Abstract # TCM2016.50

GUS gene from plant genome and it's potential uses

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Introduction: A GUS gene from rice genome has been cloned and examined for its utility. Unlike the widely used GUS from *E. coli* which is an exo-glucuronidase belonging to glycoside hydrolase family 2, plant-GUS is an endo-glucuronidase (glycoside hydrolase family 79). The gene is about 1.5kb coding for a predicted 52 kDa protein and has a signal sequence for secretion out of the cell and is homologous to heparanase group of enzyme of vertebrates.

The function of this gene in plants remains to be proven, though literature evidences suggest its involvement in cell elongation (needed for elongation of organs). In this poster, evidences which indicate the involvement of plant GUS in cell separation events (required for abscission, dehiscence, germination *etc)* are presented. Further, the utility of the plant GUS (a) as a visual marker to rapidly identify Monsanto proprietary traits in commercial fields (b) as a screenable marker *vis a vis E. coli* derived GUS (c) and as a target for suppression of cell elongation / cell separation for creating traits like dwarf plants or reduced pod shattering phenotype would be discussed.

GUS gene from rice was cloned and expressed in corn

R0 generation corn

Cob (R0 plant)

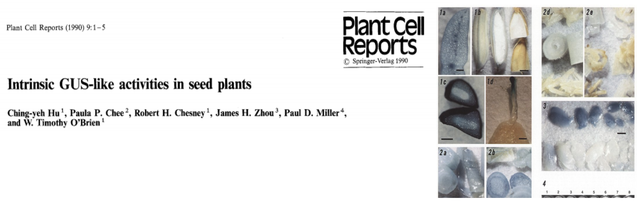
Predicted aminoacid sequence of plant-GUS

Signal sequence for secretion

MGFRVCVIVVFLGCLLLVPEKTMAQEMKRASIVIQGARRVCETDEN
FVCATLDWWPHDKCNYDQCPWGYSSVINMDLTRPLLTKAIKAFKPL
RIRIGGSLQDQVIYDVGNLKTPCRPFQKMNSGLFGFSKGCLHMKRW
DELNSFLTATGAVVTFGLNALRGRHKLRGKAWGGAWDHINTQDFLN
YTVSKGYVIDSWEFGNELSGSGVGASVSAELYGKDLIVLKDVINKVYK
NSWLHKPILVAPGGFYEQQWYTKLLEISGPSVVDVVTHHIYNLGSGN
DPALVKKIMDPSYLSQVSKTFKDVNQTIQEHGPWASPWVVGESGGAY
NSGGRHVSDTFIDSFWYLDQLGMSARHNTKVYCRQTLVGGFYGLLE
KGTFVPNPDYYSALLWHRLMGKGVLAVQTDGPPQLRVYAHCSKGRA
GVTLLLINLSNQSDFTVSVSNGINVVLNAESRKKKSLLDTLKRPFSWIG
SKASDGYLNREEYHLTPENGVLRSKTMVLNGKSLKPTATGDIPSLEPV
LRSVNSPLNVLPLSMSFIVLPNFDASACS

Transgenic corn over-expressing plant-GUS did not show any abnormal phenotype in R0 generation

plant GUS activity was previously thought to be an artifact



Plant Cell Reports (1990) 9:1–5

Plant Cell Reports
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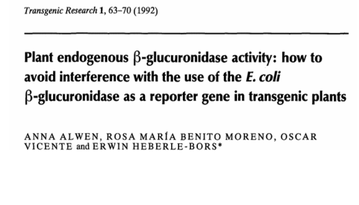
Intrinsic GUS-like activities in seed plants

Ching-yeh Hu[1], Paula P. Chee[2], Robert H. Chesney[1], James H. Zhou[3], Paul D. Miller[4], and W. Timothy O'Brien[1]



Transgenic Research 1, 63–70 (1992)

Plant endogenous β-glucuronidase activity: how to avoid interference with the use of the *E. coli* β-glucuronidase as a reporter gene in transgenic plants

ANNA ALWEN, ROSA MARÍA BENITO MORENO, OSCAR VICENTE and ERWIN HEBERLE-BORS*

Plant GUS has since been cloned but has not received much attention

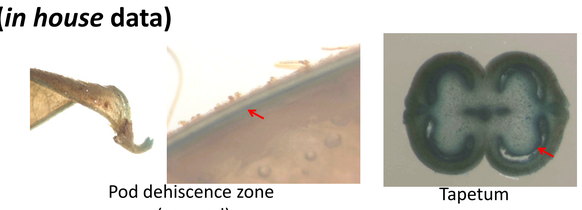
J Biol Chem (2000),275:27466–27472 from *Scutellaria baicalensis*
Plant Cell Physiol (2008). 49(9): 1331–1341 from Arabidopsis

•Unlike *E. coli* GUS (an exo-glucuronidase of 'glycoside hydrolase family 2'), plant-GUS is an endo-glucuronidase (glycoside hydrolase family 79) homologous to heparanases of vertebrates.

•The gene is about 1.5kb coding for a predicted 52 kDa protein and has a signal sequence for secretion out of the cell. **Enzyme can only be detected in acidic pH (4 – 5) assay conditions.**

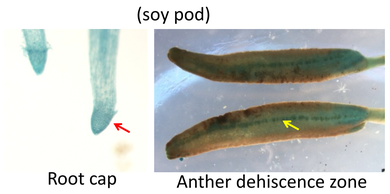
Function of plant-GUS is unknown

Plant GUS expression in wild plants is associated with cells which undergo 'cell separation' (*in house* data)



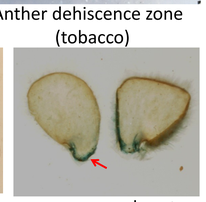
Pod dehiscence zone (soy pod)

Tapetum (tobacco)

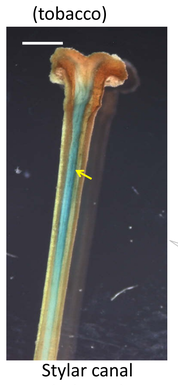


Root cap (tobacco)

Anther dehiscence zone (tobacco)



Radicle emergence zone on seed coat (chili and tomato)



Stylar canal (tobacco)

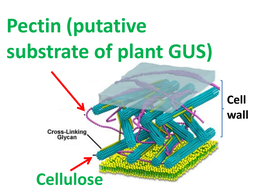
Native plant GUS expression has been linked to cells undergoing elongation' (*Planta*, *224*(4), 853)

Elongating root hairs of Brassica show high activity (a-b) which drastically reduces in mature hairs.
Elongating cotton fibers show high activity (d-e) which disappears in mature fibers (f). (g) Activity localised to elongating zone of rice stem.

Regions where cell separation occur
1. Dehiscence zone
2. Tapetum degeneration area
3. Root cap forming cells
4. Stylar canal cells
5. Radicle emergence zone of seed coat

Probably plant GUS is involved in cell wall loosening

•Cell separation and cell elongation events involve wall loosening.
•Literature indicates that glucuronic acids are constituents of pectin components of cell wall.
•Plant GUS has signal peptide for secretion, hence probably is secreted in to cell wall
Pectin cleavage by secreted plant GUS might be one of the events that is required for wall loosening during cell elongation or cell separation.



Plant cell wall

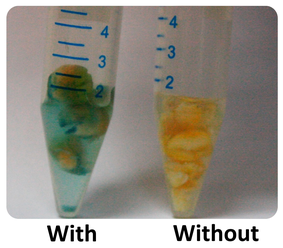
Plant-GUS has potential utility

1. As a quick 'verification tool' of Monsanto proprietary traits

Linked to Monsanto' traits, the presence or absence of traits could potentially be detected in fields or granaries within 15 mins in a cost effective manner from a large number of samples

Pros:
•Gene is of plant origin, perhaps more acceptable
• Assay cost <5 cents / data point (trait identification dip sticks cost a few dollars)
• Assay takes ~15 mins and can be conveniently carried out anywhere.

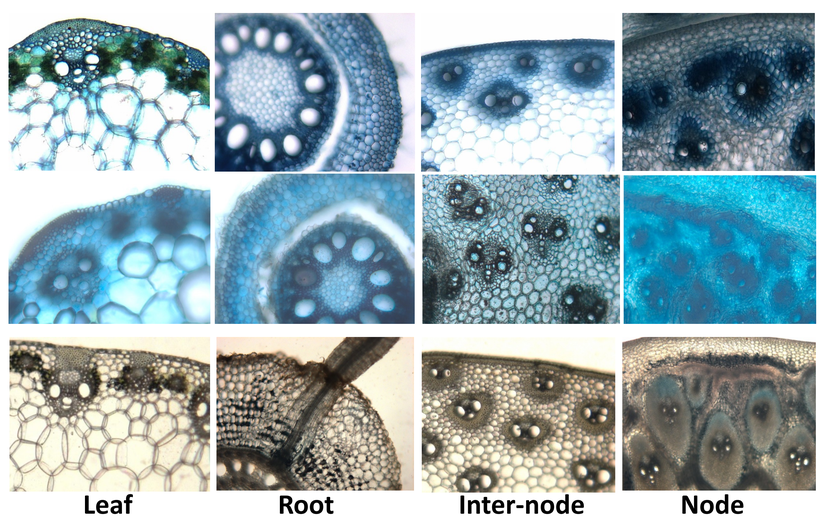
Cons:
• Cannot be used for existing products
• Creates a case of additional gene in the product



With plant GUS **Without plant GUS**

Corn seeds

2. As an alternative visual marker for gene expression studies:



Transgenic corn expressing Plant GUS under Ubiquitin promoter (mature tissue)

Transgenic corn expressing *E. coli* GUS under Ubiquitin promoter (mature tissue)

Non-transgenic control corn plant (mature tissue)

Leaf **Root** **Inter-node** **Node**

Pros: 1. Plant-GUS is secreted into cell wall offering the following advantages:
(a) accumulation of the colored product in wall enhances the delineation of individual cells (compare the sections with *E. coli* GUS above where the enzyme is localized in cytoplasm).
(b) Non –destructive assay possible (unlike with *E. coli* GUS) as the enzyme is secreted
(c) Membrane permeability issue of X-Gluc (the charged substrate) does not arise

2. Enzyme is resistant to fixatives like formaldehyde (data not shown) hence suitable for electron microscopy

Cons: Though native activity of plant-GUS is negligible in cells of mature tissues (see above), it could interfere in cells undergoing elongation or cell separation

3. As a target for changing plant morphology

• Dwarf phenotype through suppressing the plant GUS in elongation zone
• Reduced pod shatter phenotype through suppressing the enzyme in pod abscission zone

Note: Study was part of innovative efforts at MRC, Bangalore.