大豆内源的类GUS活性*

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摘 要

Jefferson 等(1986)提出用 β—葡糖苷酸酶(GUS)基因作为基因整合的标志基因。本文用组织化学和荧光定性等方法分析,检验大豆及基野生种(*Soja*)种子和植株内的类 GUS 活性。组织化学分析结果表明,栽培大豆的成熟植株和幼苗叶片以及野生种的幼苗叶片均表现为阴性反应。而不同发育时期未成熟豆荚的4个部分(荚皮、种皮、胚乳、胚)都表现强的组织化学着色反应。栽培品种和 *Soja* 种的成熟种子的子叶、胚芽、下胚轴、胚根也表现阳性反应。维管束较其周围组织表现出更强的着色反应。大豆类 GUS 活性存在于成熟干种子的任何部分。着色的深度在种子萌发初期增加,到4天后则迅速下降,到第10天,幼苗的任何部分不再呈现阳性反应。未成熟胚表现强的类 GUS 着色反应,但在培养1～2天后着色反应消失。定性荧光分析结果表明,各发育时期的大豆种子均表现阴性反应但反应强度不及阳性对照,而阴性对照无反应。用标准的 ELISA(依丽沙)方法分析结果表明,所有阳性对照的细胞具有抗 GUS 活性,而所有样本均无抗 GUS 活性。这表明大豆种子及其组织内的控制类 GUS 活性的蛋白质与大肠杆菌的 GUS 酶没有抗原相似性。因此,在转基因大豆植株里分析大肠杆菌的 GUS 活性反应是可能的。

INTRINSIC GUS—LIKE ACTIVITIES IN SOYBEAN

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* 本文于1991年2月21日收到。 This paper was received on Feb. 21, 1991.

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The *beta*—glucuronidase (GUS) gene of *E. coli* has been developed as a genefusion marker for higher plants by Jefferson et al. (1986). The objective of this report is to document and alert workers in soybean research about the presence of intrinsic GUS—like activity in cultivated soybean and its wild relative *soja*.

Seeds and plants of soybean [*Glycine max* (L.) Merr. cv.] Williams 82 and Pella and the wild relative *Glycine soja* Sieb. & Zucc P. I. 65.549 were tested. Tissues were surface disinfected and tested under aseptic conditions with histochemical and qualitative fluorometric procedures.

Experimentation and Results

Histochemical GUS Assay——Tissues from different seedlings, mature plants, young and mature seeds of each soybean cultivar and mature seeds and seedlings of *soja* were tested. Lesves were surface disinfected by dipping in 70% ethanol for one minute. After the ethanol evaporated, the leaves were cut into 1～2 mm wide strips in a laminar flow hood. Fruits and seeds were surface disinfected by soaking in 10% Clorox for ten minutes followed by rinsing with three changes of sterile distilled water in a laminar flow hood. Fruits and seeds were dissected and part of the fruit wall, seed, and embryo were cut into thin sections.

A modified Jefferson's (1987) procedure was followed. The testing solution contained 2 mM substrate, X—Glu, in a pH 7.0 phosphate buffer (29.3 mM K_2HPO_4+20.7mM KH_2PO_4) in which 10 mM EDTA and oxidation catalysts of 0.5 mM potassium ferricyanide and 0.5 mM potassium ferrocyanide were added. The sterile tissues were incubated with filter sterilized testing solution overnight in a 37°C dark incubator. The chlorophylls, if present, were cleared by soaking the tissues in 95% ethanol before data taken.

The seedling and mature plant leaves of both tested soybean cultivars and the seedling leaves of *soja* expressed negative intrinsic GUS—like reactions with histochemical tests.

The immature soybean fruits of various developmental stages showed strong histochemical staining reactions in all four tested parts: fruit walls, seed coats, endosperms and embryos. Positive reactions were also found in the cotyledons, plumules, hypoctyls, and radicals of mature seeds of soybean cultivars and *soja*. Usually, the vascular bundles expressed stronger staining activities than the surrounding tissues in both young and mature tissues.

Changes in the pattern of intrinsic GUS—like activities during seed germination——Sterile seeds of soybean cv. Williams 82 were germinated under aseptic conditions. Histochemical GUS tests performed on the seed sections or germinated seedlings at the 0,2,4,6,8,10,12, and 14th day of germination.

Table 1 Change in intrinsic GUS—like activities via histochemical test during soybean cv. Williams 82 seed germination

DAYS UNDER GERM COND	PL. PARTS			
	PLUM	COTY	HYPO	RADI
0	++	++	++	++
2	++	++	++	++
4	+++	+++	+++	+++
6	−	++	+	−
8	−	+	−	−
10	−	−	−	−
12	−	−	−	−

PLUM—plumule; COTY—cotyledon; HYPO—hypocotyle; RADI—fadical of tap root.

The intrinsic GUS—like activities existed in every part of mature dry seeds of soybean. The intensities of staining increased during the early period of the germination process then dropped rapidly after the fourth day of germination. Practically no detectable positive staining reactions were found on any part of the seedlings by the tenth day of germination (Table 1).

Changes in intrinsic GUS—like activities of immature embryos during in *vitro* culture——Immature pods of soybean cv. Pella were harvested and surface disinfected. Embryos, ranging from heart to early cotyledon stages, were excised and cultured in modified B_5 liquid medium (Hu and Sussex, 1986). Histochemical GUS tests were performed at the 0, 1, 2, 3, 5 and 7th day of culturing.

Immature embryos expressed strong GUS — like staining activities at dissection. All the staining activities of smaller embryos and most of the staining activities of larger embryos disappeared after one to two days of in *vitro* culture. By the fifth and seventh days, only small blue spots infrequently appeared on certain larger embryos.

Qualitative **Fluorometric GUS Assay**—Seeds of soybean cv. Pella at various developmental stages (0.5, 1, 2, 3, 4, 5, and 6 mm in length plus mature dry seeds) were tested along with leaves of corn (negative controls) and E. coli GUS gene containing transgenic tobacco (positive controls).

Tissue extract in MUG contaning lysis buffer was incubated at 37°C for 0 and 24 h for soybean embryos. The blue fluorescence was observed visually with a long—wave UV light box.

Soybean seeds expressed positive reactions at all tested developmental stages, but the intensities were not as strong as those of transgenic tobacco leaves (the positive control). The negative controls, the sweet corn leaves, showed no activity.

ELISA GUS Assay—Standard ELISA procedure was carried out to assay the presents of E. coli GUS in soybean tissue with a dilution series of purified E. coli GUS as the positive control.

Immature seeds of 3, 5, 7 and 12 mm in length were sampled. The detection level of the assay was as low as 7.2 ng.

Anti—GUS activity was detected in all of the positive control wells. No anti—GUS activity was detected in any of the test samples assayed.

Discussion

Fifty—two plant species, covering some Gymnosperms and all the key groups of Angiosperms, were chosen by us for surveying their intrinsic GUS—like activities (Hu et al. 1990). Histochemical (overnight incubation) and qualitative fluorometric (24 h incubation) assays indicated that, with few exceptions, such activities were detected in certain part(s) of the fruit walls, seed coats, endosperms or, especially, the embryos of the tested plants. Only 11 species shown such activities (usually quite weak) in the vegetative organs of seedlings/mature plants. The expressing of intrinsic GUS—like activities in various plant species were also reported by Wenzler et al. (1989), Plegt and Bino (1989), and Alwen et al. (1990).

The intrinsic GUS—like activities in the seeds diminished during soybean germination process. The same phenomenon was also observed in the germinating string bean seeds (Hu et al. 1990). This might be the reason that Jefferson et al. (1987) did not detect such activities in the vegetative organs of the species he tested. It is possible that this activities may not completely disappear even at the mature phase of the plant life (see the previous paragraph). High level of activities reappear at the on set of reproductive phase, as demonstrated during anther (Plegt and Bino 1990), fruit and seed developments.

In *vitro* culture conditions quickly turned off most of the intrinsic GUS—like activities in excised immature soybean embryos. But our works on histochemical GUS assay of various types cultured soybean tissues indicated that in *vitro* culture conditions did not always turn off such activities (unpublished data). These data suggested that the microenvironment around the cell played an important role in the expression of gene(s) for such activities.

Since the ELISA tests were negative, it appears that the protein(s) responsible for the intrinsic GUS—like activity in the soybean seede and other plant tissues is not antigenically similar to the *E. coli* GUS enzyme. Alwen et al. (1990) found that about 50—fold higher concentration of saccharic acid 1, 4—lactone is required to inhibit the plant GUS activity compared to the *E. coil* GUS. They also found the pH optima is 5.0 for plant GUS and close to neutral for the bacterial enzyme. We found that in soybean the histochemical staining patterns carried out at pH 7 are general and dispersed with weak intensities. The true transgenic GUS staining patterns, on the other hand, have well—defined area of transformed cells and highly intensive in colorations (Hu et al. 1990). Therefore, it should be possible to assay the *E. coli* GUS in transgenic soybean plants.

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