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Novel Plant Transformation Vectors Containing the Super-promoter

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Abstract

We developed novel plasmids and T-DNA binary vectors that incorporate a modified and more useful form of the super-promoter. The super-promoter consists of a trimer of the octopine synthase (*ocs*) transcriptional activating element affixed to the mannopine synthase 2' (*mas2'*) transcriptional activating element plus minimal promoter. We tested a super-promoter/*gusA* fusion gene in stably transformed tobacco and maize plants, and in transiently transformed maize BMS protoplasts. In both tobacco and maize, super-promoter activity was much greater in roots than in leaves. In tobacco, super-promoter activity was greater in mature leaves than in young leaves, whereas in maize activity differed little among the tested aerial portions of the plant. When compared with other commonly used promoters (CaMV 35S, *mas2'*, and maize ubiquitin), super-promoter activity was approximately equivalent to those of the other promoters in both maize BMS suspension cells and in stably transformed plants. The addition of a maize ubiquitin intron downstream of the super-promoter did not enhance activity in stably transformed maize.

Introduction

The availability of convenient vectors harboring a strong promoter that is active in all or most cells of different plant species would be useful for a variety of applications in plant molecular biology. We previously described a novel synthetic promoter consisting of a trimer of the octopine synthase transcriptional activating element (*ocs* activator) linked to the mannopine synthase 2' (*mas2'*) activator/promoter region (Ni et al., 1995). Initial studies in tobacco indicated that this promoter, called the "super-promoter", could direct expression of GUS activity to a level 2- to 20-fold higher than the commonly used "enhanced" double Cauliflower Mosaic Virus (CaMV) 35S promoter (Ni et al., 1995). The activity of the super-promoter was highest in roots, but was also high in leaves and stems.

The super-promoter was originally created by ligating three *ocs* activator fragments (positions -333 to -116 relative to the transcription start site [Leisner and Gelvin, 1988]) from the octopine synthase gene to the *mas2'* activator/promoter region (-318 to +65 relative to the transcription start site; [Ellis et al., 1984]), all from the *Agrobacterium tumefaciens* Ti-plasmid pTiA6. The construction of the original super-promoter resulted in the repeated presence within the promoter of several commonly used restriction endonuclease sites (*Bam*HI, *Eco*RI, *Hin*dIII), as well as the presence of the restriction endonuclease sites *Pst*I, and *Xho*I. This feature precluded one from easily linking genes to the promoter. In addition, it complicated further analysis of T-DNA insertions in the plant genome. We therefore modified the super-promoter, eliminating most of these internal restriction endonuclease sites. This modified super-promoter formed the basis for the construction of several novel plant expression and T-DNA binary vectors. Here, we describe these vectors and the use of the super-promoter to promote reporter *gusA* gene expression in tobacco and maize.

Results and Discussion

Construction of super-promoter cassettes in pUC119

To provide researchers with convenient tools for various applications in plant gene expression, we constructed expression cassettes containing the modified super-promoter (MSP). The plasmid pUC119 (Vieira and Messing, 1987; GenBank accession

U07650) formed the basis for these initial cassettes (the pMSP series), whose maps are shown in Figure 1.

Plasmid pMSP-1 contains the MSP followed by a multiple cloning site (mcs) and a polyA addition signal. Translational enhancers (TL) from plant viruses (e.g., the omega element from Tobacco Mosaic Virus [Gallie and Walbot, 1992] and the TL enhancer element from Tobacco Etch Virus [TEV; Restrepo et al., 1990]) may enhance the level of gene expression two- to three-fold (Holtorf et al., 1995). We constructed a second cassette, pMSP-2, with the TL enhancer element from TEV linked downstream of the super-promoter. The presence of introns within the 5' untranslated region of genes often enhances expression in maize (Mascarenhas et al., 1990) and other cereals (Cornejo et al., 1993). We therefore created a third cassette, pMSP-3, containing a maize *adh1* intron following the super-promoter for improved expression in monocot plant species.

We subsequently constructed plant transformation vectors, based upon pMSP-1, pMSP-2, and pMSP-3, in the T-DNA binary vectors pGPTV-KAN, pGPTV-HPT, and pGPTV-BAR (Becker et al., 1992). Figure 2 shows maps of these vectors.

In summary, we modified the original super-promoter (Ni et al., 1995) by eliminating most of the commonly used restriction endonuclease sites from within the promoter sequence. We constructed 21 new vectors, based upon pUC119 and the T-DNA binary vector pGPTV, containing the modified super-promoter. These vectors have the following features: (1) the modified super-promoter; (2) various plant regulatory sequences for improved gene expression (either a maize *adh1* intron for expression in monocot plant species or a translation enhancer (TL) from Tobacco Etch Virus for expression in dicot plant species); (3) a multiple cloning site with 8-16 unique restriction endonuclease sites (depending upon the vector) downstream of the promoter for cloning of genes and for more facile analysis of T-DNA insertions in the plant genome; (4) the agropine or nopaline synthase polyA addition signals; (5) markers for binary vectors for selection in plants using kanamycin, hygromycin, or phosphinothricin/Basta/Bialophos.

Activity of the super-promoter in transgenic tobacco and maize

We previously showed that in transgenic tobacco, a super-promoter-*gusA* construction was ~5-fold more active in roots than in leaves (Ni et al., 1995). We carried

out a more detailed investigation of the activity of the super-promoter in various organs of stably transformed tobacco and maize plants containing a super-promoter-*gusA* expression cassette. These tests were conducted before construction of the current series of super-promoter vectors described above; the constructs therefore did not have the exact same configuration as do the current vectors. In addition, our original constructions contained (when applicable) the maize ubiquitin intron, rather than the maize *adh1* intron incorporated into the current set of vectors. However, tests in transgenic tobacco indicated that the current super-promoter configuration behaves in a manner almost identical to that of the older version of the super-promoter (data not shown). Figure 3 shows that in tobacco, GUS activity was greatest in older (lower) leaves than in younger (upper) leaves. Interestingly, this is opposite to the expression pattern directed by the CaMV 35S promoter, which directs expression of transgenes most strongly in younger, meristematic tissues (Williamson et al., 1989). Conversely, GUS activity in young root tips was much higher than that in older portions of the roots. The pattern of expression in transgenic maize, however, was different. There was relatively little difference in GUS activity among younger or older leaves. As with the situation in transgenic tobacco, GUS activity in transgenic maize roots was considerably greater than that in leaves.

Comparison of the activities of the super-promoter and other promoters in maize

We previously showed that the super-promoter was as strong as or stronger than several commonly used promoters in transgenic tobacco (Ni et al., 1995). We therefore asked how the activity of the super-promoter compared with that of other promoters in monocots. Figure 4 shows a comparison of the relative strengths of the super-promoter, the *mas2'* promoter, the CaMV 35S promoter (-800 from pBI121; Jefferson et al., 1987), and the maize ubiquitin promoter in transgenic maize. Within a factor of two, all of these transcriptional regulatory sequences promoted approximately the same level of GUS activity in leaves, and also in roots. These data indicate that the super-promoter functions as well as other commonly used promoters in these maize tissues.

Response of the super-promoter and other promoters to the presence of introns

The presence of introns enhances the activity of promoters for gene expression, especially in monocots (Cornejo et al., 1993; Mascarenhas et al., 1990). We therefore tested whether the presence of introns would enhance the activity of the super-promoter and several commonly used promoters in maize. Figure 5 shows the constructions that we tested. Each set of constructions contains either the super-promoter, the *mas2'* promoter, the CaMV 35S promoter, or the maize ubiquitin promoter. Set I contains no intron in the *gusA* gene, Set II contains the potato ST-LS1 intron (Vancanneyt et al., 1990) in the *gusA* coding sequence, and Set III contains a maize ubiquitin intron preceding the *gusA* coding sequence. As a control, we also generated a promoter-less *gusA* construction.

We first tested these constructions in transiently transformed maize BMS protoplasts. Within a factor of 2.5, all of these constructions (except the control, promoterless *gusA* gene which yielded only a background level of activity) elicited approximately the same amount of GUS activity. Figure 6 shows the average data from four independent experiments. Thus, in transient expression assays, all of these promoter/intron combinations functioned equally well in maize suspension cells.

Promoters may have different activities depending upon whether they direct expression from non-integrated transgenes in transiently transformed cells, or from integrated transgenes that may be under transcriptional constraints of assembled chromatin (see, e.g., Frisch et al., 1995). Strong expression of a linked chloramphenicol acetyltransferase (*CAT*) reporter gene directed by a maize ubiquitin promoter plus ubiquitin leader intron in electroporated maize BMS cells has previously been described by Christensen et al. (1992). Enhancement of gene expression, resulting from increased stability of cytoplasmic mRNA, has also been reported as a result of incorporating the maize *Adh1* intron into reporter constructions (Callis et al., 1987; Luehrsen and Walbot, 1994). We therefore tested, in stably transformed maize plants, the response of each of four promoters to the presence of the maize ubiquitin intron. Interestingly, the activity of only the ubiquitin promoter increased in maize leaves in the presence of the ubiquitin intron (Figure 7). This response was up to 55-fold, indicating that, amongst the eight promoter/intron combinations tested, the strongest transcriptional regulatory sequence in maize is clearly the maize ubiquitin promoter followed by the maize ubiquitin intron.

Our results contrast with those of Callis et al. (1987) who demonstrated that introduction of the *Adh1* first intron into constructions containing either the CaMV 35S or nopaline synthase (*nos*) promoters increased activity of a linked *CAT* gene 8- and 170-fold, respectively. Addition of a maize *Bronze1* (*Bz1*) intron to the CaMV 35S promoter also stimulated CAT activity. This stimulation was associated with increased steady-state levels of *CAT* mRNA. However, these authors utilized *Adh1* and *Bz1* introns, rather than the ubiquitin intron used in our study, and they performed their analyses in transiently transfected maize BMS protoplasts. It is possible that the maize ubiquitin promoter plus intron combination functions optimally in stably transformed maize tissues, a system not tested by Callis et al. (1987).

Use of the super-promoter in various plant species

We re-configured the original super-promoter (Ni et al., 1995) such that it could easily be utilized for gene expression in a number of plant species. Over the past ~10 years, our laboratory has distributed the various super-promoter constructions to dozens of laboratories. In our laboratory, the super-promoter has routinely and effectively been used to drive both transient and stable transgene expression in tobacco plants and BY-2 cell suspensions (Ni et al., 1995; He et al., 1996; Narasimhulu et al., 1996; Kononov et al., 1997; Mysore et al., 1998; Veena et al., 2003; Lee and Gelvin, 2004), *Arabidopsis* plants and cell suspensions (Nam et al., 1997, 1998; 1999; Mysore et al., 2000; Yi et al., 2002; Zhu et al., 2003; Gaspar et al., 2004; Hwang et al., 2004; Hwang and Gelvin, 2006; Crane and Gelvin, 2007; Kim et al., 2007), maize plants and BMS cell suspensions (Narasimhulu et al., 1996), and *Brassica napus* plants (S. Johnson and S.B. Gelvin, unpublished). Others have shown that transcriptional regulatory sequences very similar to those of the super-promoter appear to be less susceptible to silencing than are other commonly used promoters, including the CaMV 35S and Cassava Vein Mosaic Virus promoters (De Bolle et al., 2003; Butaye et al., 2004).

The vectors described in this article can be obtained by contacting Dr. Stanton B. Gelvin (gelvin@bilbo.bio.purdue.edu) following completion of a Materials Transfer Agreement (MTA).

DNA sequences of the super-promoter regions of pMSP-1, pMSP-2, and pMSP-3 can be found as GenBank accessions EU181145 (pMSP-1), EU181146 (pMSP-2), and EU181147 (pMSP-3).

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Materials and Methods

Super-promoter and vector constructions

The original super-promoter contains a trimer of the *ocs* activator sequence (A*ocs*), cloned as a *Hin*dIII fragment, upstream of the *mas2'* activator plus promoter in pE1120. We modified the original super-promoter as follows:

We removed the super-promoter from pE1120 by partial digestion with *Hin*dIII plus complete digestion with *Xba*I. We cloned this fragment into the *Hin*dIII and *Xba*I sites of pBluescriptKS(-) to generate the plasmid pE1037. Digestion of pE1037 with *Hin*dIII removed the three *ocs* activator sequences from the super-promoter. The single *Hin*dIII site within this resulting plasmid (pE1048) was converted into a *Bam*HI site by filling in the overhanging nucleotides using Klenow fragment of DNA polymerase followed by annealing a *Bam*HI linker, generating pE1049. We likewise converted the *Hin*dIII sites flanking A*ocs* into *Bam*HI sites. We cloned this new A*ocs* fragment into pE1049. Screening of the resulting colonies yielded insertions of a single A*ocs* fragment (in either orientation), insertions of a dimer of the A*ocs* fragment (both in the correct or both in inverted orientation), and a trimer of the A*ocs* fragment (all three in inverted orientation; pE1054).

We continued to modify pE1054, first by removing a *Pst*I site within the super-promoter, and subsequently transferring the newly modified super-promoter region into the *Sal*I and *Xba*I sites of pUC119, generating pUΔP (pE1466). We next removed a *Xho*I site from the super-promoter region of pE1466 by filling in the *Xho*I site using Klenow fragment of DNA polymerase, generating pUΔPΔX (pE1467). We digested pE1467 with

*Hin*dIII and *Sal*I, filled-in these sites using Klenow fragment, and ligated the blunted ends to remove sites (*Hin*dIII, *Sph*I, *Pst*I, *Bsp*MI, and *Sal*I) between *Hin*dIII and *Sal*I, generating pUΔPΔX-1.

We digested pUΔPΔX-1 with *Eco*RI, filled in the overhanging ends using Klenow fragment, and ligated into this site a 401 bp *Hin*cII-*Eco*RV fragment containing the agrocinopine synthase (*ags*) terminator (ags-ter) from pTiA6, generating pUΔPΔX-2 (pE1694). We digested pUΔPΔX-2 with *Xba*I and *Sma*I, filled in the overhanging ends using Klenow fragment, and self-ligated the molecules, removing the *Xba*I, *Bam*HI, and *Sma*I sites and generating the plasmid pUΔPΔX-3 (pE1695).

We digested pBluescript KS(-) with *Eco*RV and inserted a linker containing *Bgl*II and *Bcl*I sites. This plasmid, called pBB (pE1505) contains a new multiple cloning site region. We digested pUΔPΔX-3 with *Kpn*I and *Sac*I and inserted the multiple cloning site region from pBB using *Kpn*I and *Sac*I, generating pMSP-1 (pE1578).

Constructions in *E. coli* were generated in strain DH10B. T-DNA binary vectors were introduced into *A. tumefaciens* EHA101 (Hood et al., 1986) or EHA105 (Hood et al., 1993). *E. coli* strains were grown in Luria-Bertani (LB) medium containing the appropriate antibiotics (ampicillin, 100 μg/ml; kanamycin, 25 μg/ml). *A. tumefaciens* was grown in YEP rich or AB minimal medium (Lichtenstein and Draper, 1986) containing the appropriate antibiotics (rifampicin, 10 μg/ml; kanamycin, 100 μg/ml for plates, 25 μg/ml for liquid growth).

Maize BMS Suspension culture and protoplast isolation

Maize Black Mexican Sweet (BMS) cells were grown at room temperature with shaking (150 rpm) in medium containing MS salts (Gibco), 20g/l sucrose, 2 mg/l 2,4-dichlorophenoxy acetic acid, 200 mg/l inositol, 130 mg/l asparagine, 1.3mg/l niacin, 0.25 mg/l thiamine, 0.25mg/l pyridoxine, and 0.25mg/l calcium pantothenate. Cells were isolated by centrifugation (1000 rpm for 2 min) and suspended in ISS medium (MS medium containing 0.4 M mannitol, 50 mM $CaCl_2$, 10 mM sodium acetate, 15 mM 2–mercaptoethanol, 0.5 mg/l thiamine, and 2 mg/l 2,4-dichlorophenoxyacetic acid [2,4-D]) containing 2% Cellulase (Dyadic International, Inc, Jupiter, FL), 0.1% Pectolyase

(Sigma, St. Louis), and 0.1% BSA. The BMS cells were incubated for 2 hr with shaking at 80 rpm. Protoplasts were harvested, purified by centrifugation at 1000 rpm for 10 min, and suspended in 50 ml EPS (MS plus 0.4 M mannitol, 10 mM HEPES [pH 9.6], 130 mM KCl, and 4 mM $CaCl_2$). Electroporation was carried out using a BioRad GenePulser at 200 V, capacitance 1200, and 100 msec discharge. Following electroporation, the cells were transferred to Petri dishes containing EPS with 0.5 M mannitol and incubated in the dark at room temperature until assayed for GUS activity 18 hr later.

Transformation procedures

Transgenic tobacco plants were generated and maintained as previously described (Ni et al., 1995). Transgenic maize plants (genotype Hi-II) were generated as previously described (Frame et al., 2000).

GUS activity assays

Leaf explants were generated using a paper punch and assayed for β-glucuronidase(GUS) activity fluorimetrically as previously described (Jefferson et al., 1987).

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Figure Legends

Figure 1. Maps of the pUC119-based pMSP series of plasmids. pExxxx numbers at the right of each plasmid map indicate Gelvin laboratory stock numbers. amp^r indicates that the plasmid confers resistance to ampicillin upon host bacteria. A*ocs*, octopine synthase transcriptional activating element; A*mas*P*mas*, mannopine synthase 2' activating and promoter elements; *ags*-ter, polyA addition signal from the agropine synthase gene; TL, translational leader sequence from Tobacco Etch Virus; *ADH*in, intron from the maize alcohol dehydrogenase I gene. Restriction endonuclease sites: A, *Apa*I; B, *Bam*HI; Bc, *Bcl*I; Bg, *Bgl*II; Bx, *Bst*XI; H, *Hin*dIII; N, *Not*I; Nc, NcoI; P, *Pst*I; RI, *Eco*RI; SI, *Sac*I; SII, *Sac*II; S, *Sal*I; Sm, *Sma*I; Sp, *Spe*I; Xb, *Xba*I; X, *Xho*I. Restriction endonuclease sites within parentheses are not unique to the plasmid. The sequence below pMSP-2 shows the translational reading frame following the *Nco*I site which is at the 3' end of the TEV translational leader sequence.

Figure 2. Maps of the T-DNA binary vectors based upon the pMSP series of plasmids. pExxxx numbers at the right of each plasmid map indicate Gelvin laboratory stock numbers. kan^r indicates that the plasmid confers resistance to kanamycin upon host bacteria. P*nos*, nopaline synthase promoter; tAg7, polyA addition signal for T-DNA gene 7; *hptII*, gene conferring resistance to hygromycin; *nptII*, gene conferring resistance to kanamycin; *bar*, gene conferring resistance to phosphinothricin/Basta/Bialophos. Other symbols and restriction endonuclease sites are as in the legend to Figure 1. The *bar* gene contains *Kpn*I and *Sal*I sites; therefore, these sites are not unique to T-DNA binary vectors containing the *bar* gene (indicated by [K] and [Sl]). (A) T-DNA binary vectors based upon the pMSP-1 series of plasmids; (B) T-DNA binary vectors based upon the pMSP-2 series of plasmids; (C) T-DNA binary vectors based upon the pMSP-3 series of plasmids.

Figure 3. GUS activity (pmole/min/mg protein) directed by the super-promoter in various tobacco and maize tissues and organs. L1,2…etc., Leaf number; MF, maize flower; R, root; R-o, old root section; R-y, young root section; S, stem section. Numbers

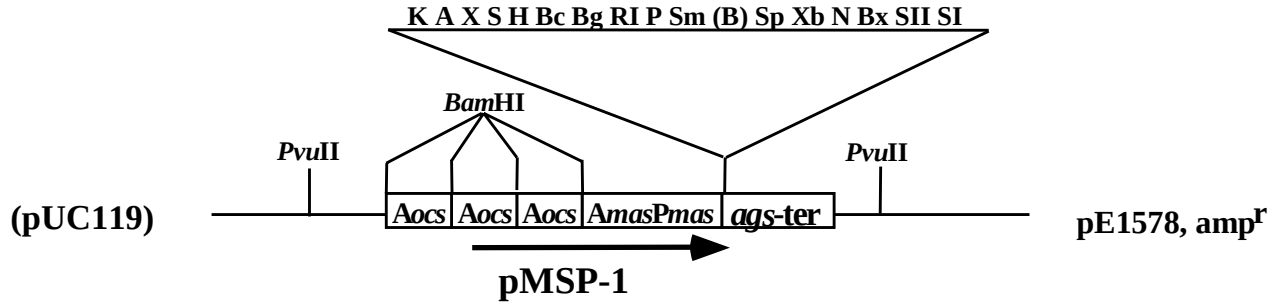
in parentheses indicate the number of samples analyzed for each tissue or organ.

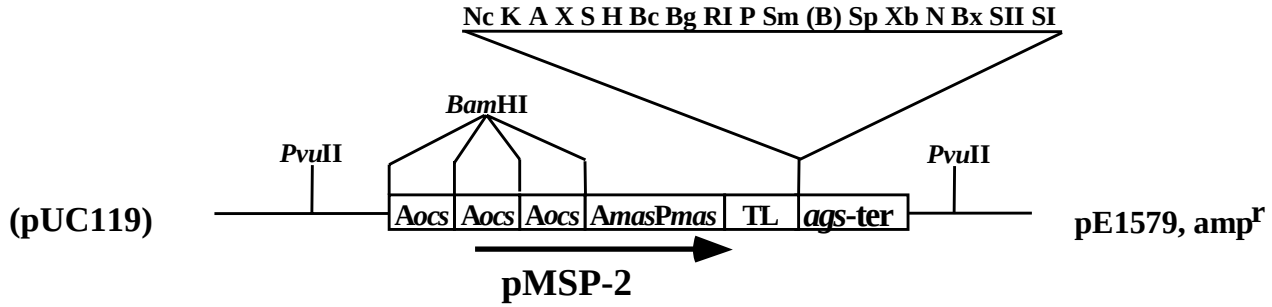
Figure 4. Comparison of the relative strengths of various promoters in the leaves and roots of transgenic maize plants. *Ubi*, maize ubiquitin promoter. n, number of individual transgenic plants analyzed. The large standard deviation most likely reflects the "position effect" of T-DNA integration upon transgene activity in independent transgenic events.

Figure 5. Constructions used for maize BMS protoplast transfection experiments. *nos*, nopaline synthase polyA addition signal sequence; p*Ubi*, maize ubiquitin promoter.

Figure 6. Comparison of the relative strengths of various promoters (lacking or containing introns in the *gusA* gene) in transiently transfected maize BMS protoplasts. Numbers represent the average of four independent experiments, with standard deviation. C, control construction lacking a promoter; *mas*, *mas2'* promoter, 35S, CaMV 35S promoter; sp, super-promoter.

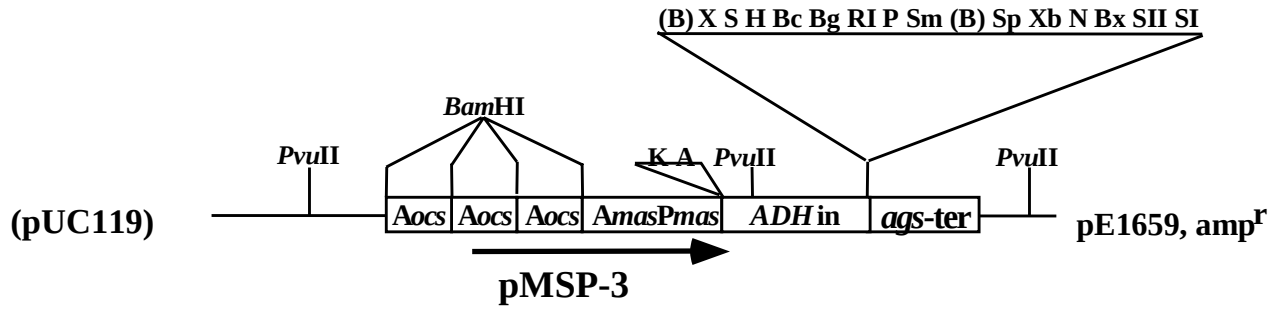
Figure 7. Effect of the maize ubiquitin intron upon promoter activity in stably transformed maize leaves. n, number of individual transgenic plants analyzed. -, constructions lacking the maize ubiquitin intron; +, constructions containing the maize ubiquitin intron. The large standard deviation in the data from maize plants containing the maize ubiquitin promoter plus intron construction most likely reflects the "position effect" of T-DNA integration upon transgene activity in independent transgenic events.

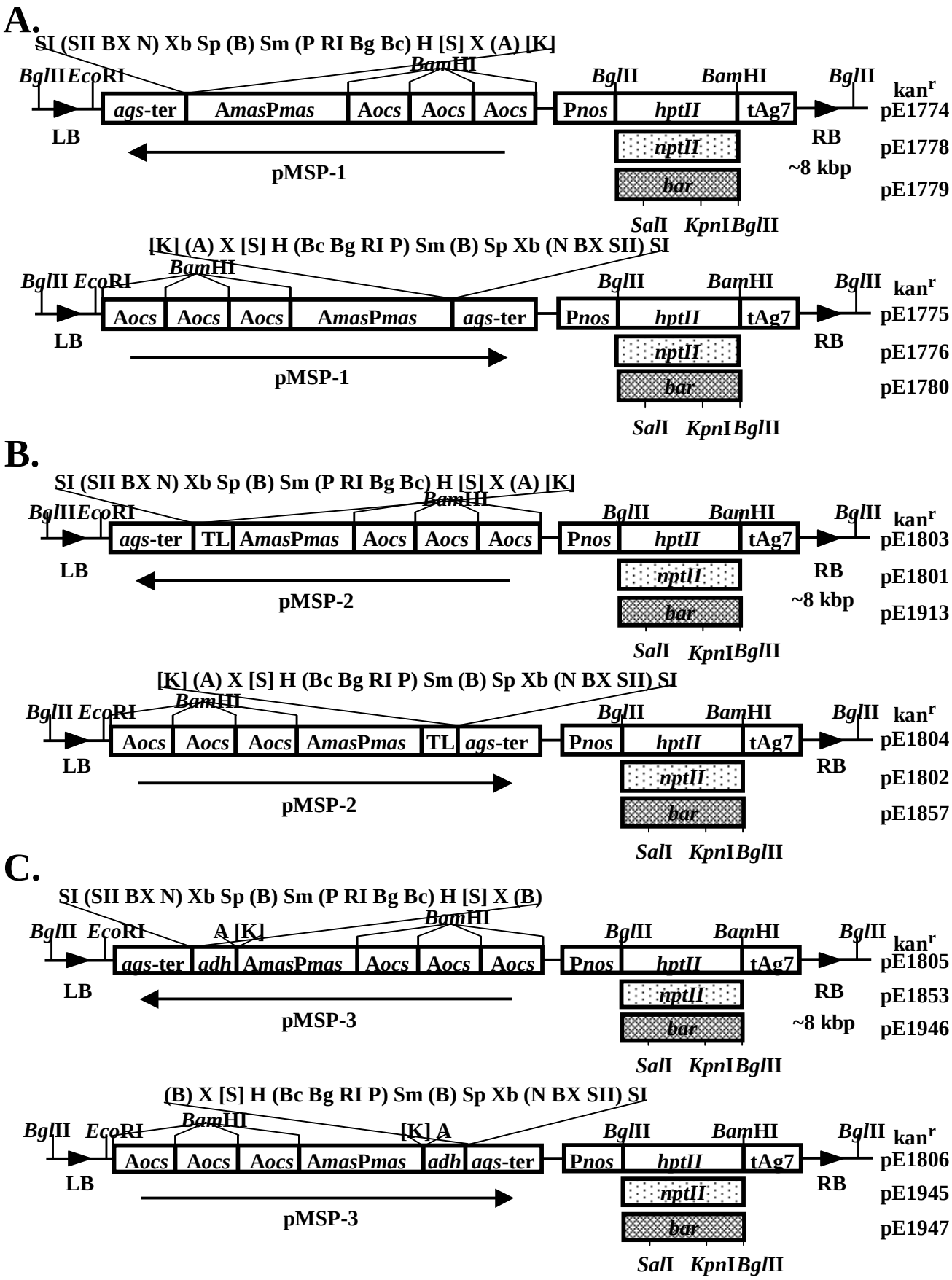




NcoI ApaI XhoI SalI HindIII BglII
GCC ATG GTA CCG GGC CCC CCC TCG AGG TCG ACG GTA TCG ATA AGC TTG ATC AGA TCT GAT
KpnI BclI

EcoRI PstI SmaI (BamHI) SpeI XbaI NotI SacII SacI
CGA ATT CCT GCA GCC CGG GGG ATC CAC TAG TTC TAG AGC GGC CGC CAC CGC GGT GGA GCT C
BstXI




A.
SI (SII BX N) Xb Sp (B) Sm (P RI Bg Bc) H [S] X (A) [K]
BglII EcoRI
BamHI
BglII
BamHI
BglII
kanr
ags-ter
AmasPmas
Aocs
Aocs
Aocs
Pnos
hptII
tAg7
pE1774
LB
RB
nptII
pE1778
pMSP-1
~8 kbp
bar
pE1779
SalI
KpnI
BglII
[K] (A) X [S] H (Bc Bg RI P) Sm (B) Sp Xb (N BX SII) SI
BglII EcoRI
BamHI
BglII
BamHI
BglII
kanr
Aocs
Aocs
Aocs
AmasPmas
ags-ter
Pnos
hptII
tAg7
pE1775
LB
RB
nptII
pE1776
pMSP-1
bar
pE1780
SalI
KpnI
BglII
B.
SI (SII BX N) Xb Sp (B) Sm (P RI Bg Bc) H [S] X (A) [K]
BglII EcoRI
BamHI
BglII
BamHI
BglII
kanr
ags-ter
TL
AmasPmas
Aocs
Aocs
Aocs
Pnos
hptII
tAg7
pE1803
LB
RB
nptII
pE1801
pMSP-2
~8 kbp
bar
pE1913
SalI
KpnI
BglII
[K] (A) X [S] H (Bc Bg RI P) Sm (B) Sp Xb (N BX SII) SI
BglII EcoRI
BamHI
BglII
BamHI
BglII
kanr
Aocs
Aocs
Aocs
AmasPmas
TL
ags-ter
Pnos
hptII
tAg7
pE1804
LB
RB
nptII
pE1802
pMSP-2
bar
pE1857
SalI
KpnI
BglII
C.
SI (SII BX N) Xb Sp (B) Sm (P RI Bg Bc) H [S] X (B)
BglII EcoRI
A [K]
BamHI
BglII
BamHI
BglII
kanr
ags-ter
adh
AmasPmas
Aocs
Aocs
Aocs
Pnos
hptII
tAg7
pE1805
LB
RB
nptII
pE1853
pMSP-3
~8 kbp
bar
pE1946
SalI
KpnI
BglII
(B) X [S] H (Bc Bg RI P) Sm (B) Sp Xb (N BX SII) SI
BglII EcoRI
BamHI
[K] A
BglII
BamHI
BglII
kanr
Aocs
Aocs
Aocs
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adh
ags-ter
Pnos
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bar
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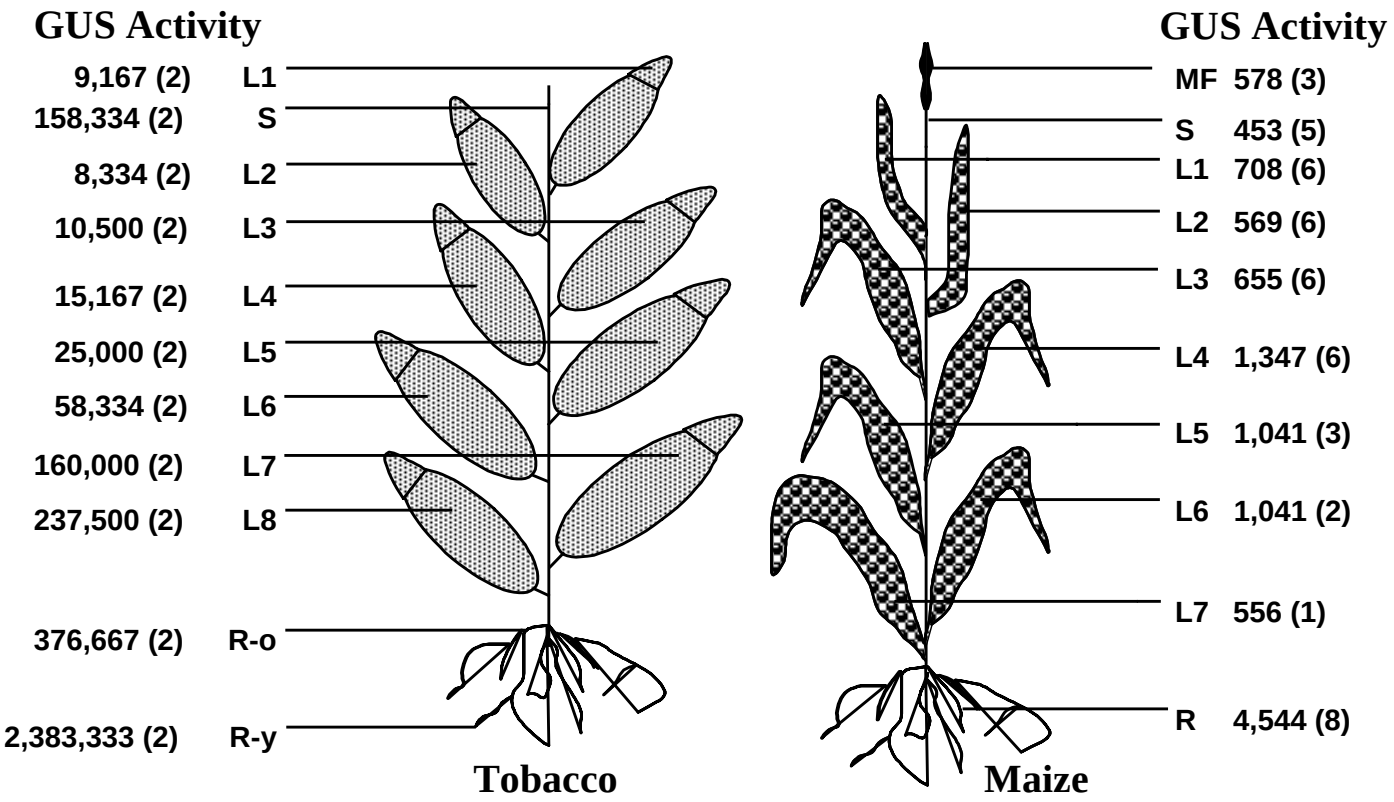
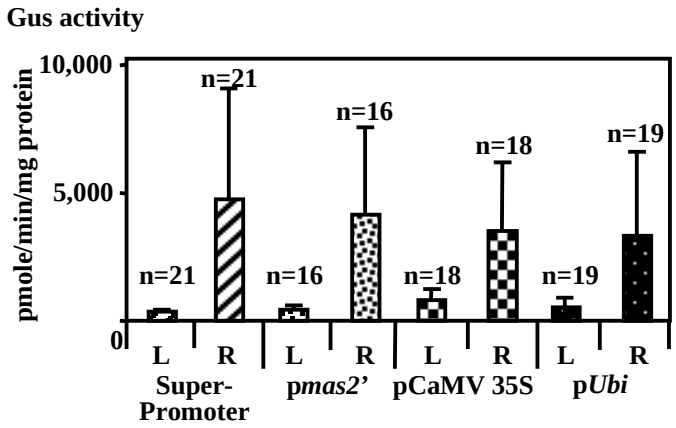
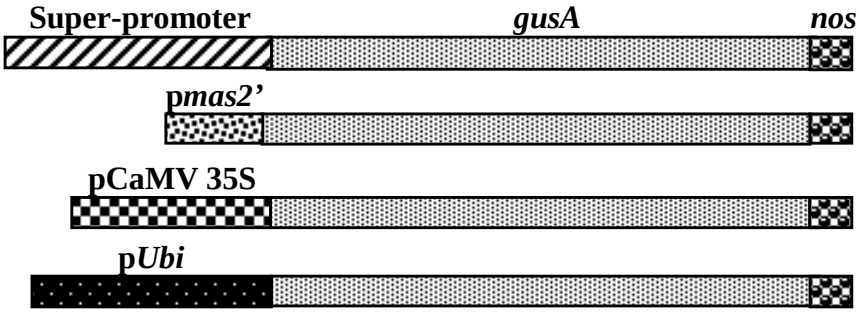
SalI
KpnI
BglII

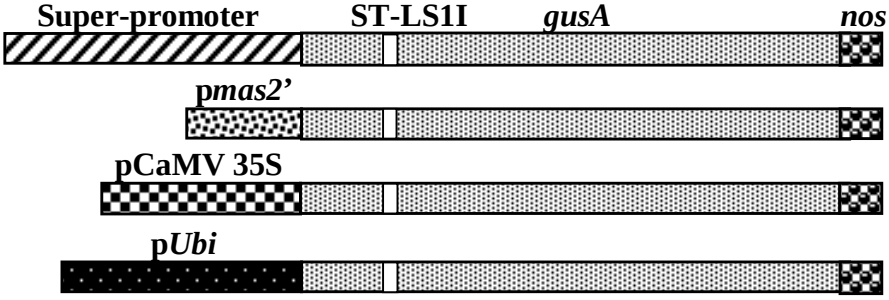

GUS Activity
9,167 (2) L1
158,334 (2) S
8,334 (2) L2
10,500 (2) L3
15,167 (2) L4
25,000 (2) L5
58,334 (2) L6
160,000 (2) L7
237,500 (2) L8
376,667 (2) R-o
2,383,333 (2) R-y
Tobacco
GUS Activity
MF 578 (3)
S 453 (5)
L1 708 (6)
L2 569 (6)
L3 655 (6)
L4 1,347 (6)
L5 1,041 (3)
L6 1,041 (2)
L7 556 (1)
R 4,544 (8)
Maize


Gus activity
pmole/min/mg protein
10,000
5,000
0
n=21
n=21
n=16
n=16
n=18
n=18
n=19
n=19
L
R
L
R
L
R
L
R
Super-Promoter
p*mas2'*
pCaMV 35S
p*Ubi*

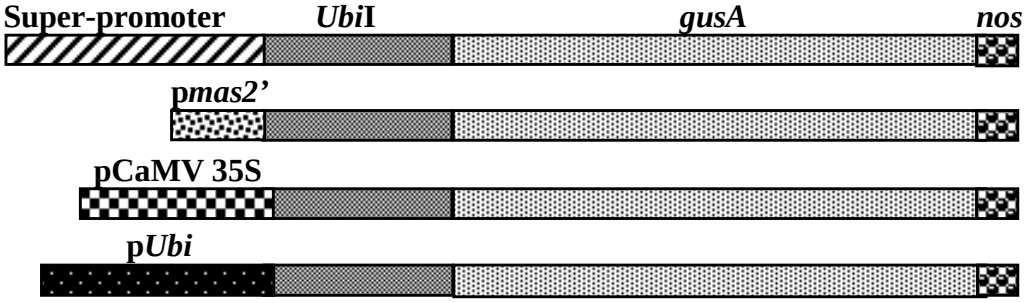
I. No Intron



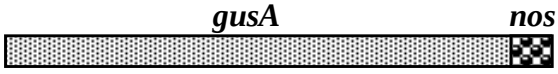
II. ST-LS1 Intron

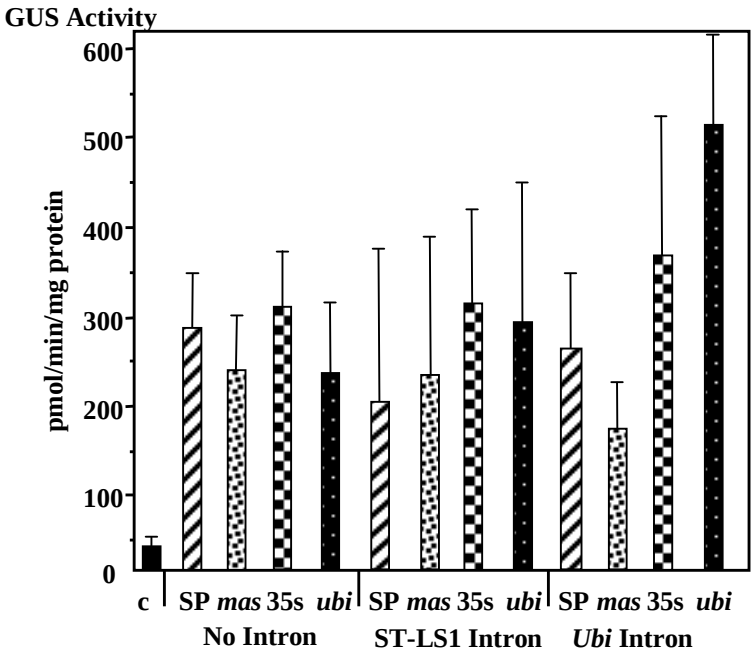


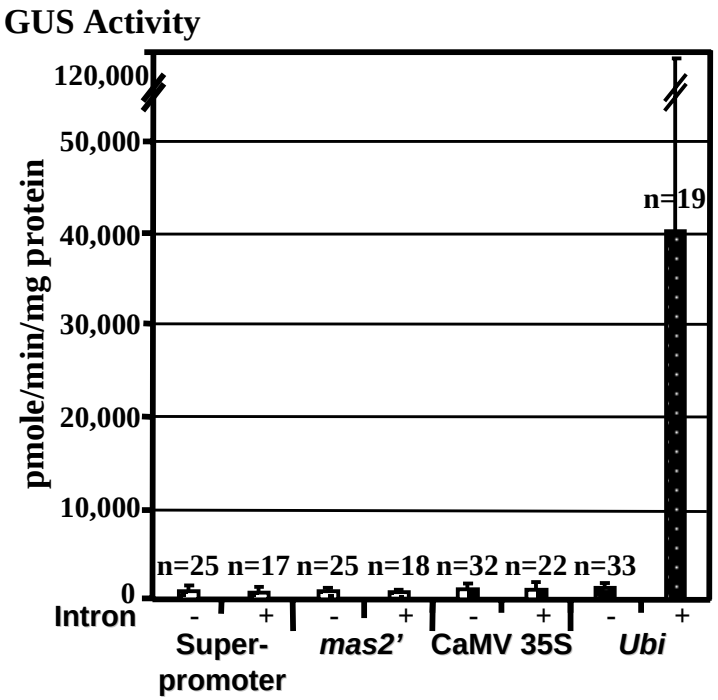
III. Ubiquitin Intron



IV. Promoterless *gusA*




GUS Activity
pmol/min/mg protein
600
500
400
300
200
100
0
c
SP
mas
35s
ubi
SP
mas
35s
ubi
SP
mas
35s
ubi
No Intron
ST-LS1 Intron
Ubi Intron


GUS Activity
120,000
50,000
40,000
30,000
20,000
10,000
0
pmole/min/mg protein
n=19
n=25 n=17 n=25 n=18 n=32 n=22 n=33
Intron
- + - + - + - +
Super-promoter
mas2'
CaMV 35S
Ubi