ABSTRACT: 277

TRANSFORMATION OF HRPZPSTA GENE TO IMPROVE SOYBEAN DISEASE RESISTANCE

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In the long process of evolution, phytopathogenic bacteria could infect plants. Some non-host plants and resistant host plants can resist infection by a hypersensitive reaction. Harpin protein coded by hrp genes was thought to be an elicitor to elicit a hypersensitive reaction in non-host plants so the plants can obtain a broad-spectrum resistance to phytopathogenic bacteria. *Phytophthora* root rot is a major disease which can cause soybean seedling lodging, root and stem decay of mature plants. In order to improve the soybean's ability of resistance to the pathogen, the hrpZpsta gene was cloned and transformed into soybean via *Agrobacterium* mediation and the pollen tube pathway.

The hrpZpsta gene with a size of 423 bps was amplified from the genome of *Pseudomonas syringae* pv. *tabacand*. The betaine-aldehyde dehydrogenase gene (badh) was used as a selected marker gene to construct the safety recombinant plant expression vector of pCAMBIAhrpZpsta. The hrpZpsta gene was transformed into soybean recipients of Jinong 17, Jinong 28, Jilin 30, and DE2259 via *Agrobacterium*-mediated transformation and the pollen tube pathway. Transgenic plants were screened with 200 mmol/L NaCl in the phase of shoot induction, and detected by PCR, RT-PCR and Southern Blot in T1, T2 and T3 generations. The transformation efficiency was 2.5% for *Agrobacterium*- mediation and 3.9% for the pollen tube pathway, respectively.

Detecting transgenic plants by PCR, we found 138 positive transgenic plant rows in the T1 generation, including 30 and 20 rows from Jilin 30 and DE2259, respectively via *Agrobacterium*-mediation, and 36, 32 and 20 rows from the pollen tube pathway from Jilin 30, DE2259 and Jinong 28, respectively. We found 210 plant rows in the T2 generation, including 40 and 32 rows from Jilin 30 and DE2259, respectively via *Agrobacterium*-mediation; and 70, 38 and 30 rows from Jilin 30, DE2259 and Jinong 28, respectively via the pollen tube pathway. The results of Southern Blot and RT-PCR show that the hrpZpsta gene had integrated into the soybean recipient genome and was expressed. In resistance identification, several promising lines with high resistance to *Phytophthora* root rot have been selected.

Transformation of HrpZpsta Gene to Improve Soybean Disease Resistance

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1. Introduction

Soybean disease is the major restriction for yield and quality improvement in northeast China soybean production.

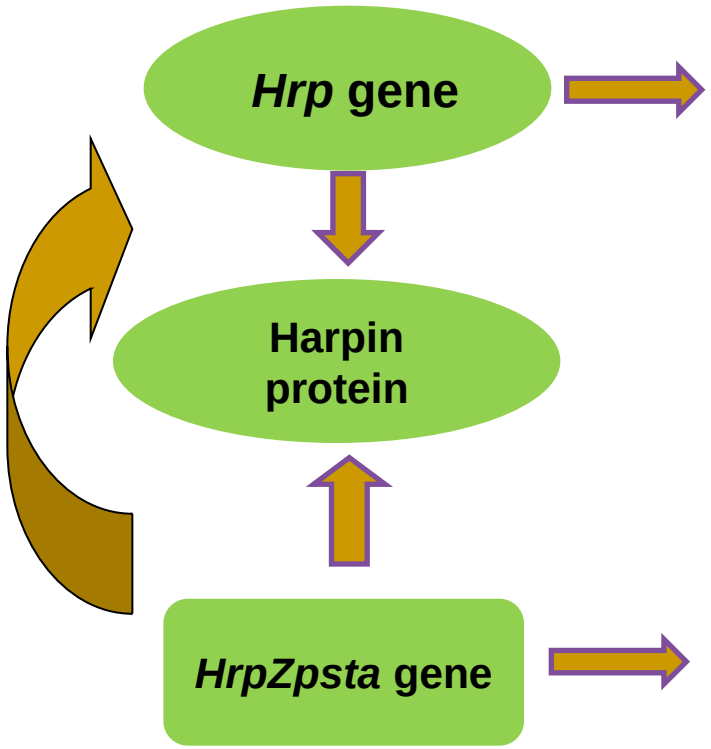
The damage of Phytophthora root rot:

- occur in whole growth period, the pathogenic bacteria can infect soybean root, stem, leaf and pod.
- Lead to suddenly lodging for seeding
- Decay for roots and stems of grown-up plant, then wilt and die
- Decrease the content of seed protein and yield loses as high as 25% ~ 50%.

The damage of Cercospora sojina Hara

- Infect plant leaf, stem, pod and seed
- Lead to serious losses for yield and quality.
- The yield lose is about 5% ~ 10% in generally and 30% ~ 50% in seriously.
- Caused decreases of 100 seed weight, protein and oil content.

In the long process of evolution, phytopathogenic bacteria could infect plants. Some of non-host plants and resistant host plants can resist the infection by hypersensitive reaction(HR). Harpin protein coded by hrp genes was thought to be an elicitor to elicit hypersensitive reaction in non-host plants. So the plants can obtain broad-spectrum resistance to phytopathogenic bacteria.



1.Be comprised of several transcription units, and exist in cluster

2.The molecular weight is around 25~30k Da, for 6~8 transcription units

3. Exist in phytopathogen broadly

4.Be related to HR reaction of induced host

1.One of the members in Hrp gene family

2.Identified in tobacco wildfire pathogens

3.Be able to encode the corresponding Harpin protein

4.Be able to induce HR reaction in host

Objective

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- **To clone the *hrpZpsta* gene and construct plant expression vector**
- **To transfer the expression vector into recipient soybean**
- **To investigate the resistance performance of transformed soybean lines.**
- **To select transformed resistance lines**

2. Methods

- The *hrpZpsta* gene was cloned by PCR

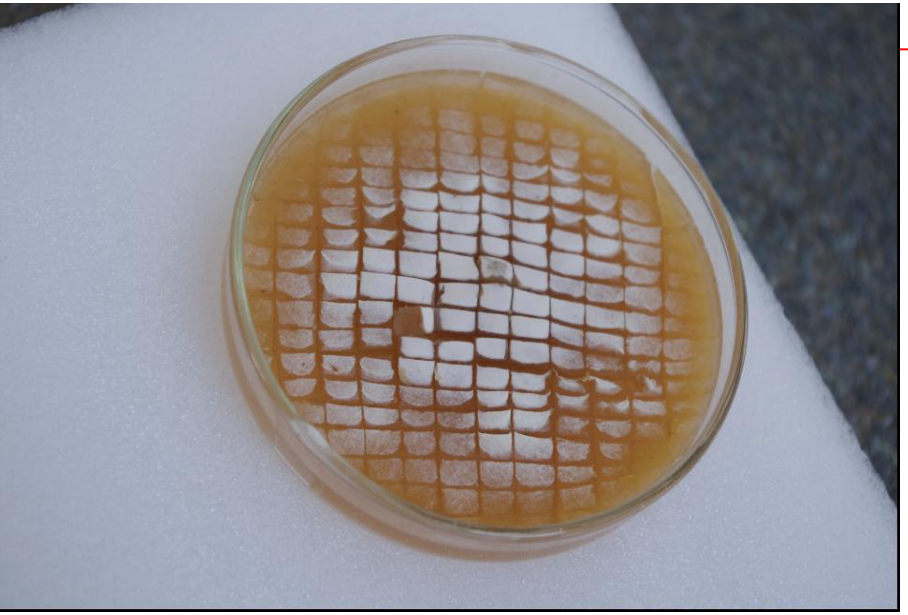
- The *hrpZpsta* gene was transformed into recipient soybean via Agrobacterium mediation and pollen tube pathway.

- Detection of Phytophthora root rot

Inoculation temperature: 24-25℃, humidity: >80% ,

5 days after inoculation to investigate disease index.

Disease index	**Resistance level**
0	immune (IM)
≤2.0	high resistant (HR)
2.1 ~ 15.0	resistant (R)
15.1 ~ 40.0	moderately resistant (MR)
40.1 ~ 60.0	moderately susceptible (MS)
60.1 ~ 80.0	susceptible (S)
≥80.1	high susceptible (HS)



▫ Detection of Cercospora sojina Hara

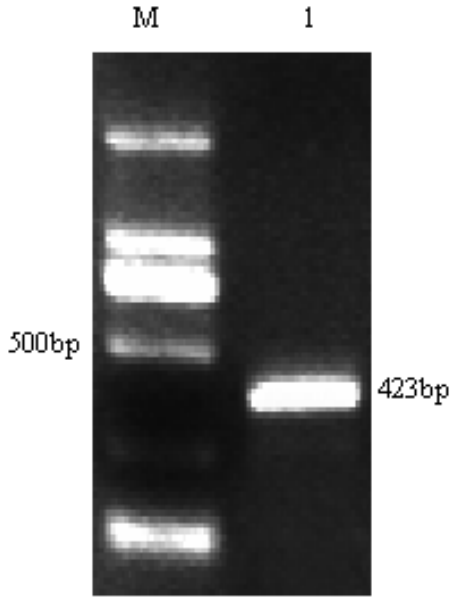
Inoculation: to spray spore liquid on blade surface;
Inoculation temperature: 24-25℃, humidity: >80% ,
15 days after inoculation to investigate disease index.

Disease index	Resistan level
0	immune (IM)
≤2.0	high resistant (HR)
2.1 ~ 15.0	resistant (R)
15.1 ~ 40.0	moderately resistant (MR)
40.1 ~ 60.0	moderately susceptible (MS)
60.1 ~ 80.0	susceptible (S)
≥80.1	high susceptible (HS)

3. Results

3.1 The cloning of *hrpZpsta* gene and construction of plant expression vector

choosed genome DNA of tobacco wildfire pathogen as templater, fragment of 423 base-pair had been amplified by PCR reaction



PCR of hrpZpsta gene
M ： DNA Marker 1： PCR product

The complete DNA sequence of hrpZpsta gene is:

atgcagagtctcagtcttaacagcagcacgctgcaaagcccggcg
atggcgctcgttctgatccgtcctgaaaccgagacaaccgggccga
gtacatccagccgggcgcttcaggaagtgattgcgcagcttgccca
ggagctgactcacaatggtcaactggacgagagttcgccattgggc
aagttgctcggcaaagcgatggccgccagtggcaaggctggcggc
ggcctcgaggacatcaaggctgcgctggacacgcttatccacgaa
aagctgggcgacaattttggcgcttctgccgacaacgcctcggatac
cggacaacccgacttgatgacacaggtgctcaatggcctggccaa
gtcgatgctgaatgatcttctgacccggccccgccagcaacagcaa
ttccaatggtga

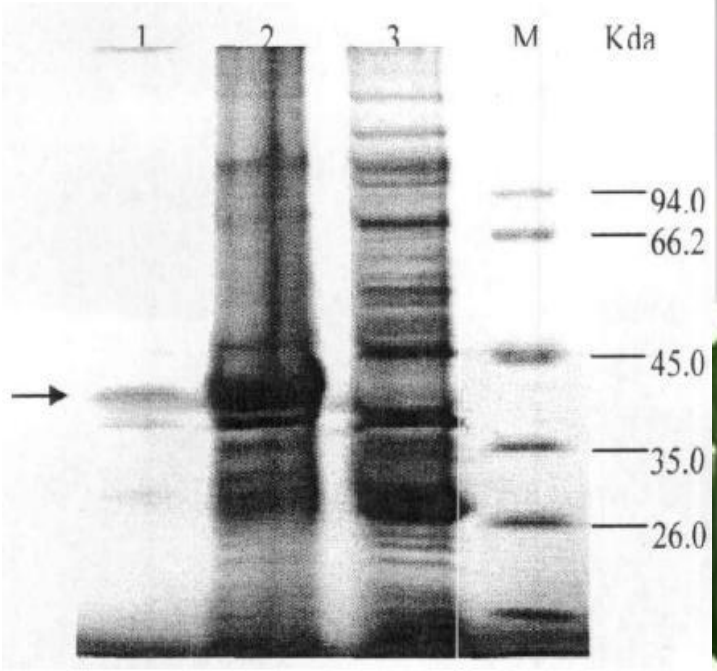
The deduced amino acid sequences is:

MQSLSLNSSMLQSPAMALVLIRPETETTGPSTS
SRALQEVIAQLAQELTHNGQLDESSPLGKLLGK
AMAASGKAGGGLEDIKAALDTLIHEKLGDNFGA
SADNASDTGQPDLMTQVLNGLAKSMLNDLLTR
PRQQQQFQW

Detection of hrpZpsta ability of inducing plant hyper-sensitive reaction with leaf injection

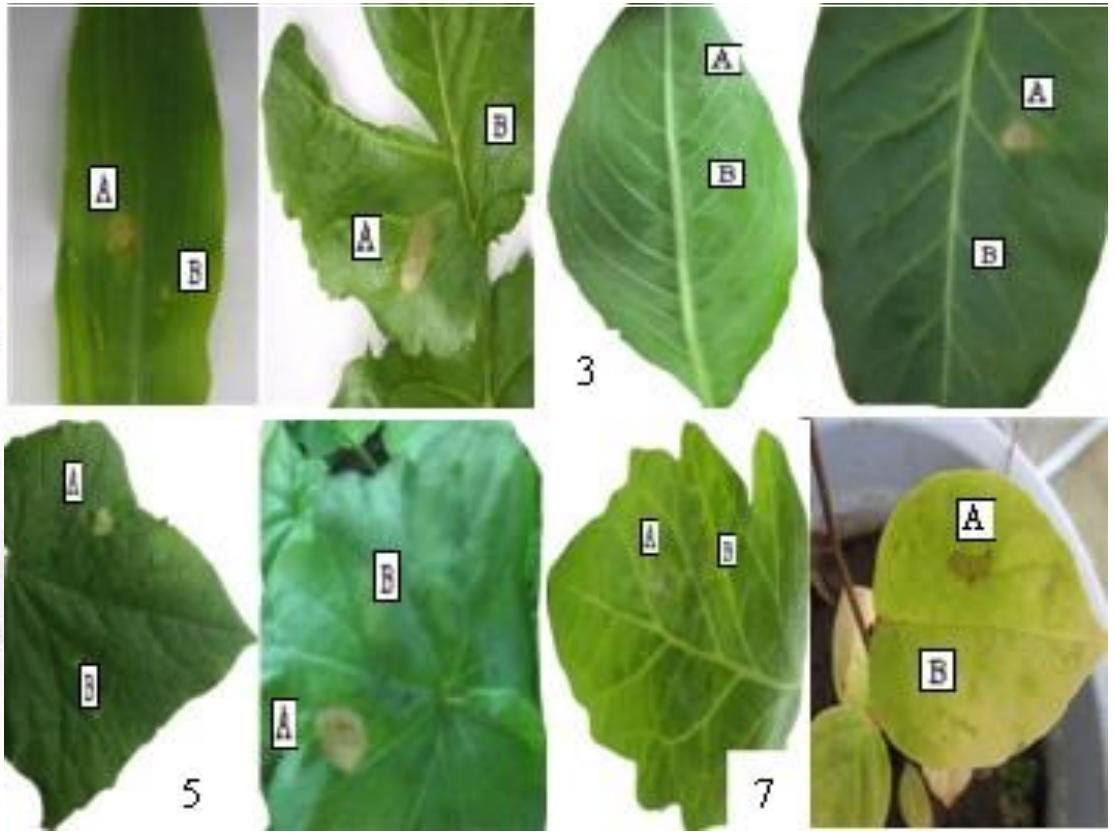
After 12 hours, atrophy initially occured at the injuction part , After 24 hours, hollow performance initially emerged, after 48 hours, wither blot with semi-transparent traits appeared initially, non-performance existed in control.

SDS-PAGE shows , target protein efficiently expressed in the recombinant strain with molecular weight 41.2kD.



SDS-PAGE

M ： DNA Marker
1:supernatant; 2：precipitate
3：CK

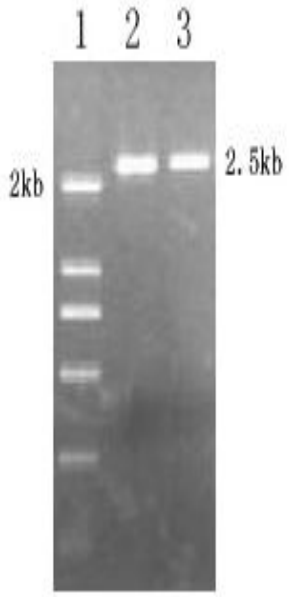


Hypersensitive reaction induced by *hrpZpsta* in plant

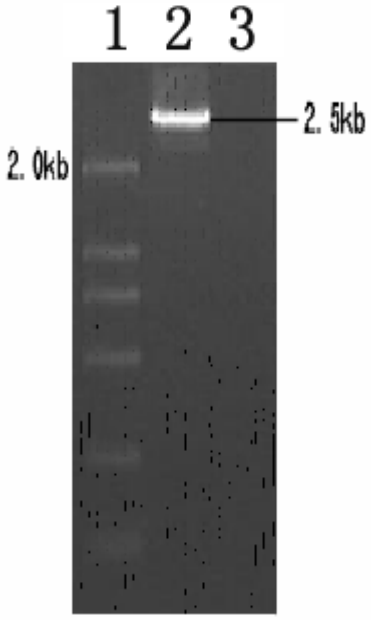
1.corn ; 2.radish ; 3.evening primrose ; 4.tobacco ; 5.cucumber ; 6.geraniu
7.tomato ; 8.Schisandra ; A.with *hrpZpsta* ; B.CK

3.2 Cloning of specific promoters

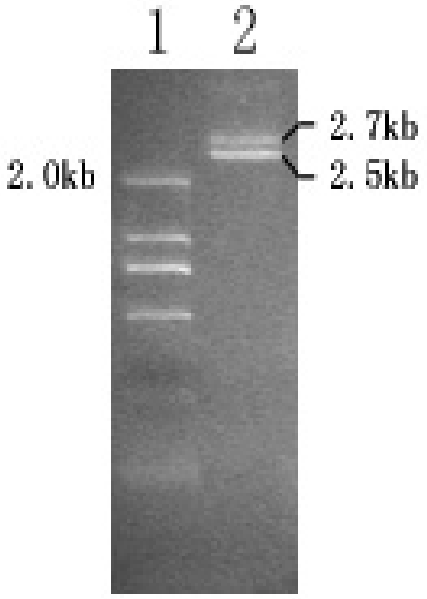
Cloning of the root specific promoter RSP



PCR amplification of RSP
1:DNA Marker
2、3 ： PCR product



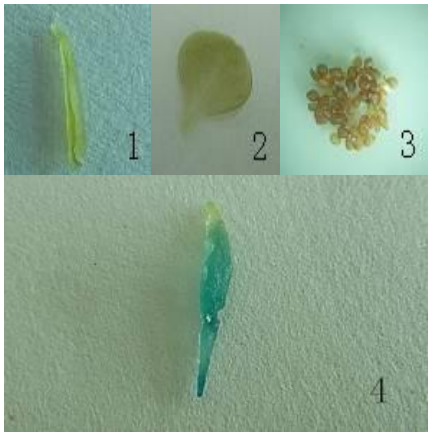
Identification of PCR product
pMD18-T-RSP
1:DNA Marker 2 :PCR product
3 ：water control



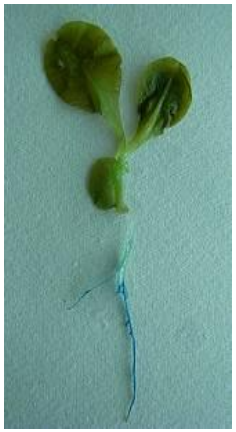
Restriction identification of pMD18-T-RSP
1: DNA Marker
2:pMD18-T-RSP digested by *Pst*I and *Nco*I

There are many kinds of general promoter elements like TATA box, CAAT box and primary regulatory elements of root specific expression in RSP.

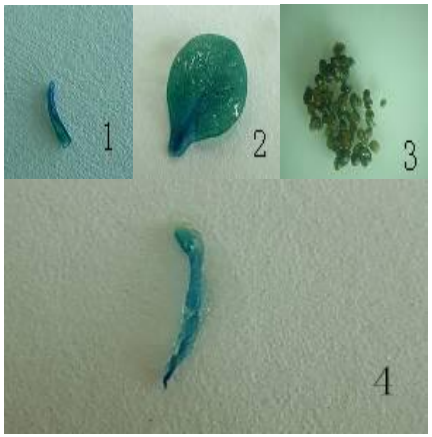
Detection of GUS histochemical staining:



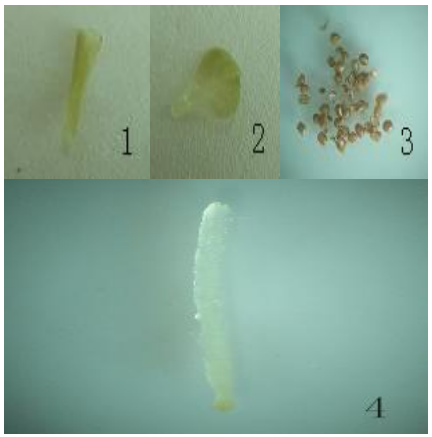
A



B



C



D

GUS histochemical staining result of transgentic tobacco

A GUS staining result of transgentic tobacco with pRSP-*GUS* *in different organizations*

B GUS staining result of transgentic tobacco with pRSP-*GUS* *in the whole plant*

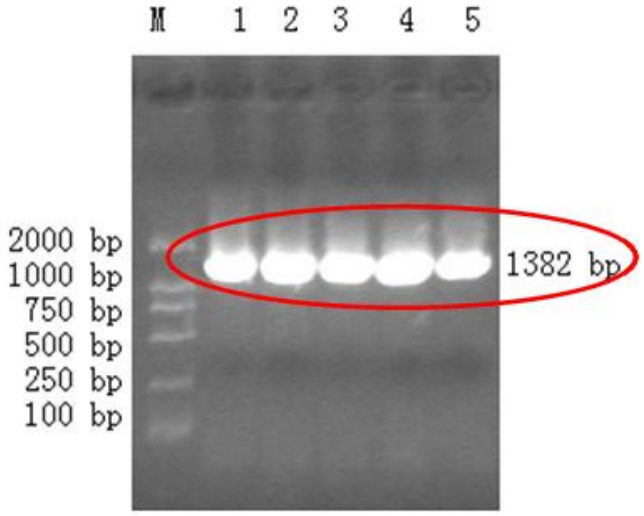
C GUS staining result of transgentic tobacco with pBI121 *in different organizations*

D GUS staining result of non-transgentic tobacco in different organizations

1：detection of GUS activity in stem；2：detection of GUS activity in leaf；

3：detection of GUS activity in seed；4：detection of GUS activity in root

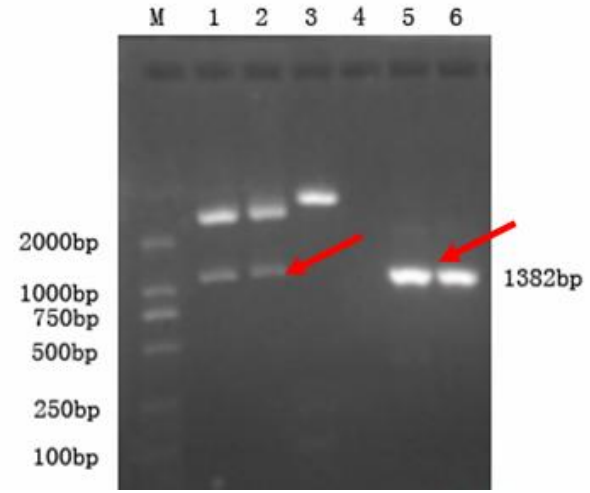
Cloning of the seed specific promoter *7αP*



Electrophoretic analysis of PCR product

M：DNA Marker

1-5：PCR product of target fragment



Restriction enzyme and PCR analysis of recombinant vector

M：DNA Marker ；

1-2: Restriction enzyme digestion product digested by *BamHI* and *HindIII;*

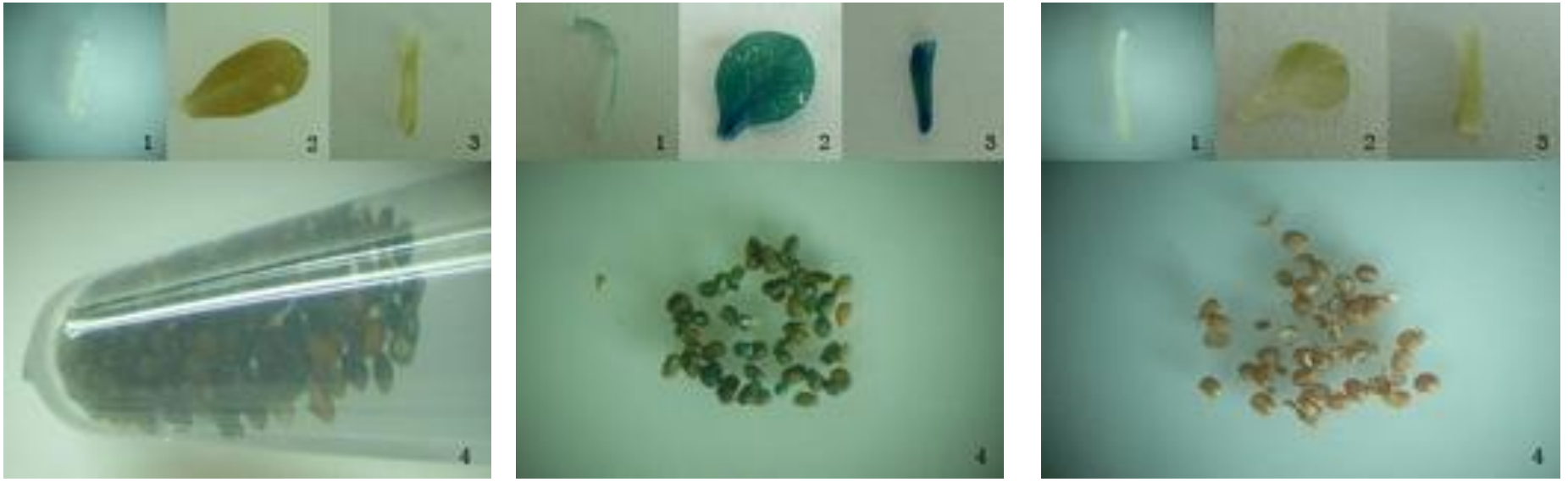
3：the plasmid of pMD18-T-7αP;

4：negative control; 5-6：PCR product


1 tgcttggatt tggaccagac ttgaatttta atttaatgat attataatat gtgaatatat
61 ttttgagaca attgtaaatt tcagataaaa aaataatgta attaaaattg taataactat
SEF4-motif E-box
121 atcgtatact taattaatta ttaaatgtga caaaaaagat atacatcaaa acttaatgtt
181 tcatgacttt ttttttaatg tgtgtcctaa atagaaatta aaaataaaaa ttattatatc
W-box
241 caaatgaaaa aaacatttaa tacgtattat ttaagaaata acaatatatt tatattttaa
GT-1-motif AACA ACGT SEF1-motif
301 tatgtattca catgtaaatt taaaaacaaa aacaaaattt ctcttttatt gattaattaa
361 aataatttta taactacatt tattttctat tattatcaat tttcttctgt ttttttattt
421 ggcatatata cctagacaag tcaaaaaatg actattcttt aataatcaat cattatcctt
481 acatattgtt cgaactacga gttatgaagt gtcaattgca ccttagtgtt ttgataggcc
541 tccatttgcc gctcattaat tagtttgata acagccgtac cgatcaatta cttatgcttc
601 ttccatcgta attatatgca tgtcggttct tttaatcttg gtactctcga atgccaccac
661 aacactgact agtctcttgg atcatgagaa aaagccaaag aacaaaaaag acaacataaa
721 gagtatcctt tgcaaaaaat gtctaagttc ataaaataca aacaaaaacg caatcacaca
GCN4 (CA)n
781 cagtggaccc aaaagccatg cacaacaaca cgtactcacc aaggtgcaat cgtgctgccc
RY
841 aaaaacattc accaactcaa tccatgatga gcccacacat ttgttgtttg taaccaaatc
901 tcaaacgcgg tgttctcttt gggaagcaac catatcagca tatcacacta tctagtctct
961 tggatcatgc atgcgcaacc aaaagacaac acataaagta tcctttcgaa agcaatgtcc
1021 aagtccatca aataaaattg agacaaaatg caacctcacc ccacttcact atccatggct
BoxIV
1081 gatcaagatc gccgcgtcca tgtaggtcta aatgccgtgc acatcaacac gtactcaaca
BoxIII BoxII
1141 tgcagcccaa attgctcacc atcgctcaac acatttcttg ttaatttcta agtacactgc
1201 ctatgcgact ctaactcgat cacaaccatc ttccgtcaca tcaatttttgt tcaattcaac
BoxI CAAT box
1261 acccgtcaac ttgcatgcca cccatgcatt gcaagttaac aagagctata tctcttctat
DPBF
1321 gactataaat acccgcaatc tcggtccagg ttttcatcat cgagaactag ttcaatatcc
TATA box +1 transcription start site
1381 ta

Detection of GUS histochemical staining:

A B C



The GUS histochemical staining results of transgentic tobacco

A. GUS staining result of transgentic tobacco with p7αP-GUS in different organizations

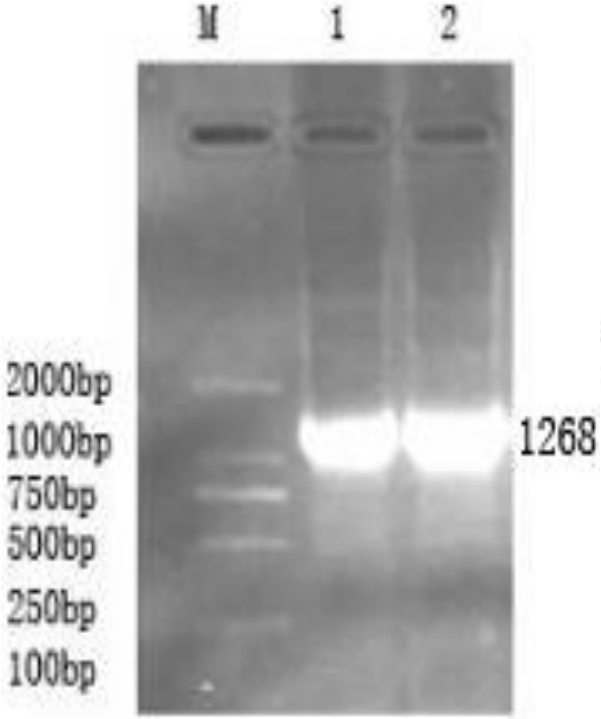
B. GUS staining result of transgentic tobacco with pBI121 in different organizations

C. GUS staining result of non-transgentic tobacco in different organizations

1.detection of GUS activity in root；2.detection of GUS activity in leaf；

3.detection of GUS activity in stem；4.detection of GUS activity in seed

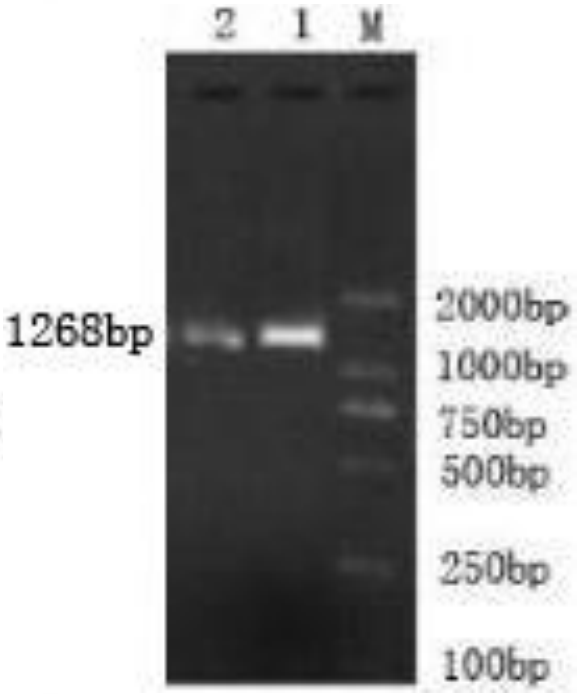
Cloning of the testa specific promoter scsp



Electrophoretic analysis of PCR product

M：DNA Marker

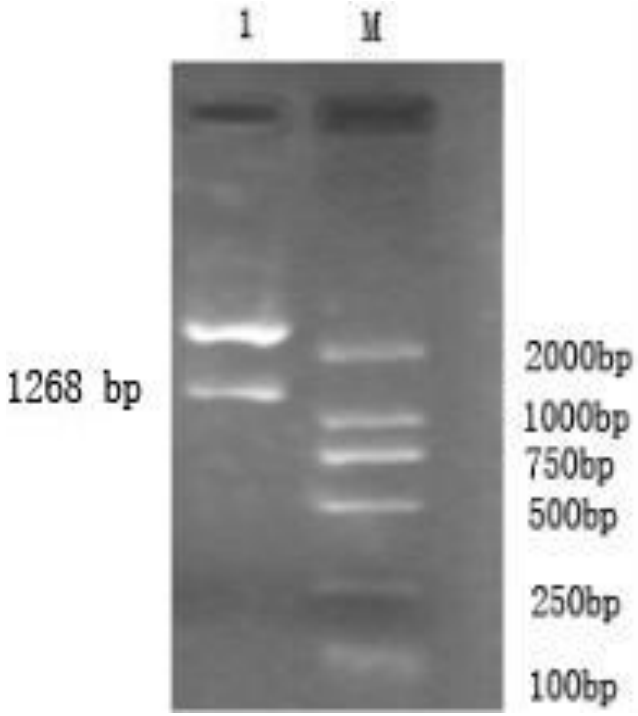
1-2：PCR product of target fragment



Electrophoretic analysis of pMD18-T-SCSP PCR product

M: DNA Marker

1-2: PCR product



Restriction identification of pMD18-T-SCSP

M: DNA Marker

1: product digested by *BamHI* and *HindIII*


1 tctttgtaat ctcacctttt tcatttaaat acatttctac tttttaagtt ctatattttc
61 tctcaatttt cttcgataaa ccatgaaatt taacatggta tatcagcgat accacccact
CAATBOX1 GATABOX S1FBOXSORPS1L21 CACTFTPPCA
121 ttgaaaagcca tgtatggcta gtatgggcag ccaaaatttg ccctggttca agcaaaagcaa
DOFCOREZM EBOXBNNAPA
181 gtgtttatat agatgtgact tttgttgagg aactcatgcc aatggtactg attgtgaaac
ARR1AT
241 tgagaaaact aatttggaga atttgaatta tgatcattaa atactcctct cctgactacc
POLLEN1LELAT52 W-BOX
301 ttcgtccctc aaatttgtac catcattatt tcccaaaaat ttgattacaa tgcactaatt
CURECORECR TATABOX5 CARGCW8GAT
361 aatgaatgtt tcttacatta tcatatttatc atatctgaca ttttgttttt actttttata
SEF4MOTIFGM7S
421 ataattattt taaaaagtca tacatgcaaa taatttttta atagtttaca gttaaatttt
RY
481 tacagtaaaa atgcatgaaa attaaactt tatttttccaa gtcatcattt agtcaaatcc
541 caaaacaatg attatttttt gcaaatgaat gtttattgaa catttaaatg tagcctaatt
AACA E-BOX L1-BOX
601 aattctggtt atggtgtcaa tgttccaaaa cctaatgcaa gatcttagca agtacataca
661 tagatctaat tttaaactta tctttacgca agagatatta agattataca tctagtttta
GATA-BOX
721 aacattaact tttgttttttg tgttaaaaaaa cagtaacatt ttcttaattt tgtagagtga
781 cgtgctccaa ccatattaac gaagattta attggtattc aagttcatga acttagtaaa
841 taagttttgg tcttcagttt tcaattttca ttacaacatt tatgtaaaat atcaacgttt
ACGTTBOX
901 tctgaaattt gttgcttgtg tgctccaacc acatttaaga gatttatagaa attaatttc
961 aagaagataa tgattcctac tcttgctggc cctaccatag tacaataaat ccactcataa
1021 atcaacaagt cgtcgtcata ggcaattggg catcatatca taaacaatac gtacgtgata
1081 ttatctagtg tctctcagtt tactttatga gaaattattt ttctttaaaa aaagttaatt
ARFAT
1141 aataaaaaca tttgcgatac cgtgagttac aagaaatccg ccgaattcat ctctataaat
TATABOX2
1201 aaaaggatct atatgagagg taaaatcata ttaactcaaa atgggttcca tgcgtctatt
POLASIG1
1261 agtagtgg

Detection of GUS histochemical staining:

GUS staining result of non-transgentic tobacco in different organizations

1.detection of GUS activity in stem
2.detection of GUS activity in leaf；
3.detection of GUS activity in root；
4.detection of GUS activity in flower

GUS staining result of transgentic tobacco with pSCSP-*GUS*

A B C

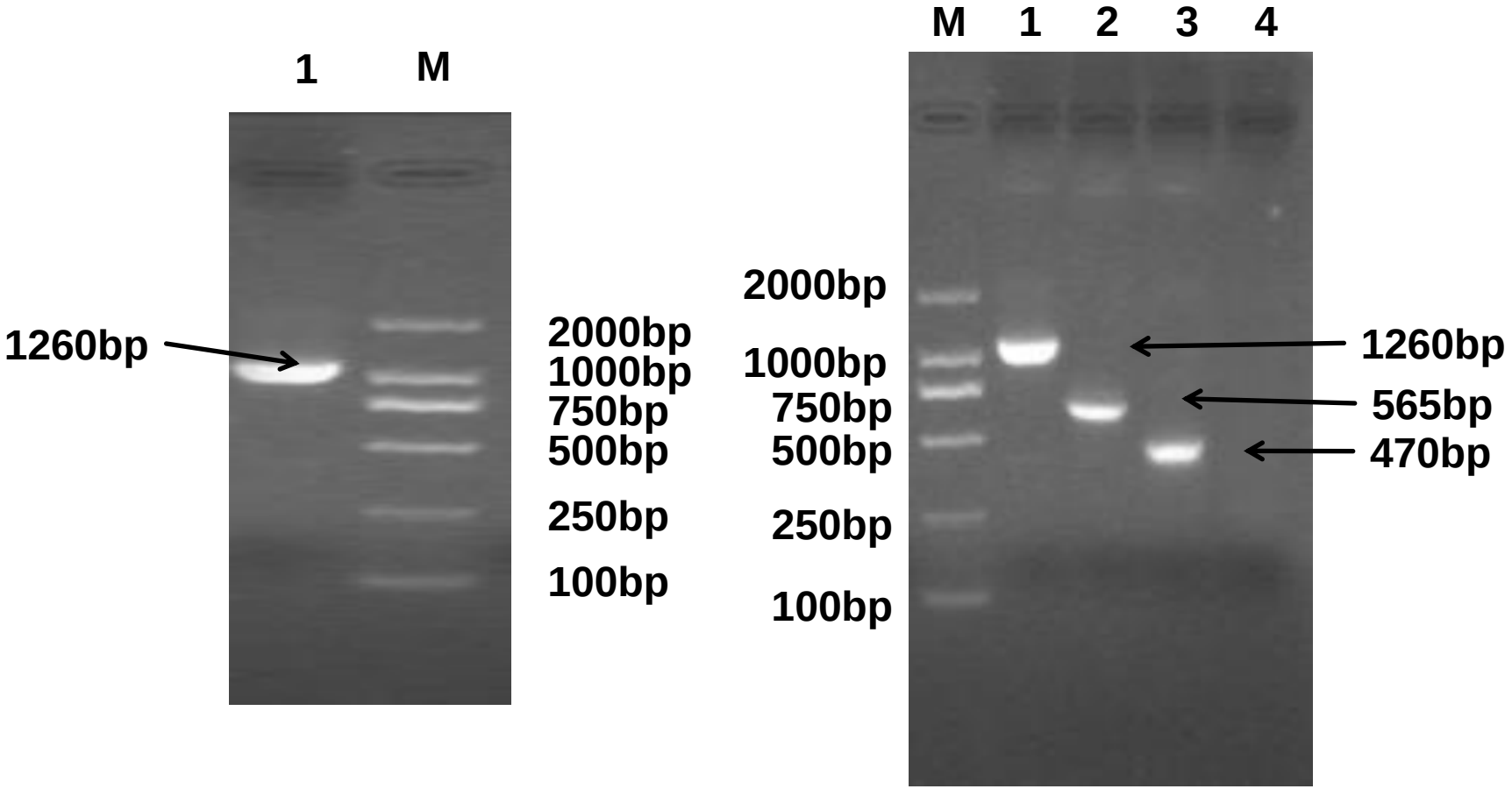
Pictures of GUS staining in seed with pSCSP-GUS

A，B：1，2 GUS staining result of transgentic tobacco in seed and seed coat with pSCSP-GUS

3.GUS staining result of non-transgentic tobacco in seed coat

C: GUS staining result of transgentic tobacco in seed with pBI121(35Spromoter)

Cloning of the droughty-inducible promoter *CBF4A*



PCR results of intact promoter sequence 4A of transcription factor CBF4 and 5` partial fragment 4B and 4C

Cis-element	Consensus sequence	Fuction
ABRE	TACGTG	cis-acting element involved in the abscisic acid responsiveness
CAAT-box	CAAT	common cis-acting element in promoter and enhancer regions
CCGTCC-box	CCGTCC	cis-acting regulatory element related to meristem specific activation
G-box	CACGTG	cis-acting regulatory element involved in light responsiveness
MBS	CAACTG	MYB binding site involved in drought-inducibility
TATA-box	TATAAA	core promoter element around -30 of transcription start
TCT-motif	TCTTAC	part of a light responsive element
as1	TGACGTCA	cis-acting regulatory element involved in the root-specific expression

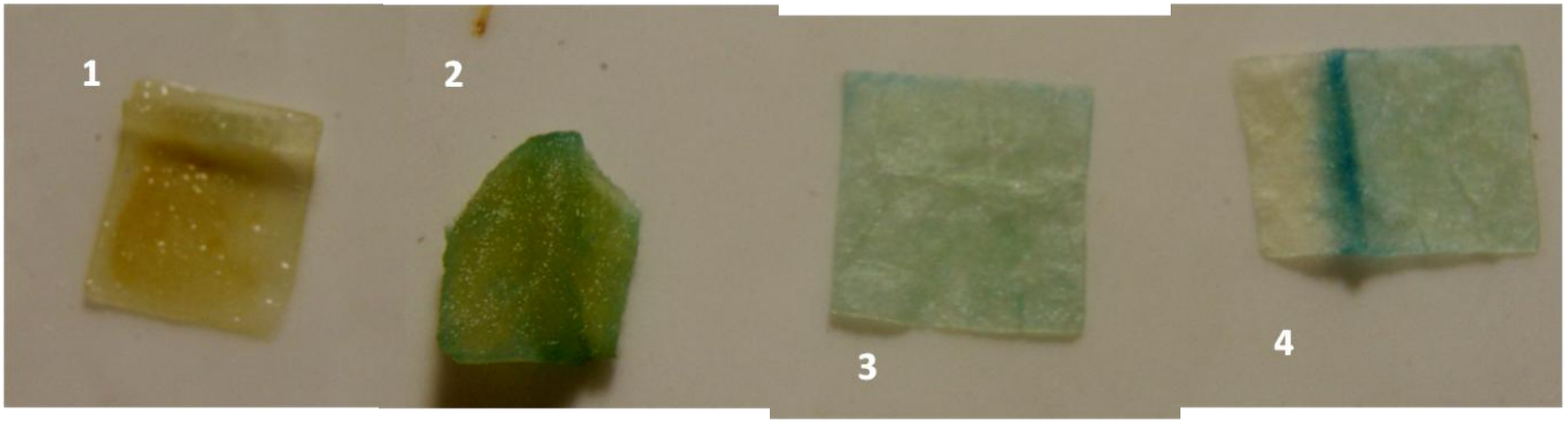
1 RIGINAGAGA ACCAAAGTTC CAGTTCCTTA TATGGAAGAT CCCAACACAA
TTATTTGTTA TAGTGAAAAA
71 AAAACATCTT ACATGGGACC AAAGGCTTTG CACCTTTGTT GTCTCAGGGC
TTTACTTGGT CTTTAATTGA

141 ATGTACAAAG TTGATGTAAA AGCTTTGGCT CCATCGTTCA TGGAGTCTAG
TACATTGTTT TGGGTTTTAA
211 AAGTTTTCTG TGTTAAAGCT CGTCGTCTAA TTGATGTTGT GTTTGGTTTT
AGTTGAAAGG TTTAGACGTT
281 TAGTAAAAAA GTTACTCAAA TTCTTGAAAT CTCCTTGGCC TTGCTGATTA
TAAAATCAAA TATTCATTTA
351 TAAAATAATC CAAAAGGGTT CAATTATTTT TGAAACCGAA TCGAATGAAG
ACAACTCCAT TTTGATTGAT
421 TTTTTTTTTG TTGTATTAAA GAATAAAAAA CTCGACTGGT TCAACAAGAC
TGGATTGTCT TTAAACTACT
491 GGTTCAATGT ATTTTGGGTA CATAATGAAA AAAATACACA ATCCGCCTTG
GCCACGTATC CAATTTTGGC
561 TTAGTTGATG ATGATAATAC TTTTGTTTTT TGGTAAGGCT GATGATGATA
ATAGTTAGAA GAAGTTCATA
631 GAATAATCCA ATGGATTTCC AATAATAAAA CACAGAAATA AATATAATCC
AAACAATTGC CAAAATAAAT

701 GAATAAGAAA AGGGACCCAC ACCAAAAGCT AAAAGCGCGT GGGTCAGATT
ACAAAAAGCG AAACCCCAAA
771 CCGTGGCTAG ACAGCGGACG AACCCGTCCC TTCAAACGTG GCTCACCTTT

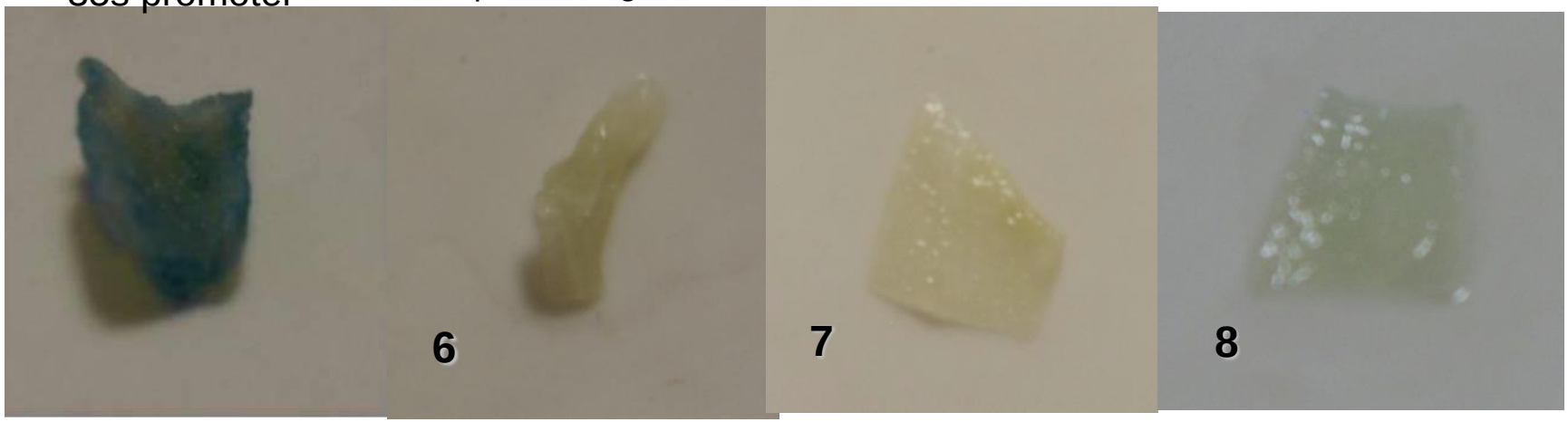
TCT-motif
CAAT-box
CAAT-box
CCGTCC-box
ABRE
MBS
as1
ABRE
TATA-box

Detection of GUS histochemical staining:



1. Tobacco without 35s promoter

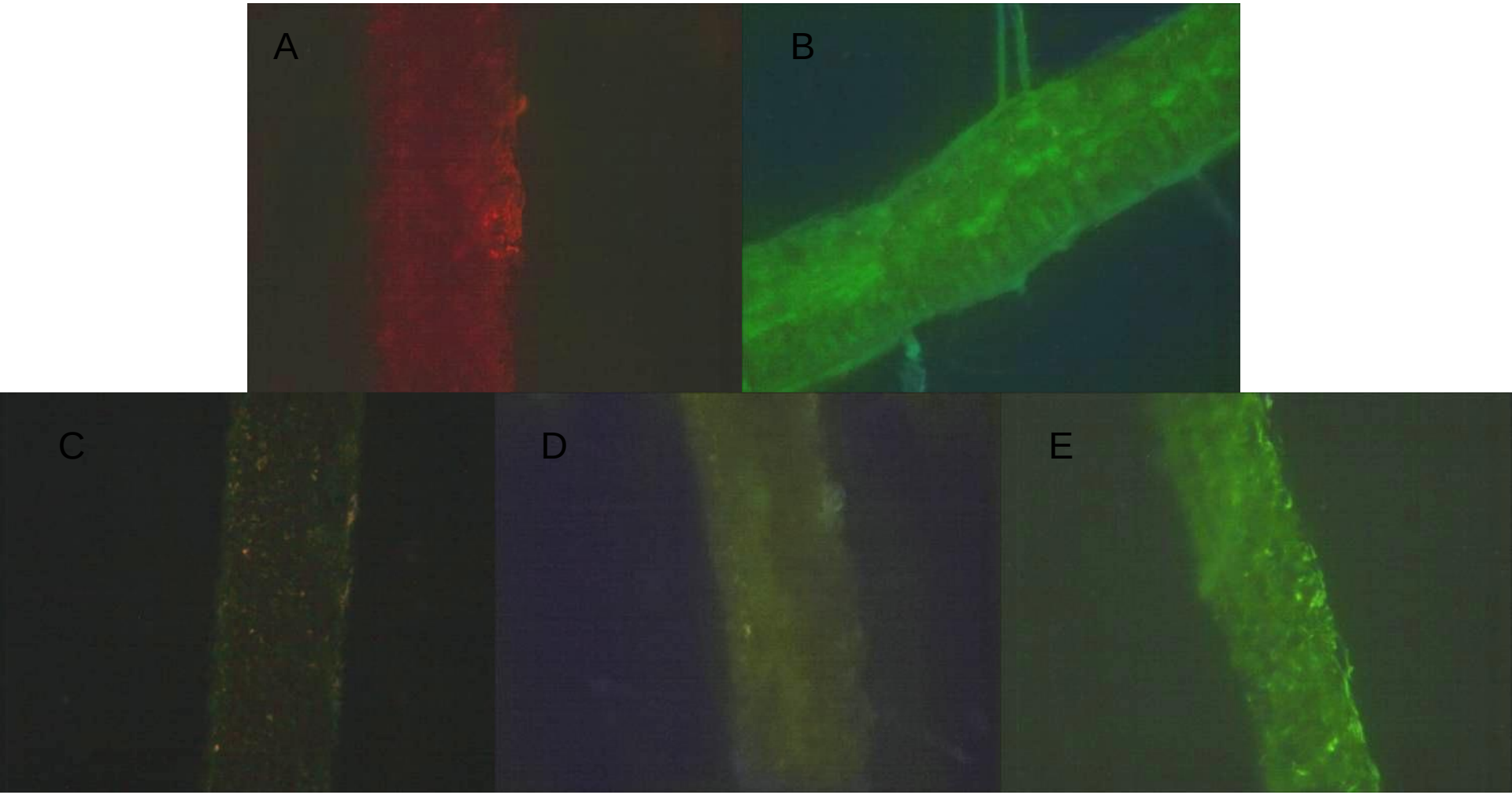
2,3,4. The tobacco of transformed 4A, 4B, and 4C with drought processing.



5. The tobacco with 35s promoter

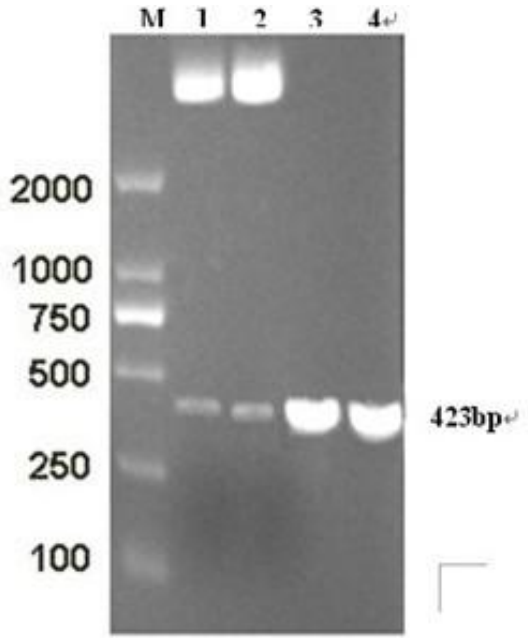
6,7,8. The tobacco of transformed 4A, 4B, and 4C without drought press

Detection of fluorescence microscope:



A：non-GMO tobacco，B：tobacco with p1301-35s-GFP，C：tobacco with p1301-4A-GFP，D：tobacco with p1301-4B-GFP, E：tobacco with p1301-4C-GFP

Construction plant expression vector

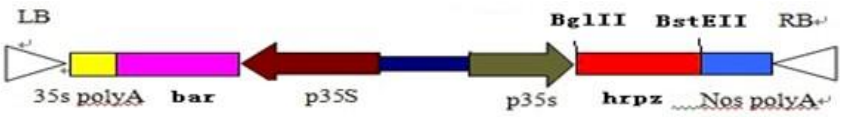


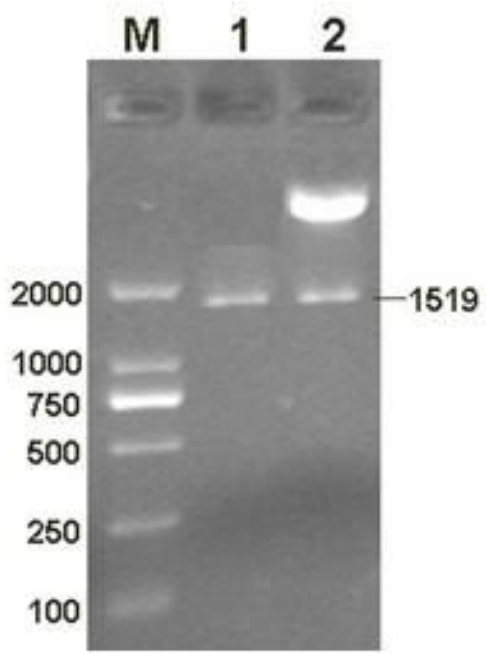
pCAMBIA3301-hrpZpsta-bar

M: DNA Marker DL-2000;

1-2: enzyme-digested products

3-4: PCR products





pCAMBIA1301-hrpZpsta-badh

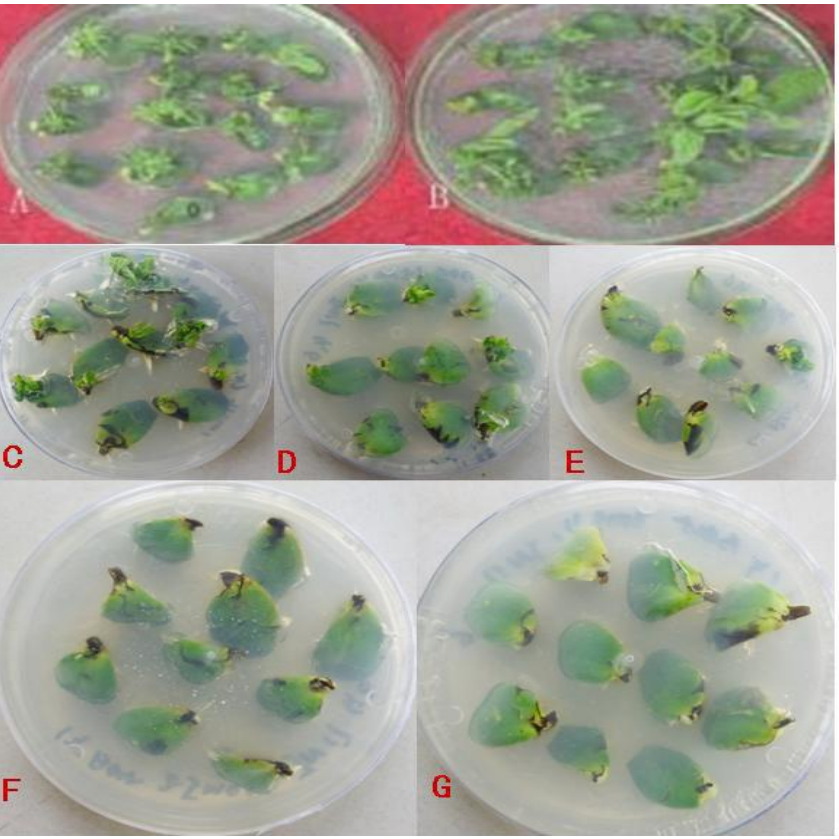
M: DNA Marker DL-2000;

1: PCR product

2: enzyme-digested products

3.3 Transformation of the plant expression vector

Transformation via Agrobacterium mediation:



Growth situation of soybean cotyledon under various NaCl concentration

A. 0 mmol·L^{-1}; B. 50 mmol·L^{-1}; C. 100 mmol·L^{-1}; D. 150 mmol·L^{-1}; E. 175 mmol·L^{-1}; F. 200 mmol·L^{-1}; G. 225-250 mmol·L^{-1}

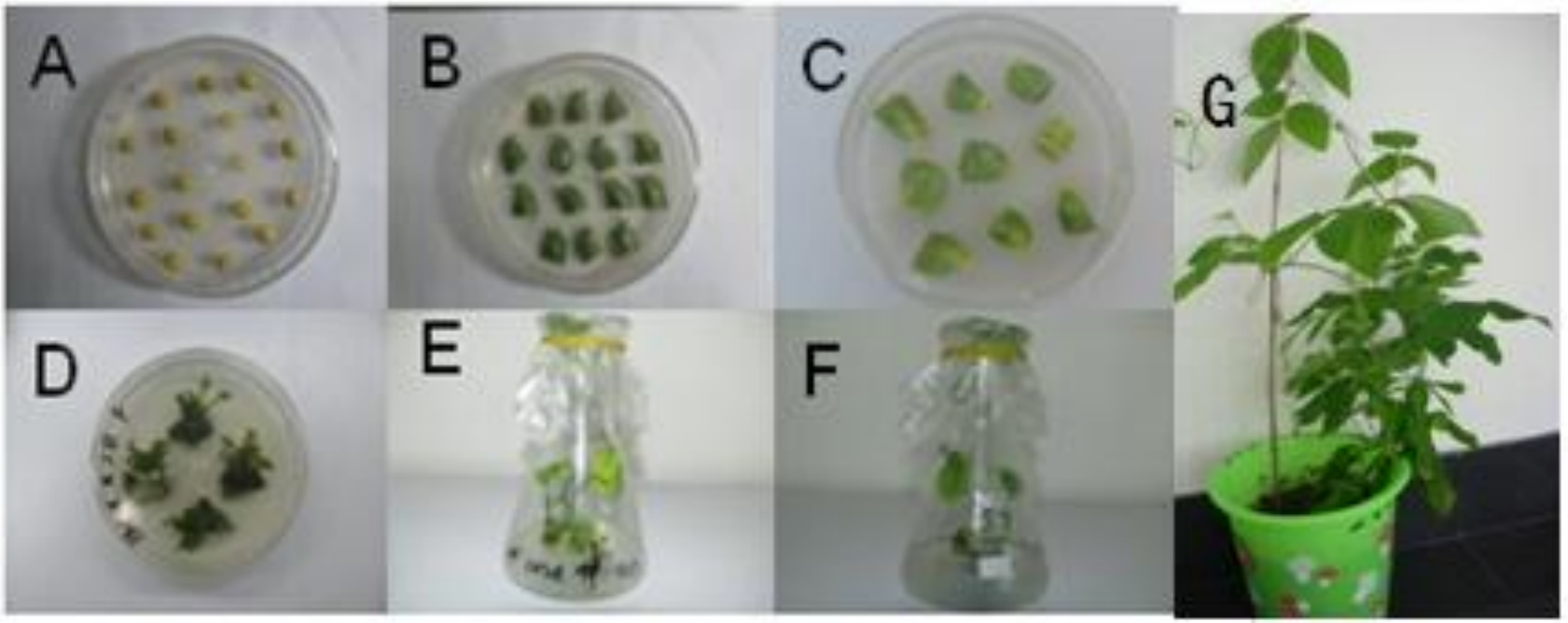
The differentiation rate of cotyledon buds at different NaCl concentration

Genotype	NaCl concentration/ mmol·L^{-1}							
	0	50	100	150	175	**200**	**225**	250
JN 27	100	96.7	76.6	37.7	22.3	**11**	**2**	0
J 17	100	93.3	71	35.7	23.3	**9**	**1**	0
JL30	100	92.3	65.7	32.3	24.3	**5.7**	**0**	0
DE2259	100	92.2	63.3	33.3	21.1	**4**	**0**	0
JN 28	100	91.1	58.9	23.3	4	**0**	**0**	0

at 200 mmol/L: brown stains emerged at the surface of cotyledon;
at 250mmol/L: the colour of cotyledon from various genotypes turn to brown and black,;
So,,200mmol/L concentration was used as the selected pressure.

Acceptor genotypes：
Jilin 30, Jinong17, Jinong9901-11, Jinong27 and Jinong28

We have got 50 positive transgenic plant rows in T1 generation, including 30 and 20 rows from Jilin30 and Jinong27 respectively. The transformation efficiency was 2.5% .



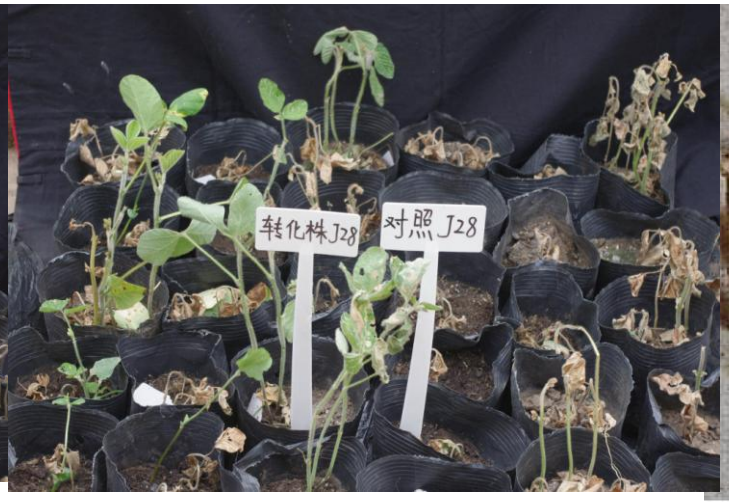
A..GerminationB.PrecultureC.Co-culture D.Selective culture

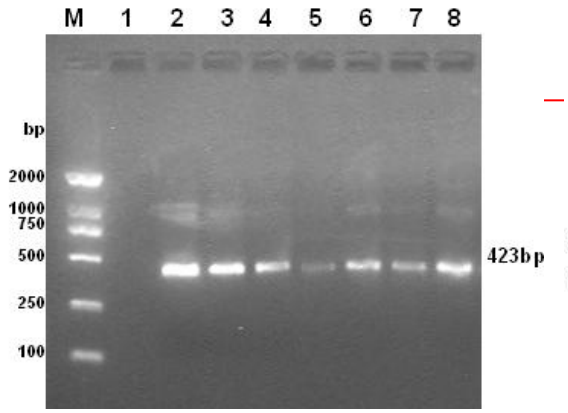
E.Elongation culture F.Rooting culture G.In greenhouse

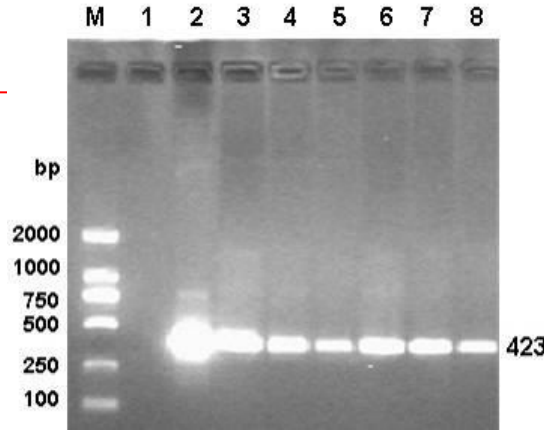
pollen-tube pathway

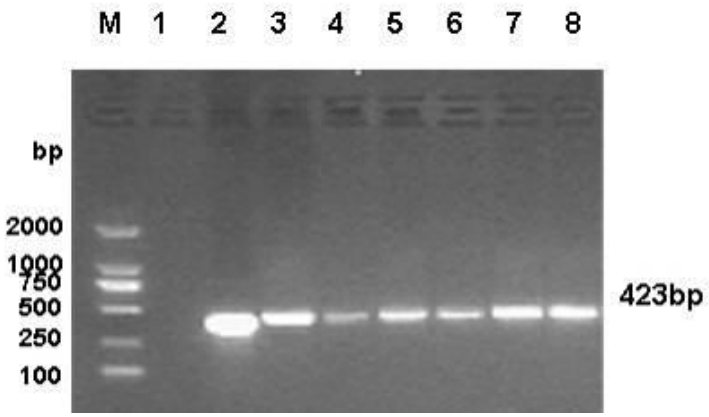
Acceptor genotypes：Jilin30，Jinong27，T43，Jinong18，Jinong17，Jinong2001-241，Jinong2002-D4 and Jinong2001-254.

We have got 88 positive transgenic plant rows in T1 generation, including 36, 32 and 20 rows from Jilin30, Jinong18 and Jinong27 respectively.



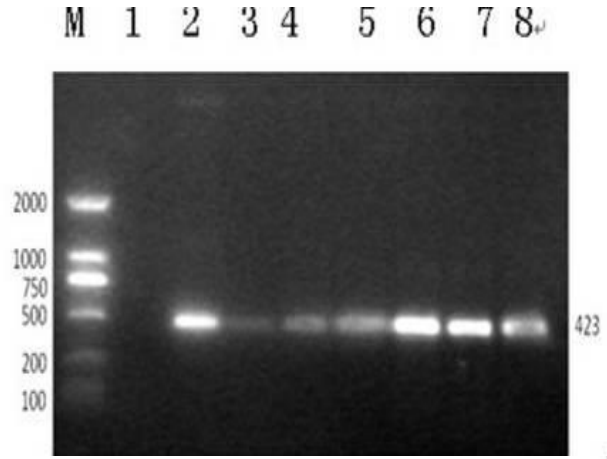


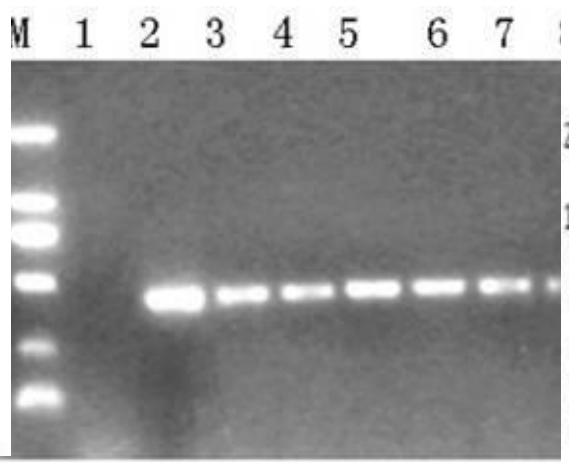


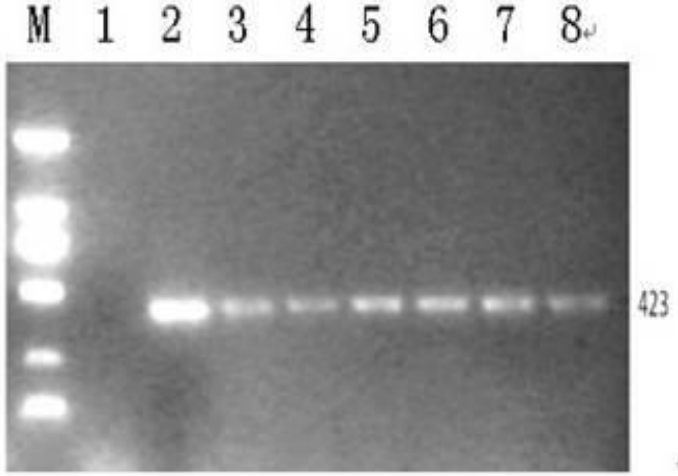


PCR detection of transgenic plants with hrpZpsta in generation of T1,T2 and T3
（ Agrobacterium-mediation)

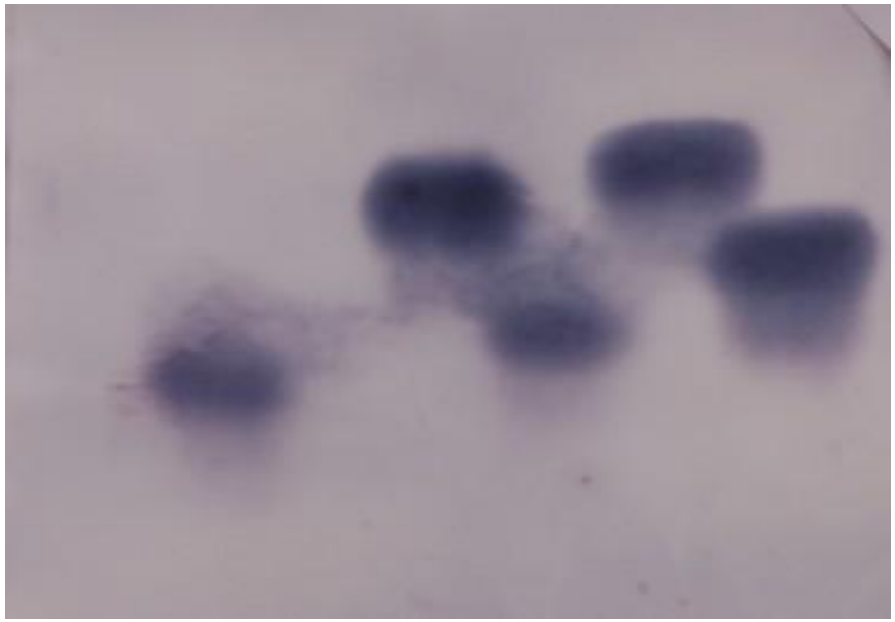
M、DNA Marker（DL2000）；1、Negative control；2、positive control；
3、transgenetic lines of STH17-1；4、transgenetic lines of STH17-2；5、transgenetic lines of STH17-3；
6、transgenetic lines of STH17-4；7、transgenetic lines of STH17-5；8、transgenetic lines of STH17-6



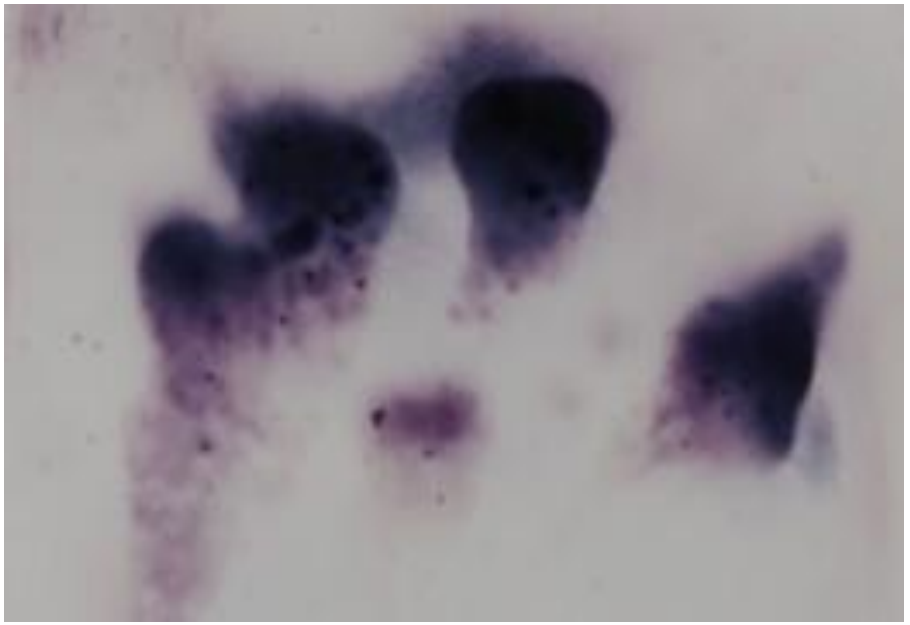




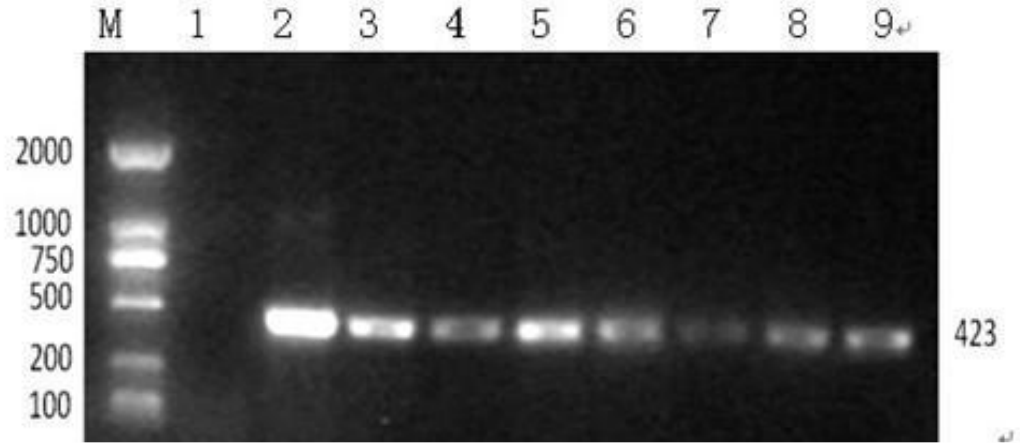
PCR detection of transgenic plants with hrpZpsta in generation of T1,T2 and T3（pollen-tube way）



Southern blot hybridization of
T1 generation transgenetic plant

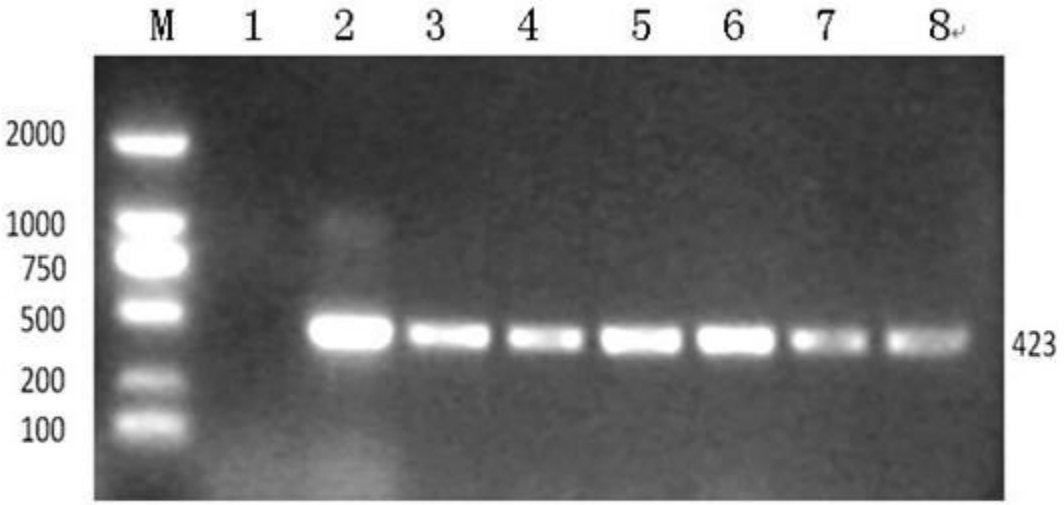


Southern blot hybridization of
T2 generation transgenetic plant



RT-PCR detection of transgenic plants with hrpZpsta in generation T3 (Agrobacterium-mediation)

M、DNA Marker（DL2000）; 1、Negative control;; 2、positive control; 3-9、transgenetic lines.



RT-PCR detection of transgenic plants with hrpZpsta in generation T3 (Pollen tube pathway)

M、DNA Marker（DL2000）; 1、Negative control;; 2、positive control; 3-9、transgenetic lines.

We have got 210 plant rows in T2 generation, including 40 and 32 rows from Jilin30 and Jinong27 respectively via Agrobacterium-mediation and 70, 38 and 30 rows from Jilin30, Jinong27 and Jinong28 respectively via pollen tube pathway. The results of Southern blot, RT-PCR show that *hrpZpsta* gene had integrated into soybean recipient genome and expressed.

3.4 Identification of disease resistance for transformed lines

Resistance detection of Phytophthora root rot

Genotypes	Untransformed lines(wt)		Transformed lines(mt)		WT-MT
	Disease index	Disease level	Disease index	Disease level	
Jinong29	72	S	34	MR	38**
Jinong18	64	S	30	MR	34**
Jinong17	56	MS	20	MR	36**
Jilin30	54	MS	20	MR	34**

Detection of transformed plants: 1.Jinong18; 2. Jinong17; 3. Jilin30; 4. Jinong29

1

2

3

4

Resistance detection of Cercospora sojina Hara

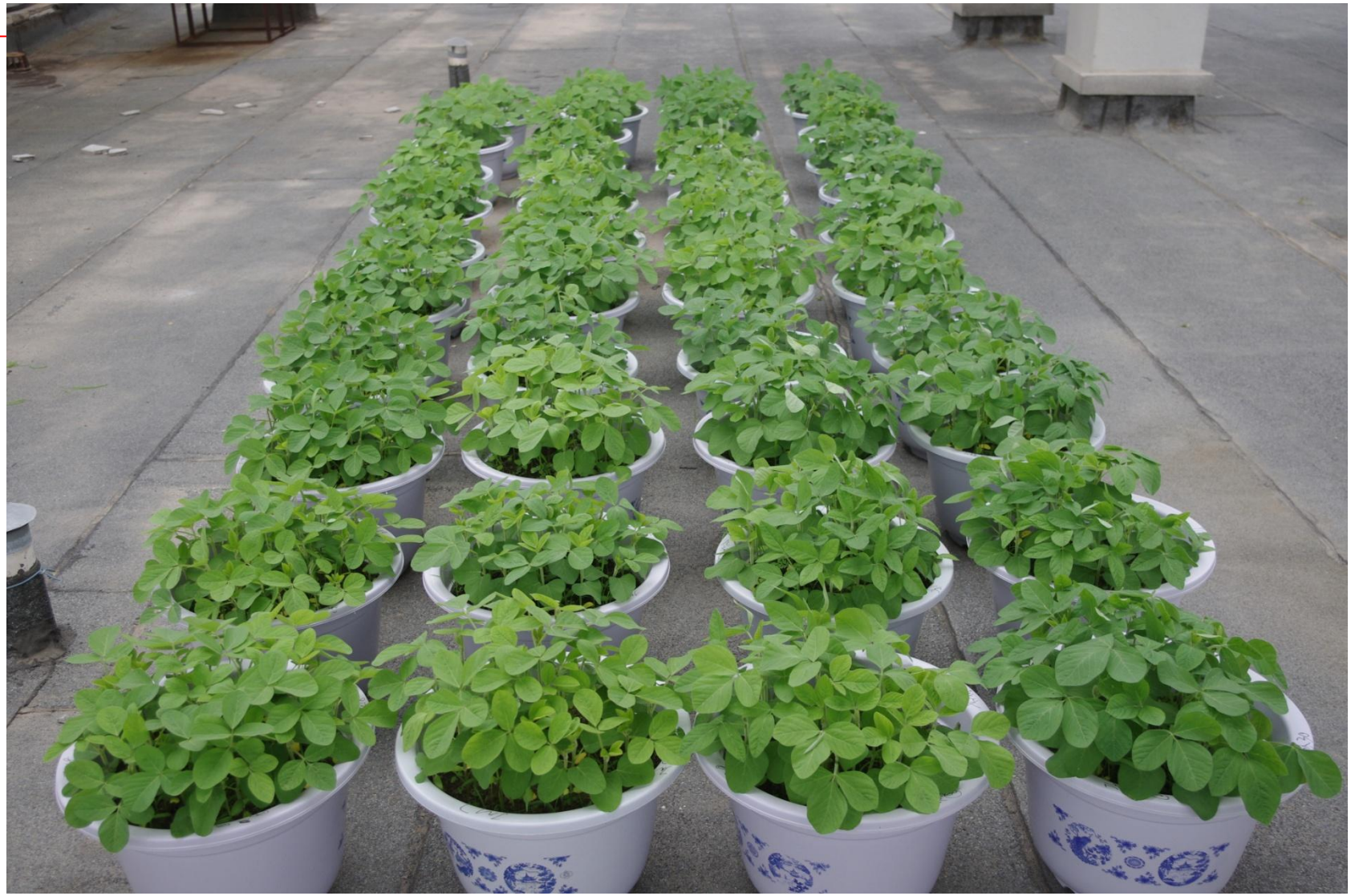
Untransformed lines:

Genotypes	No.of plants investigated	Disease level						Disease index	Resistant level
		0	1	3	5	7	9		
Jinong18	50	45	5	0	0	0	0	10.00	R
Jinong17	50	17	8	25	0	0	0	55.33	MS
Jinong29	50	0	2	3	6	38	1	70.22	S
Jilin30	50	6	7	3	28	6	0	56.57	MS

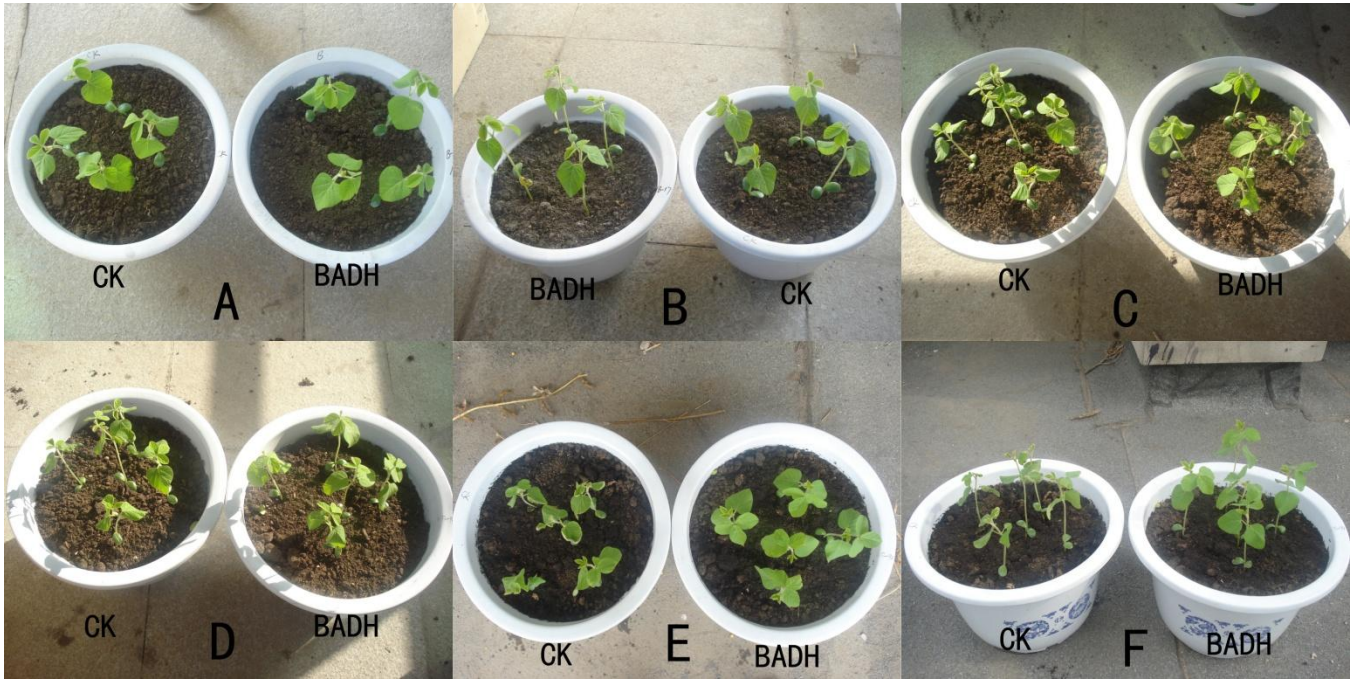
Transformed lines:

Genotypes	No.of plants investigated	Disease level						Disease index	Resistant level
		0							
Jinong18	50	49	1	0	0	0	0	2.00	HR
Jinong17	50	39	11	0	0	0	0	22.00	MR
Jinong29	50	3	11	27	9	0	0	54.80	MS
Jilin30	50	12	21	15	2	0	0	30.00	MR

Detection of transformed plants: 1.Jinong18; 2. Jinong17; 3. Jilin30; 4. Jinong29



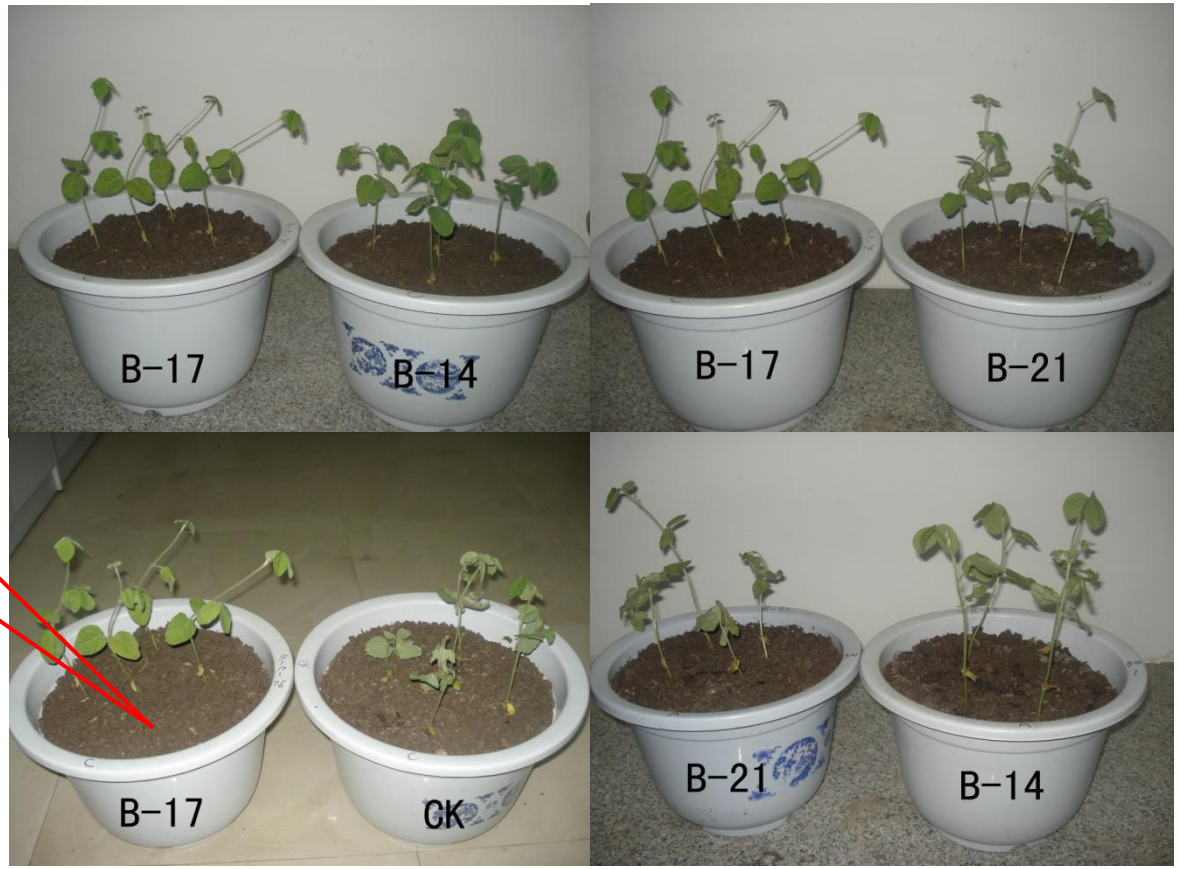
3.5 Drought-tolerance evaluation of transformed Jinong17 with *badh* as select gene



A.10 days in drought condition（re-water），B.13 days in drought condition，C.16 days in drought condition，D.19 days in drought condition，E、F.22 days after re-water

Between 10th and 19th day during drought-tolerance, re-water one time. Then drought-tolerance lasted during 28th and 31th day, wilt performed seriously.

The drought-tolerance of BADH-17 is the best



Drought-tolerance(20 days):BADH-17 > BADH-14 > BADH-21 > CK

3.6 Yield test of transformed lines

Receptor	Plant lines	Yield(kg/pot)	Yield increased(%)	Growth Period (days)	Receptor	Plant lines	Yield(kg/pot)	Yield increased(%)	Growth Period (days)
Jinong18	STH18-3	3.416 a	15.0	126	Jinong17	STH17-5	2.790 a	12.8	135
	STH18-2	3.279 ab	10.4	125		STH17-3	2.687 ab	8.6	137
	STH18-6	3.256 ab	9.6	126		STH17-2	2.604 bc	5.3	136
	STH18-1	3.125 bc	5.2	125		STH17-4	2.504 cd	1.2	135
	CK	2.970 cd	--	125		吉农17CK	2.475 cd	--	134
	STH18-4	2.906 d	-2.2	125		STH17-6	2.431 d	-1.7	137
	STH18-5	2.667 e	-10.2	125		STH17-1	2.390 d	-3.4	135

Receptor	Plant lines	Yield(kg/pot)	Yield increased(%)	Growth Period (days)	Receptor	Plant lines	Yield(kg/pot)	Yield increased(%)	Growth Period (days)
Jilin30	STH30-7	2.523 a	10.5	138	Jinong29	STH29-6	2.882 a	10.7	135
	STH30-5	2.470 ab	8.2	137		STH29-2	2.816 ab	8.2	134
	STH30-4	2.432 ab	6.5	138		STH29-1	2.788 abc	7.1	133
	STH30-2	2.362 ab	3.5	136		STH29-4	2.737 abc	5.1	133
	STH30-1	2.348 ab	2.8	136		STH29-3	2.633 bcd	1.2	133
	吉林30CK	2.283 ab	--	137		吉林29 CK	2.603 cd	--	133
	STH30-6	2.250 b	-1.4	137		STH29-5	2.547 d	-2.2	135
	STH30-3	2.249 b	1.5	136					

The transformed lines have been approved to do the field experiment.

4. Conclusion

4.1. In our experiment, the hrpZpsta gene from tobacco wildfire pathogen was cloned and transformed into 4 recipient soybean varieties via Agrobacterium-mediation and pollen tube pathway. The results of Southern blot, RT-PCR show that hrpZpsta gene had integrated into soybean recipient genome and expressed.

4.2. The disease resistance of transgenic soybean lines to Phytophthora root rot and Cercospora sojina Hara were increased obviously. Several promising lines with high resistance and yield had been selected.

4.3. We used *badh* as select gene and established new select system for soybean cotyledon node transformation via Agrobacterium mediation. This system can be used both in screening transformed explants and in increasing drought tolerance of transgenic plants.

Acknowledgements

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-Junqi Yin(Master Student)
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Thank you for your attention