCAAGGAAACATACTTTCTAACCGATA
                                W-box
-105  CTCAATCGATCTGCACCTACCACGAAATTACCAATAAGAGTCTGTTTGATATAATTTGCAGGGCAGATGTGTTTGTCTAT
                                CAAT-box                                      TATA-box
 -25  ATACACATGAACTTCTGATTCGATTTGAACACCCCATTTCTCCGATTCAATAACTTCTGTTGCAAAACTTCTCTCTCCGA
                                  ▲
                                 (+1)
 +55  TTGCTGCAATTTCATAGCTAACTCTTTGTTATACAGAGGTATATATGGCTAACTCTTCTTTGCTTCTTTCTTGTTTTAAT
+135  TTCATGTCTTCTACCAATAACAATAATGTGAAAGCTTTGCTTGTTTCATGTTTAACTGTGGTTGCTGTTGTCTAATGCTC
+215  TCTCATTATTAAGATTAATTGCTGTATTATCTTCGATGATCACTTCACATGTATCAGCAATTGCTAATTGATTGTTCCTT
+295  AAATTCTGCAGCTGAATGGCAGAACCAGAGTCTTCTCGTTCTATACCAGAAGGGGACTATGATGTCTTCTTGAGCTTTAG
                M  A  E  P  E  S  S  R  S  I  P  E  G  D  Y  D  V  F  L  S  F  R
+375  AGGAGAAGATACTCGCAAGACGTTTACTGGTCATCTATATGCTGCCTTAGATGATGCAGGAATCCGCACTTTTCTAGATG
                G  E  D  T  R  K  T  F  T  G  H  L  Y  A  A  L  D  D  A  G  I  R  T  F  L  D
+455  ATAATGAACTTCCTAGAGGAGAAGAAATCTCCGAGCATCTTCTCAAGGCAATTCGAGAATCAAAGATATCCATAGTTGTC
                D  N  E  L  P  R  G  E  E  I  S  E  H  L  L  K  A  I  R  E  S  K  I  S  I  V  V
+535  TTCTCAAAAGGATATGCTTCTTCTAGATGGTGTCTCAATGAACTTGTAGAGATTCTAAAGTGCAAAAGGAAGAAAACCGG
                F  S  K  G  Y  A  S  S  R  W  C  L  N  E  L  V  E  I  L  K  C  K  R  K  K  T  G
+615  TCAGATTGTTCTTCCTATATTCTATGACATTGATCCTTCAGATGTGAGAAAACAGACTGGCTGTTTTGCAGAGGCATTTG
                Q  I  V  L  P  I  F  Y  D  I  D  P  S  D  V  R  K  Q  T  G  C  F  A  E  A  F
+695  ATAAGCATGAAGAGTGTTTTGAAGAGAAGTTGGTGAAGGAGTGGAGAAAAGC
                D  K  H  E  E  C  F  E  E  K  L  V  K  E  W  R  K
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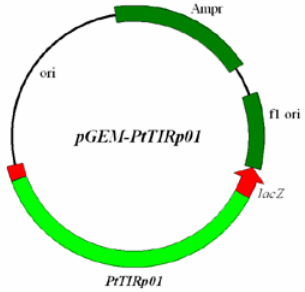
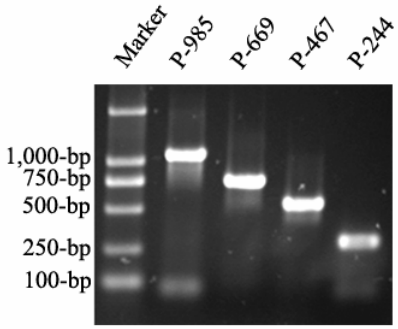
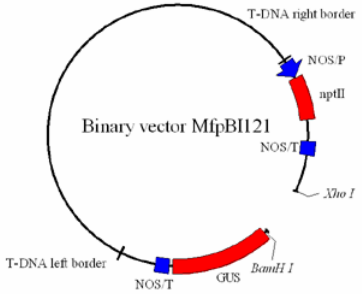
Zheng et al. *For.Stud.China*, 2007

Function analysis of the *PtDrl02* promoter

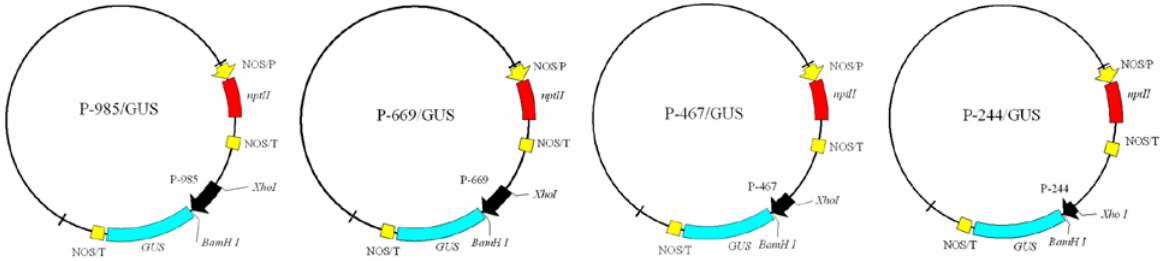


PCR

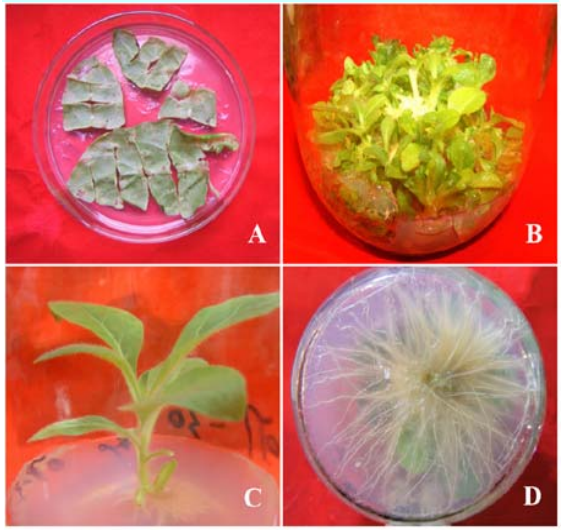




5′ - *Xho* I- P-985-*BamH* I - 3′
5′ - *Xho* I- P-669-*BamH* I - 3′
5′ - *Xho* I- P-467-*BamH* I - 3′
5′ - *Xho* I- P-244-*BamH* I - 3′

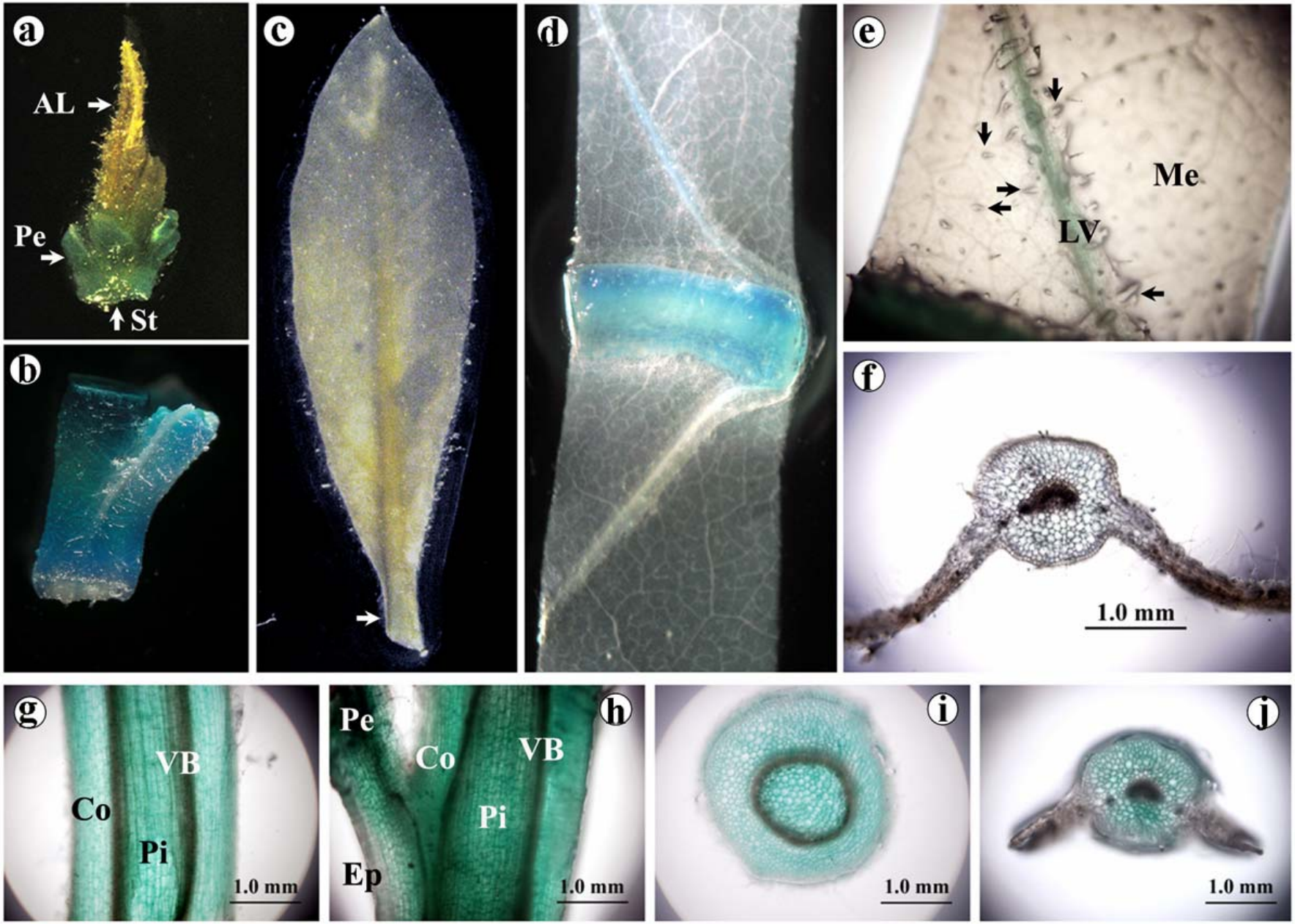


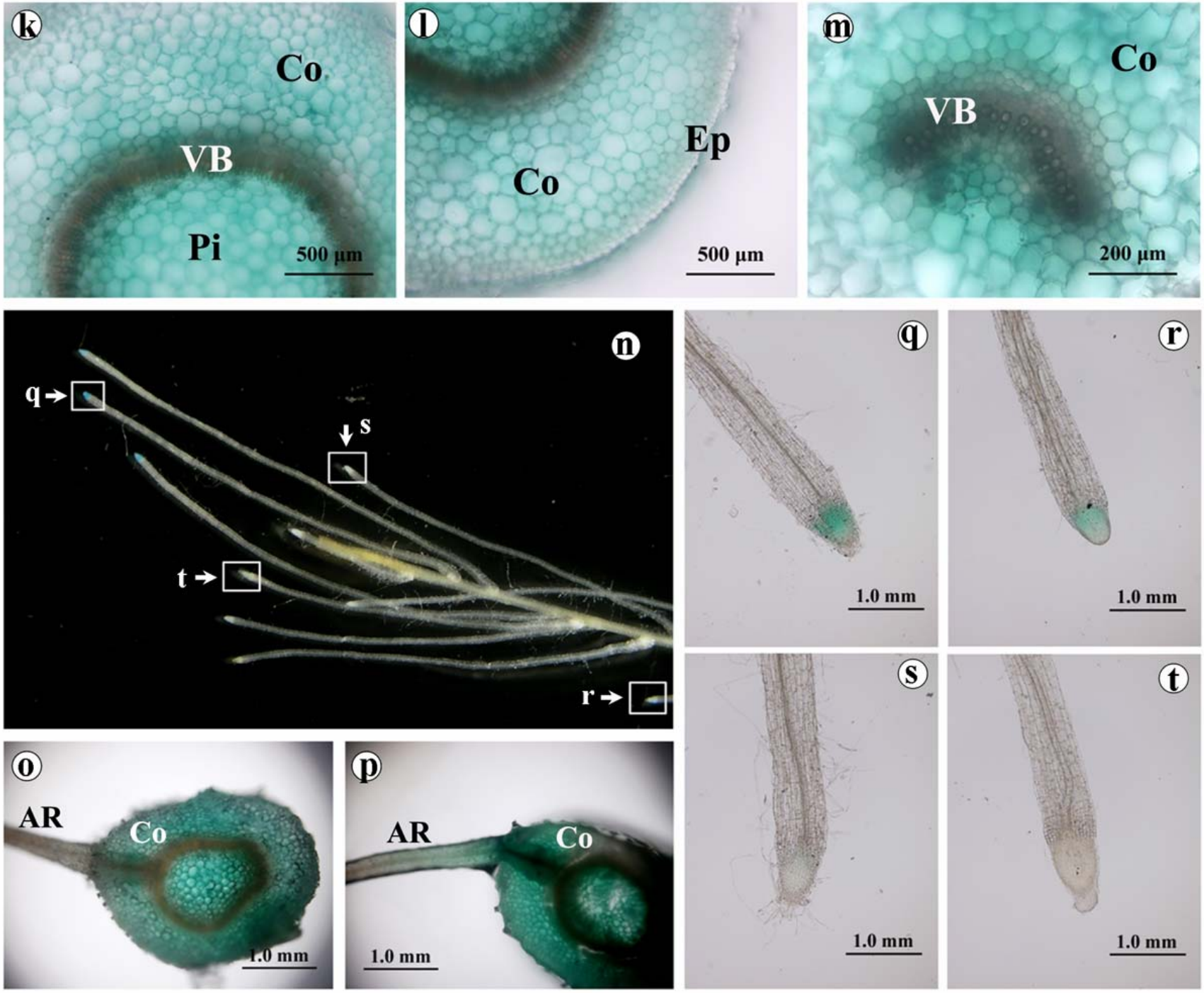
Tobacco transformation



Transformant	Transgenic lines (n)	GUS staining
P-985	61	High
P-669	50	Medium
P-467	45	Low
P-244	50	Low
CaMV 35S	60	Strong

- **Tissue-specific expression pattern of the *PtDrl02* promoter**




k
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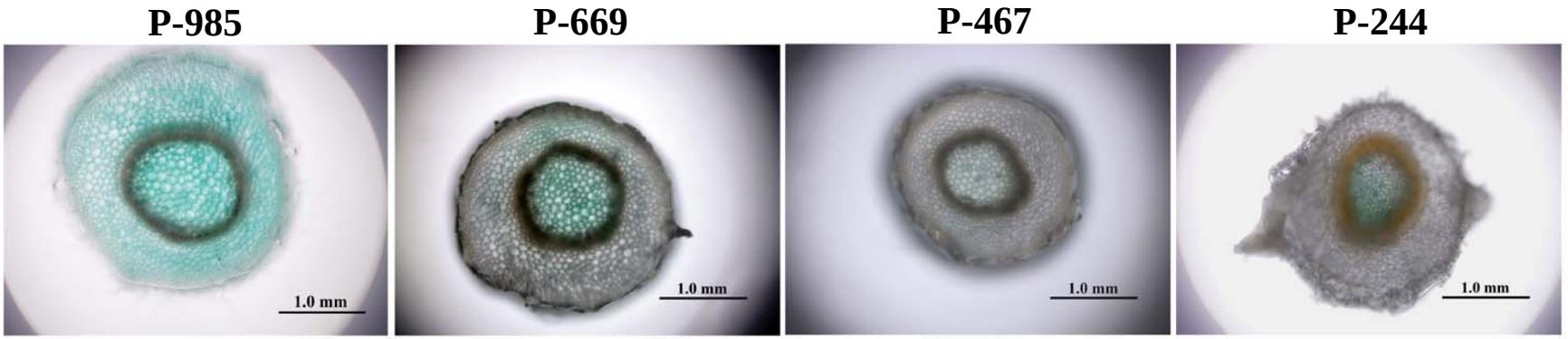
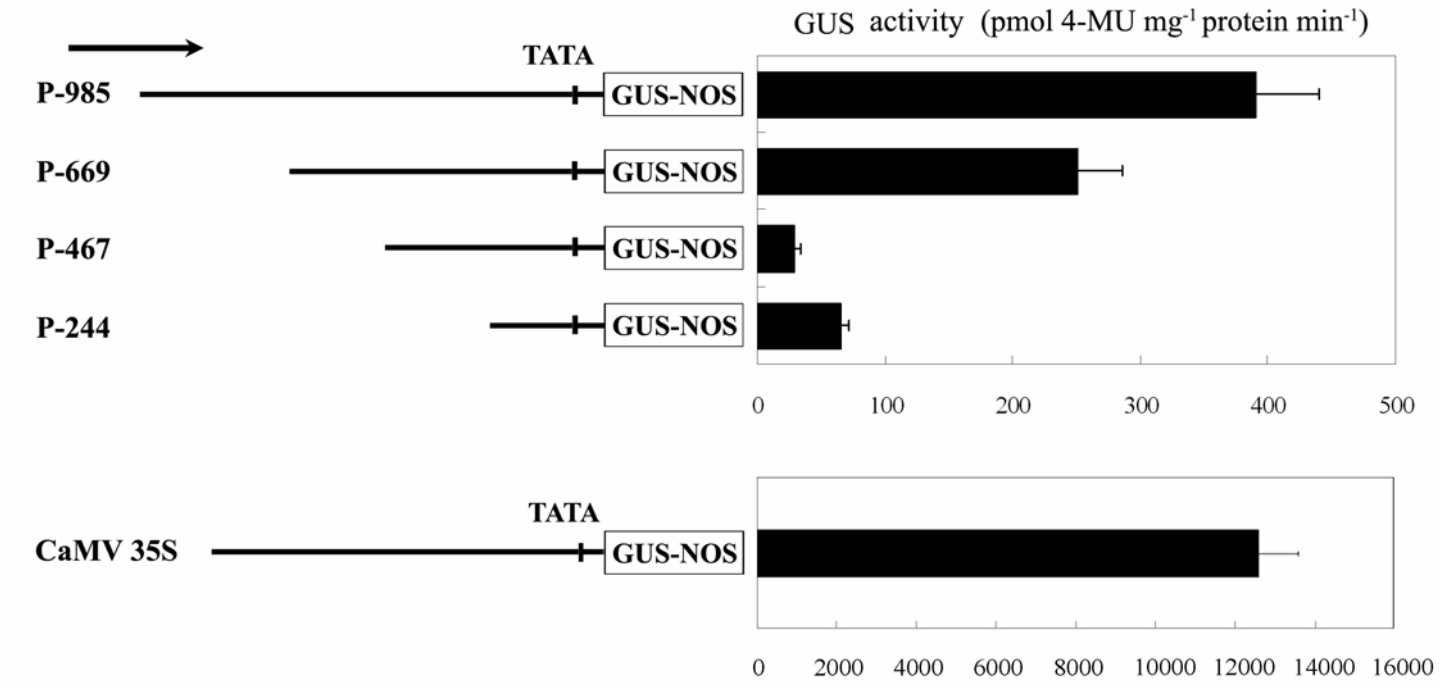
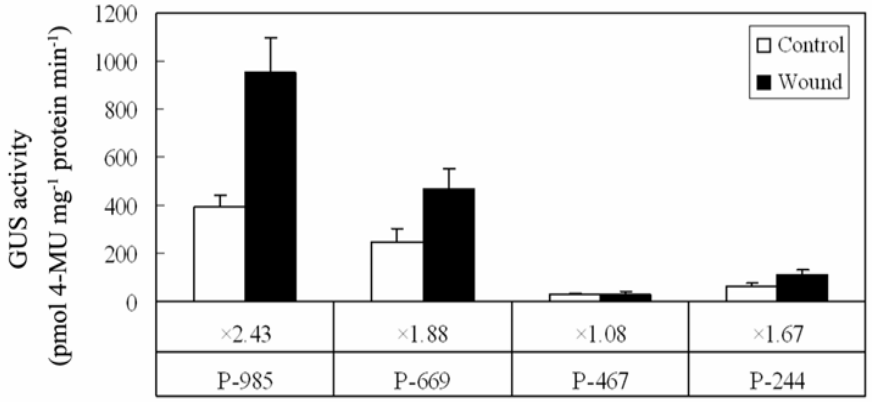
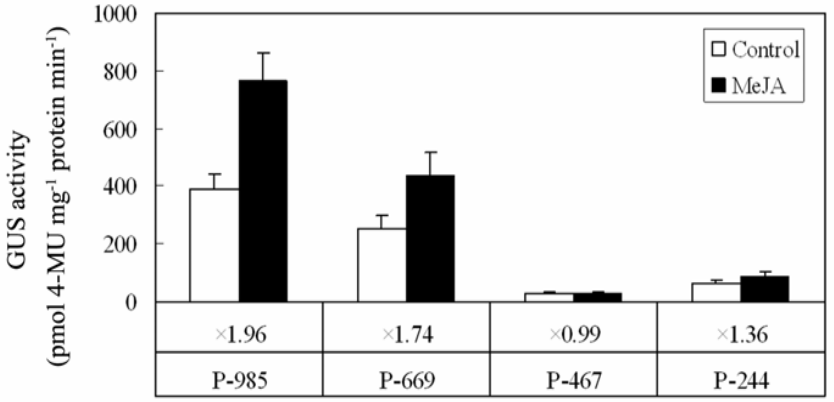
• Deletion analysis of the *PtDrl02* promoter

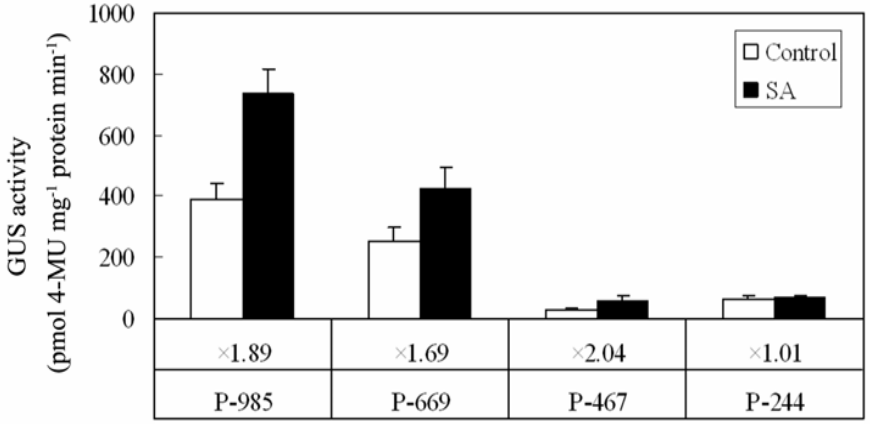


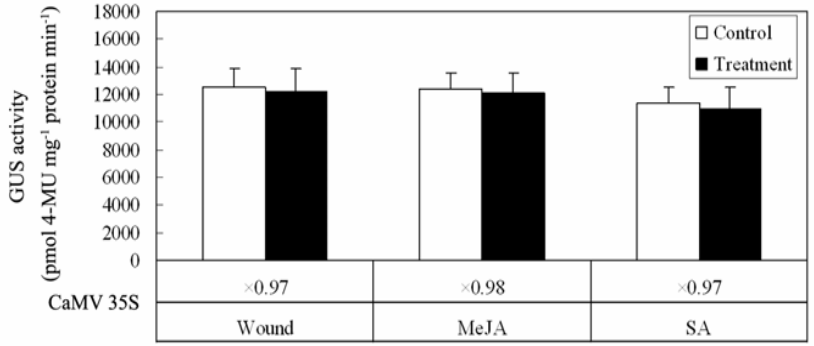


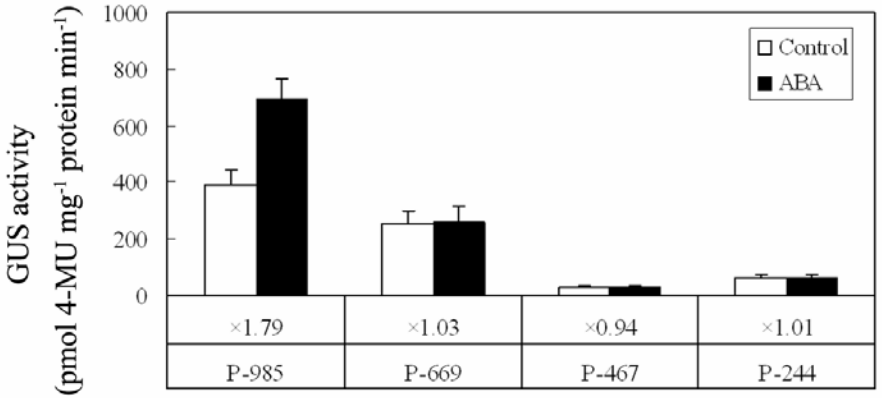
• Activation of the *PtDrl02* promoter

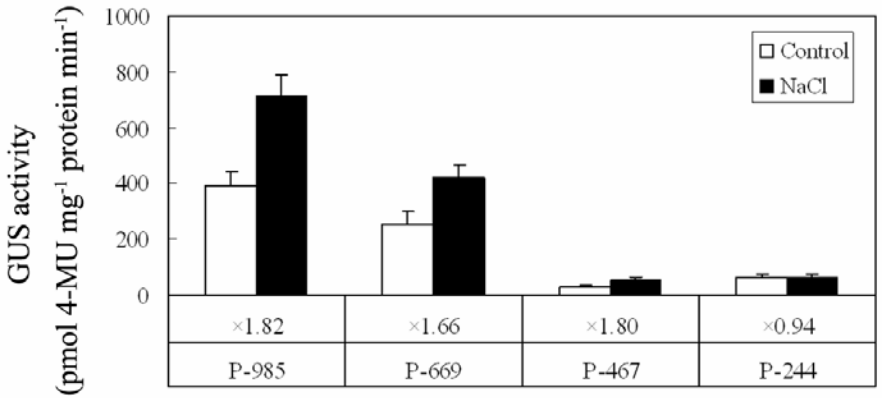


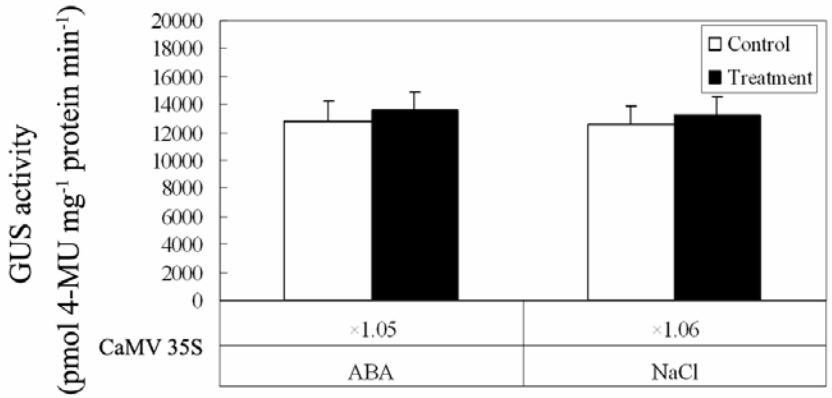








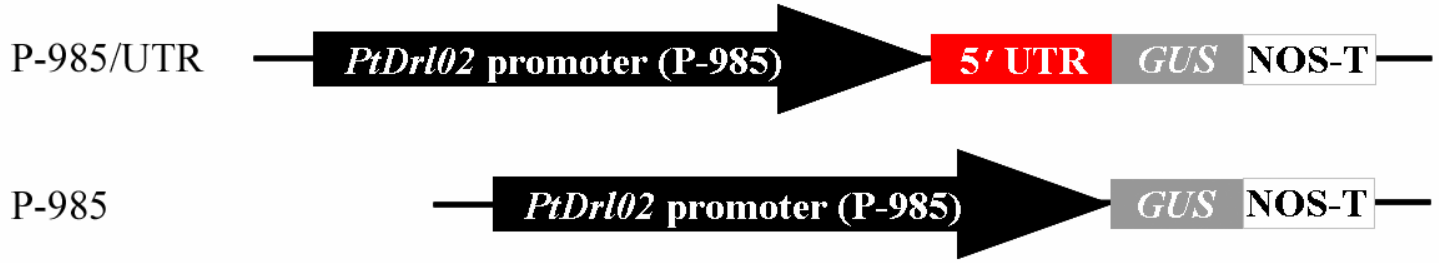


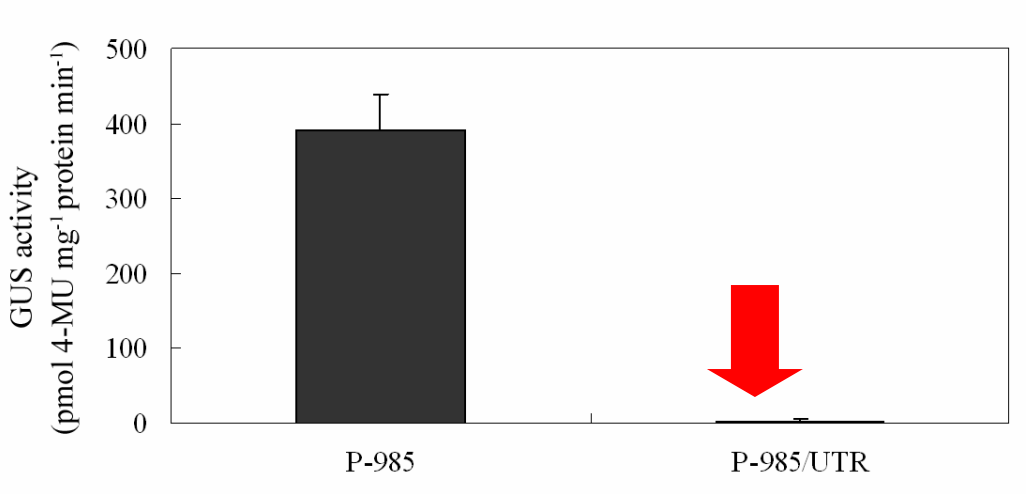


Brief summary

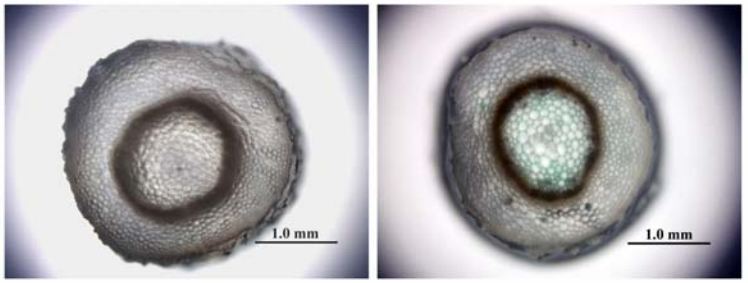
Regulatory region	Inducers	Potential *cis*-elements
-985/-669	ABA	ABRE-motif
-669/-467	Wound, MeJA	W-box
-467/-244	SA, NaCl	GT-1-motif
-244/0	Wound, MeJA	W-box

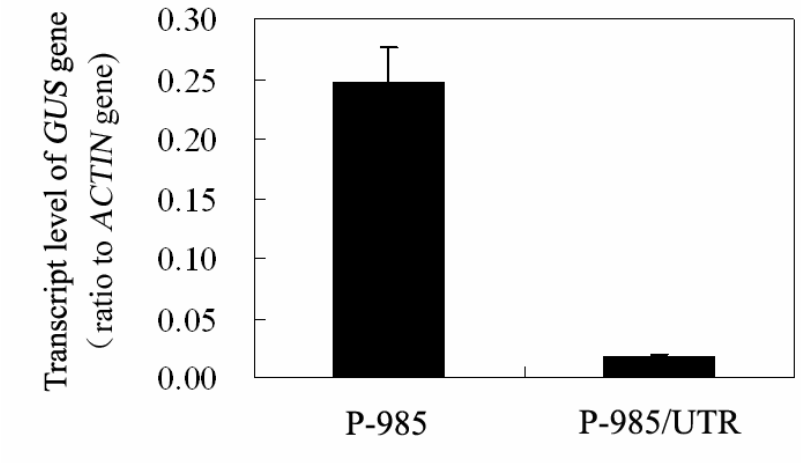
• The *PtDrl02* promoter activity is affected by its 5′ UTR

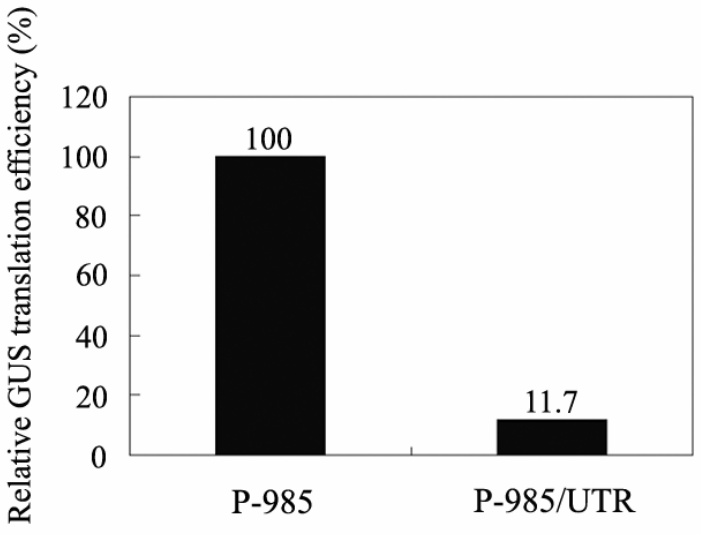




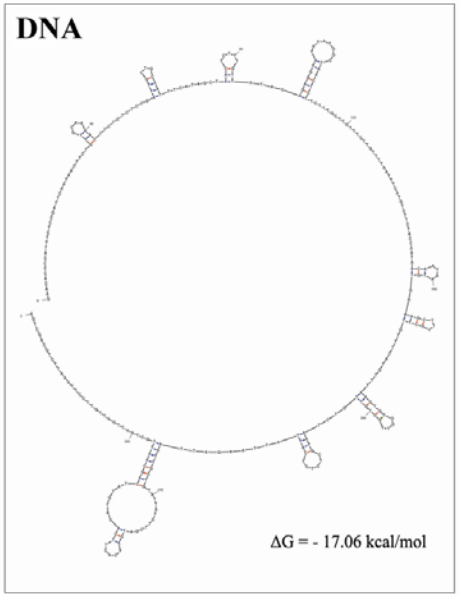
GUS staining of P-985/UTR

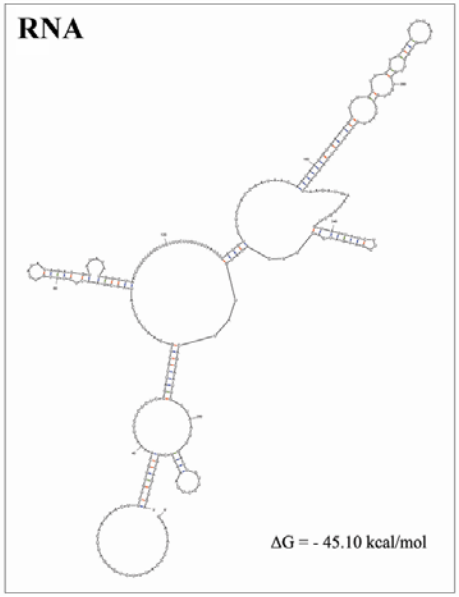






Predicted secondary structure of the *PtDrl02* 5′ UTR





Future work

- Analysis of the molecular interaction between *PtDrl02* promoter and its candidate transactivator PtWRKY1
- Examination of the orientation of *PtDrl02* promoter/cis-acting regulatory elements
- Testing of the *PtDrl02* promoter activity in the transgenic poplar (*P. tomentosa*) plants

Acknowledgement

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