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EFFECT OF MONOCOT INTRONS ON TRANSGENE EXPRESSION IN DICOT *Nicotiana* PLANTS

I. O. Nitovska
M. Yu. Vasylenko
B. V. Morgun

Institute of Cell Biology and Genetic Engineering
of the National Academy of Sciences of Ukraine, Kiyv

E-mail: molgen@icbge.org.ua

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The aim of the work was to study the effect of introns of the rice *OsAct1* and the maize *hsp70* genes on transgene expression in *Nicotiana* plants in order to find out their use in the verification of vectors containing these monocot introns. The following methods were used: *Agrobacterium*-mediated transformation of leaves of greenhouse *N. benthamiana* and *N. tabacum* plants by vector pCB271 containing the introns of cereals, light fluorescence microscopy and fluorimetry of Green Fluorescent Protein (GFP). The presence of transgenes was detected by polymerase chain reaction. The transient GFP expression was observed in infiltrated tissue of *N. benthamiana*. Transgenic plants of *N. tabacum* resistant to kanamycin were obtained. Fluorescence of GFP in extracts of some transgenic tobacco lines was shown. The impairment of the transgene expression in some *N. tabacum* transformants has been observed. Eventually, transgenes, containing introns from the *hsp70* corn or from the *OsAct1* rice genes downstream the promotor, were expressed in *Nicotiana* plants. Thus, *N. benthamiana* and *N. tabacum* plants can be used to test vector constructs for cereals transformation. It has been shown that the monocot introns can have negative impact on the transgene expression in *Nicotiana* plants.

Key words: monocot introns, *Nicotiana*, *Agrobacterium*-mediated transformation, GFP, genetically modified organisms.

Genetic transformation of cereals is a powerful tool to improve existing varieties, to increase their economic attractiveness in cultivation [1]. One of the ways to boost the efficiency of transformation of monocotyledonous plants is to create adapted vector designs with special promoters and regulatory elements aimed at enhancing the expression of transgenes. It is shown that the use of regulatory elements that work well in dicotyledonous plants is not sufficiently effective in monocotyledons [2–4]. Therefore, a number of promoters for the expression of transgenes in monocot have been isolated and been adopted, namely: corn ubiquitin gene promoter (*ZmUbi1*) [5], corn sucrose synthase gene promoter (*sh1*) [6], promoters of rice genes of actin 1 (*OsAct1*) [2] and actin 2 (*OsAct2*) [7] and others [8]. Often, for effective transgene expression, promoters of monocotyledonous, as promoters of corn genes *adh1*, *ubi1*, *sh1* and rice *OsAct1*, require the presence of their first intron in a 5′ non-translated region [5, 6, 9]. The monocot introns themselves, embedded after "non-native" promoters, can also act as separate regulatory elements to increase the expression of transgenes in monocotyledonous plants [10, 11]. This phenomenon known as IME (intron-mediated enhancement) was observed not only for monocots, but also for dicots, and in general for a wide range of organisms, including mammals and invertebrates [12]. Increasing levels of transgene expression due to introns may be more than 100 times [6], but more often it is 2–10 times.

One of the first regulatory elements of the gene expression that was used to create effective vectors for the genetic transformation of monocots was the 5′-region of the rice actin 1 gene (*OsAct1*) [2]. It has been shown that the first intron of the *OsAct1* rice gene can regulate the expression of genes under the control of another promoter in the

absence of the "native" one, even with greater efficiency [9].

Before using genetic constructs to transform target species, it is desirable to test them on model objects, such as *Nicotiana tabacum* or *N. benthamiana*. *Nicotiana* species are dicotyledonous plants, so it is likely that the expression of vectors created for the transformation of monocots will not happen in *Nicotiana* plants. For example, there was a decrease in expression levels of transgenes that were under the control of transcriptional elements of cereals in tobacco cells, as compared to cells once obtained after transformation with vectors that did not contain these elements [6, 10, 11, 13]. Thus, the purpose of our work was to study the possibility to use *Nicotiana* species to test vectors that contain the intron of the maize *hsp70* (heat shock protein 70) gene and the intron 1 from the rice *OsAct1* gene.

In the investigation we utilized the pCB271 binary vector, containing in its T-DNA *npt*II (neomycin phosphotransferase II) gene and *S65Tpgfp* (green fluorescence protein) gene, both under the control of the enhanced e35S promoter of the cauliflower mosaic virus, as well as the regulatory elements between the promoter and the coding part of the genes to elevate their expression in monocotyledons, namely: intron of the corn *hsp70* gene before *npt*II and intron 1 of the rice actin 1 gene (*OsAct1*) before the *S65Tpgfp* gene. To investigate the activity of the vector in *Nicotiana* plants, we have been carried out an agroinfiltration of leaves of *N. benthamiana* greenhouse plants to further study the transient expression of GFP, and have been conducted an *Agrobacterium*-mediated transformation of *N. tabacum in vitro* plants to obtain stable tobacco transformants and to study the presence and expression of *npt*II and *S65Tpgfp* transgenes in them.

Materials and Methods

Plant material. Greenhouse plants of *N. benthamiana* of the wild type 5–6 weeks old, grown under conditions at 20–25 °C and 14-hour photoperiod, were used in the experiments on agroinfiltration. For *Agrobacterium*-mediated transformation, *N. tabacum* plants cv. Petit Havana, grown under aseptic conditions on hormone free MS medium [14] at 25 °C and 16-hour mode of the illumination, were used.

Plasmids and bacterial strains. The binary vectors pCB271, pICH5290 and rICH6692 [15], which are in the collection of the Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine, were used in this work. The test vector pCB271 in its T-DNA region contained a selective neomycin phosphotransferase II (*npt*II) gene, a mutant reporter *S65Tpgfp* gene of green fluorescence protein (GFR) for the screening of transformation events, both under the control of the enhanced e35S promoter of the cauliflower mosaic virus, as well as regulatory elements between the promoter and the coding part of the named genes to enhance their expression in monocotyledons, namely: intron of the corn *hsp70* gene before the *npt*II gene and the intron 1 of the rice actin 1 gene (*OsAct1*) before *S65Tpgfp* (Fig. 1).

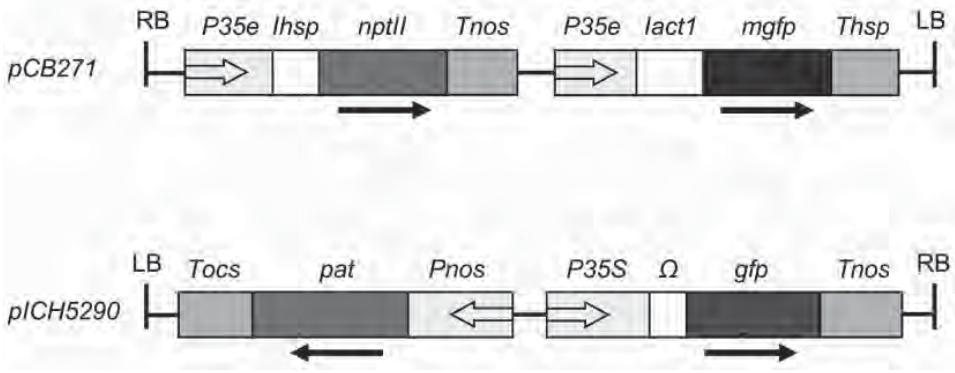


Fig. 1. **T-DNA regions of the plasmids used in the study:**
RB, LB — right and left T-DNA borders, P35e — enhanced 35S RNA gene promoter of the cauliflower mosaic virus, Ihsp — the corn *hsp70* gene intron, *npt*II — gene of neomycin phosphotransferase II, Tnos — the nopaline synthase gene terminator, Iact1 — the intron 1 of the rice *actin 1* gene, *mgfp* — mutant *S65Tpgfp* gene of the green fluorescence protein, Thsp — the wheat *hsp* gene terminator, Tocs — the octopine synthase gene terminator, *pat* — the phosphinothricin acetyltransferase gene, Pnos — the nopaline synthase gene promoter, P35S — 35S RNA gene promoter of the cauliflower mosaic virus, omega (Ω) — leader of TMV virus to enhance the translation, *gfp* — gene of green fluorescent protein wild type

The T-DNA of the control vector, pICH5290 (Fig. 1), had a selective phosphinothricin acetyltransferase gene (*pat*) and a wild type *gfp* reporter gene under the control of the 35S cauliflower mosaic virus promoter (CaMV35S). The vector pICH6692 contained a gene *p19* from tomato bushy virus, which encodes p19 protein, a suppressor of post-transcriptional gene silencing [16]. Plasmids were in *Agrobacterium tumefaciens* strain GV3101, which is derived from the strain C58 [17].

Agroinfiltration. In the experiments on agroinfiltration the night culture *A. tumefaciens* was used. The bacteria were grown on an orbital shaker at 28 °C, 200 rpm in 20 ml with LB medium [18] containing antibiotics, depending on the plasmid: vector pCB271 (50 mg/l kanamycin, 50 mg/l spectinomycin); vectors pICH5290 or pICH6692 (50 mg/l rifampicin, 25 mg/l gentamycin, 50 mg/l carbenicillin). The night culture of the bacteria was precipitated by centrifugation (5000×g, 5 min) and after removal of the supernatant was suspended in an infiltration buffer (10 mM $MgSO_4$, 10 mM MES pH 5.5) to an optical density (OD600) of 0.7–0.8 units. Bacterial suspensions containing the vectors pCB271 or pICH5290 were mixed with the bacterium containing the vector pICH6692 in equal proportions. Bacterial mixtures were injected into different parts of one leaf of *N. benthamiana* plant [15]. The plants continued to grow in greenhouse for 10 days after agroinfiltration.

Fluorescence analysis of GFP. The GFP accumulation in the infiltrated sections of the leaves was visually evaluated under UV illuminations (UVP, Upland, USA) over 3–7 days after infiltration. The fluorescing leaf sectors were marked to facilitate the identification of the infiltrated sections without UV light. Fluorometric measurements of GFP were carried out using a spectrofluorometer Fluorat-02-Panorama of the Lumex Company (St. Petersburg, Russia) both from the leaf surface and in plant extracts obtained from infiltrated areas. The excitation wavelength was 395 nm for the wild type GFP and 485 nm for the mutant GFP S65T [19]. The emission wavelength was 510–515 nm. The fluorescence results were fixed in standard units.

Preparation of plant extracts. The leaf tissue of 300 mg was milled in 3 ml of a cooled buffer (50 mM Tris-HCl, pH 8.0) and centrifuged at 4 °C, 16 000×g for 20 min. The supernatant was used for fluorometric measurements.

Fluorescence microscopy of GFP. Microscopic studies of leaf tissue and protoplasts isolated from infiltrated sectors of the *N. benthamiana* leaf were performed using a microscope Axiophot-35 (Carl Zeiss Microscopy, USA) with an excitation channel of 400–488 nm. Protoplasts for cytological studies were obtained by cultivating leaf strips at a width of 1 mm in a mixture of enzymes: 0.6% Onozuka R-10 (Duchefa, Netherlands), 0.6% Macerozyme R-10 (Duchefa, Netherlands) and 0, 2% Cellulysine (Calbiochem, USA) in 0.5 M sucrose, pH 5.7, at 27 °C overnight.

Transformation. The *Agrobacterium*-mediated genetic transformation of aseptic *N. tabacum* plants was carried out using "leaf discs" method [20], followed by regeneration on selective MS media containing 1 mg/l BAP, 0.1 mg/l NAA and antibiotics: 500 mg/l cefotaxime (Cx) for suppressing bacterial growth and 100 mg/l kanamycin sulfate (Km) for the selection of transgenic plants after transformation with the test pCB172 vector or 5 mg/l of phosphinothricin instead of kanamycin after transformation by the control pICH5290 vector. The cultivation of explants was carried out under aseptic conditions in a 16-hour photoperiod at 25 °C. The explants were transferred to a fresh regenerative medium every three weeks. After 8 weeks culturing, we calculated the frequency of regeneration (RF) in percent, which was equal to the number of explants that regenerated plants on the selective medium, to the total number of explants. The regenerants were placed in jars with the hormone free MS medium containing 500 mg/l Cx and 100 mg/l Km or 5 mg/l of phosphinothricin, depending on the vector used for transformation.

PCR analysis of regenerants for the presence of the transgenes. From the leaves of tobacco regenerants obtained after *Agrobacterium*-mediated transformation, as well as the untransformed plant have been isolated the total DNA using CTAB and PVP-40 [21]. To exclude the contamination of the plant material by *A. tumefaciens*, an amplification of the bacterial *vir*-D1 gene was carried out prior to the PCR assay on the transgenes [22]. The PCR on the *gfp* gene was performed using the following pair of primers: F — 5′-GACGT GAACG GCCAC AAGTT CA-3′ and R — 5′-CGATG CGGTT CACCA GGGTG T-3′, and on the *npt*II gene was used a pair of primers that was described earlier [23]. The amplification product of the part of the *vir*-D1 gene sequence should have been 432 pairs of

nucleotides (bp), gfp — 311 bp, nptII — 700 bp. The reaction mix for PCR (20 µl) contained 0.5 units of FIREPol® DNA polymerase (Solis BioDyne, Estonia), 2 µl of 10× buffer B, 1.6 µl of 25 mM $MgCl_2$, 200 µM of each dNTP, 0.1 µM of each forward and reverse primer and 30 ng of purified total DNA. As a positive control for *gfp* and *npt*II, the total DNA of the tobacco plants transformed by these genes was used, and for the *vir*-D1, the total DNA of *A. tumefaciens* was applied. The amplification program for the detection of the *gfp* gene was given as follows: the first cycle at 94 °C for 4 minutes followed by 34 cycles (denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, elongation at 72 °C for 19 s). The amplification program for detecting the sequence of the *npt*II gene was generated using the descending PCR technique: starting denaturation — 4 min at 94 °C; 9 cycles: 30 s at 94 °C, 45 s at 68 °C, 30 s at 72 °C; 26 cycles: 30 s at 94 °C, 30 seconds at 60 °C, 30 s at 72 °C. The final elongation was 5 min at 72 °C followed by rapid cooling to 22 °C. PCR was performed using the Mastercycler personal (Eppendorf, Germany) amplifier. The amplification products were separated by electrophoresis in 0.8% agarose gel with bromine ethidium (0.5 µg/ml) in LB buffer [24] at 6 U/cm for 90 min.

Statistical processing of the results was carried out according to the standard method [25].

Results and Discussion

The transient GFP expression in N. benthamiana plants. The effect of the first intron of the rice actin 1 gene (*OsAct1*) on the transient expression of GFP into the leaf blade of *N. benthamiana* after the agroinfiltration was investigated. The upper part of the *N. benthamiana* leaf was infiltrated with the test vector pSV271, which contained this transcriptional element between the promoter e35S and the nucleotide sequence of the mutant S65Tpgfp gene (Fig. 1). The control vector pICH5290, which did not contain the monocot introns before the coding sequence of *gfp* gene was injected into the tip of the leaf blade. After 3 to 6 days following the infiltration, a green fluorescence of GFP protein was observed in infiltrated areas of the leaf under ultraviolet illumination (Fig. 2).

The bright fluorescence of GFP in leaf sections infiltrated with the control vector was observed on the third day after agroinfiltration, whereas for the test vector, the fluorescence peak was on the 5–6 days. Under UV illumination, the GFP fluorescence in the leaf areas infiltrated by the test vector was less bright compared with zones infiltrated by the control vector (Fig. 2, *B*).

As a result of the fluorescence microscopy GFP, the expression of the vector pCB271 in the tissues (Fig. 3, *A*) and the protoplasts isolated from the *N. benthamiana* leaves infiltrated by the test vector was shown (Fig. 3, *B*).

GFP fluorescence in the leaf tissues agroinfiltrated with pCB271 vector evidenced an expression of the mutant *S65Tpgfp* gene in dicotyledonous *N. benthamiana* plants, despite the presence of the first inron of the rice *OsAct1* gene in its transcriptionally active region. After infiltration of the *N. benthamiana* leaves, we observed a delay of 2–3 days of expression of the test vector compared to the control, possibly due to the presence of a transcriptional element of cereals in the vector pCB271.

The GFP fluorescence of the leaf surface and the plant extracts of infiltrated zones was measured using a spectrofluorimeter. As a result of the fluorimetric measurements from the leaf surface, using the optimal excitation wave for each GFP (395 nm for wild GFP and 485 nm for mutant GFP S65T [19]), it was shown that the wild-type GFP fluorescence was higher compared to the mutant (Fig. 4). When measuring the GFP fluorescence of leaf extracts, we recorded a greater GFP fluorescence levels for extracts obtained from the leaf zones infected *A. tumefaciens* with the

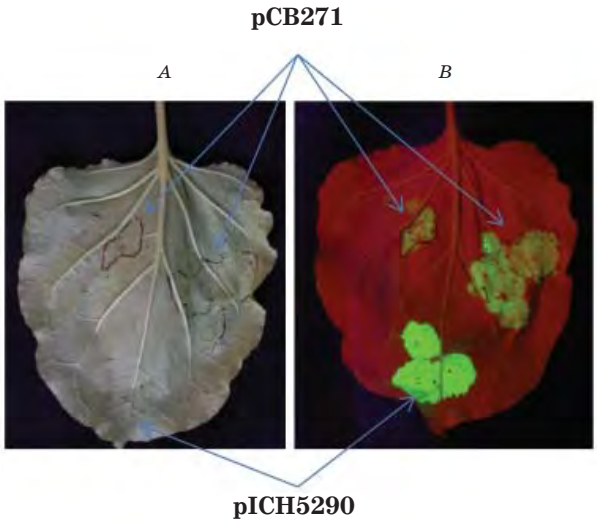


Fig. 2. **The appearance of the *N. benthamiana* lower leaf surface in 6 day after agroinfiltration by vectors pCB271 and pICH5290 in white (*A*) and ultraviolet (*B*) light**

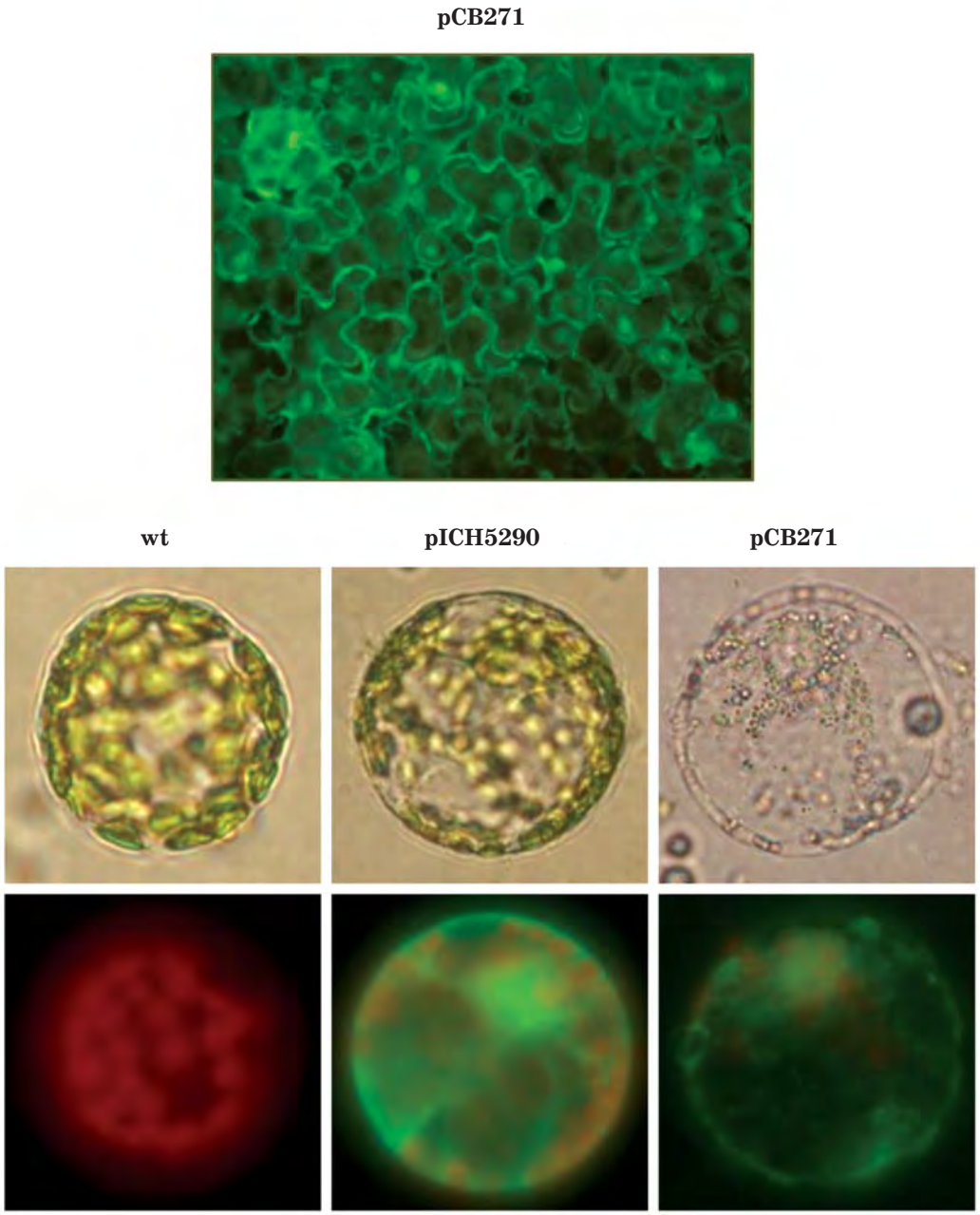
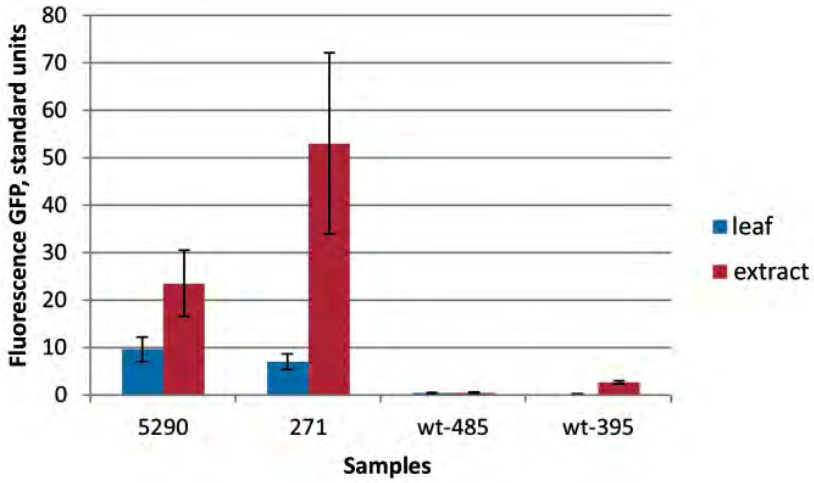


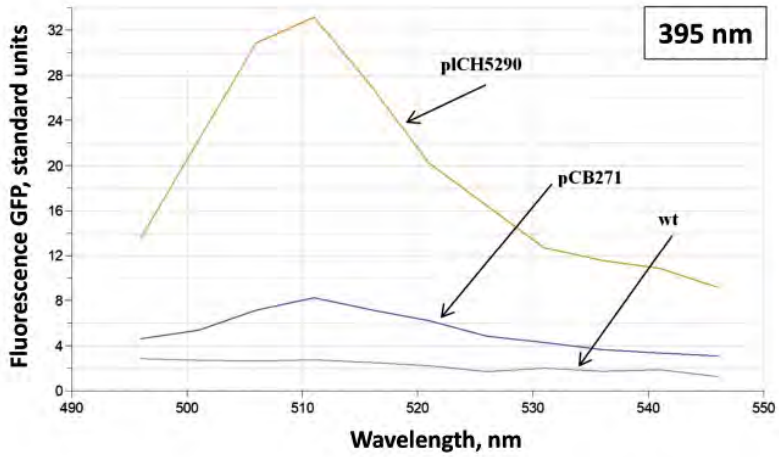
Fig. 3. **Microscopic studies of transient expression of GFP in *N. benthamiana* plants:**
A — a leaf tissue after infiltration *A. tumefaciens* with vector pCB271in UV light; *B* — protoplasts isolated from non-infiltrated sections of the leaf (wt) and from leaf areas infiltrated by the vector pICH5290 or pCB271 in ultraviolet (bottom) and white (top) light

test vector relative to the extracts obtained from the zones infiltrated by the control vector (Fig. 4, *A, B*).

According to the literature, the mutant form of the GFP S65T protein had 100 times more fluorescence than wild-type GFP when using a wavelength of excitation of 490 nm [19]. In our study, when using the excitation wavelength optimal for a mutant GFP, its fluorescence was only 5–7 times greater than the wild type GFP (Fig. 4, *B*), possibly due to the presence of rice intron in the transcriptionally active region of the mutant gene. In addition, the *S65Tpgfp* gene is controlled by the enhanced CaMV35S promoter, which should further increase the level of transgene expression. When fluorometric measurements were conducted from the leaf surface, using the excitation wave optimal for each protein, the fluorescence



A



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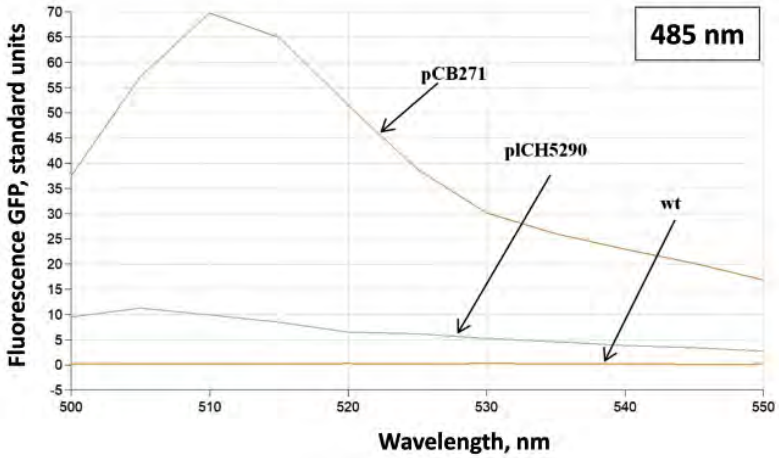

Fig. 4. **Flourimetry of GFP from leaf surface (*A*) and in leaf extracts (*A*, *B*) of *N. benthamiana* 6 days after agroinfiltration:**
5290 — leaf zones, infiltrated *A. tumefaciens* with vector pICH5290; 271 — leaf areas, infiltrated *A. tumefaciens* with vector pCB271; wt-395 — the fluorescence of wild-type non-infiltrated tissue at a wavelength of excitation of 395 nm; wt-485 — the fluorescence of non-infiltrated tissue at a wavelength of excitation of 485 nm. Diagram A shows the average values of GFP fluorescence and confidence interval at $P < 0.05$

of the mutant GFP correlated with such the wild type protein or was smaller, and only in the extracts we observed a slight increase (2–3 times) of the fluorescence of the mutant GFP compared with GFP of the wild type. In our opinion, low expressions of mutant GFP compared to the expected ones are probably due to the presence of a monoclonal transcriptional element above the encoding region of the gene and its negative effect on the expression of GFP in dicotyledonous *N. benthamiana* plants. Consequently, according to our data, the presence of the first intron of the rice *OsAct1* gene in the transcribed region of the vector worsens the expression of the gene in dicot plants of *N. benthamiana*, but it occurs, so this model object may be used for the preliminary testing of vectors that contain the named regulatory element, important for the successful heterologous expression of genes in monocotyledons.

Agrobacterium-mediated genetic transformation of tobacco. The next step of investigation was the obtaining transgenic plants using *A. tumefaciens* containing the vector pCB271 to study the effect of monocot introns on the transgene transcription in tobacco transformants. Two experiments on *Agrobacterium*-mediated transformation of tobacco were carried out (Table). The multiple regeneration of tobacco plants (from 3 to 10 per explant) on the selective media was observed after 4 weeks following transformation. Total DNA of 50 lines of regenerants resistant to kanamycin were tested for the availability of *npt*II and *gfp* genes by PCR method (Table). The presence of fragments of the expected size (311 bp or 700 bp) for both the *gfp* and *npt*II genes was shown (Fig. 5), as an absence of bacterial contamination of the investigated plant material.

In experiments on *Agrobacterium*-mediated transformation, a significant number of regenerated plants became sensitive to kanamycin during further cultivation on the selective medium (Table), which is not typical for tobacco transformation. Sensitive plants were light green or completely discolored, poorly grown on the selective nutrient medium, did not form roots. Sensitive tobacco regenerants, obtained in the second experiment, were investigated for the availability of transgenes (Fig. 5). The presence of the *npt*II gene in 13 of the 16 analyzed plants was shown, and 12 of them contained both genes (Table).

Hence, 81.3% of sensitive regenerants were transgenic. This fact unlikely is due to silencing transgenes, because we did not remove selective pressure after transformation during the plant material cultivation. Therefore, we believe that the emergence of a significant number of transgenic plants that become susceptible to the selective agent during further cultivation is the result of the negative effect of the of the corn *hsp70* gene intron on the expression of the *npt*II gene in tobacco. In the control experiment with pICH5290 vector, which did not contain the monocot nucleotide sequences between the promoter and the coding part of the gene, all obtained transgenic plants did not lose resistance to the selective agent during further cultivation and contained both transgenes. The obtained results correlate with the literature data regarding the reduction of the level of gene expression in tobacco cells

Table. **Tobacco transgenic plants obtaining using *A. tumefaciens* containing the vector pCB271**

Experiment, N	RF, %	Planted regenerants				PCR-analysis of regenerants											
						nptII						*gfp*					
				S		R			S			R			S		
		T, pcs	R, pcs	pcs	%	T, pcs	"+"		T, pcs	"+"		T, pcs	"+"		T, pcs	"+"	
							pcs	%		pcs	%		pcs	%		pcs	%
1	78.5	329	231	97	29.6	33	25	75.8	ni	ni	ni	33	18	54.5	ni	ni	ni
2	55.6	45	25	20	44.4	17	15	88.2	16	13	81.3	15	8	53.3	13	12	92.3

Notes: RF — regeneration frequency; T — total; R — resistant to the kanamycin; S — sensitive to the kanamycin; "+" — the presence of a positive signal, ni — not investigated

after transformation with vectors containing monocot introns as regulatory elements of gene expression in comparison with constructions that do not contain them [6, 9, 11, 13]. Thus, the embedding of the *OsAct1* first intron into the transcription region between the promoter CaMV35S and the *uidA* gene sequence resulted in a decrease in GUS activity after transformation of 4-fold compared with the use of a vector without an intron and no activity of GUS was detected in tobacco cells after transformation by vector containing the promoter of the rice gene *Act1* with and without the first intron [9]. This may be due to the fact that dicot cells often do not recognize monocot introns and, accordingly, their splicing is not always correct [11].

The expression of the S65Tpgfp gene in transgenic N. tabacum plants obtained by *A. tumefaciens* with the vector pCB271 examined via flourimetric measurements from leaf surface and leaf extracts. Fluorescence of GFP during measurements from the leaf surface was not detected for

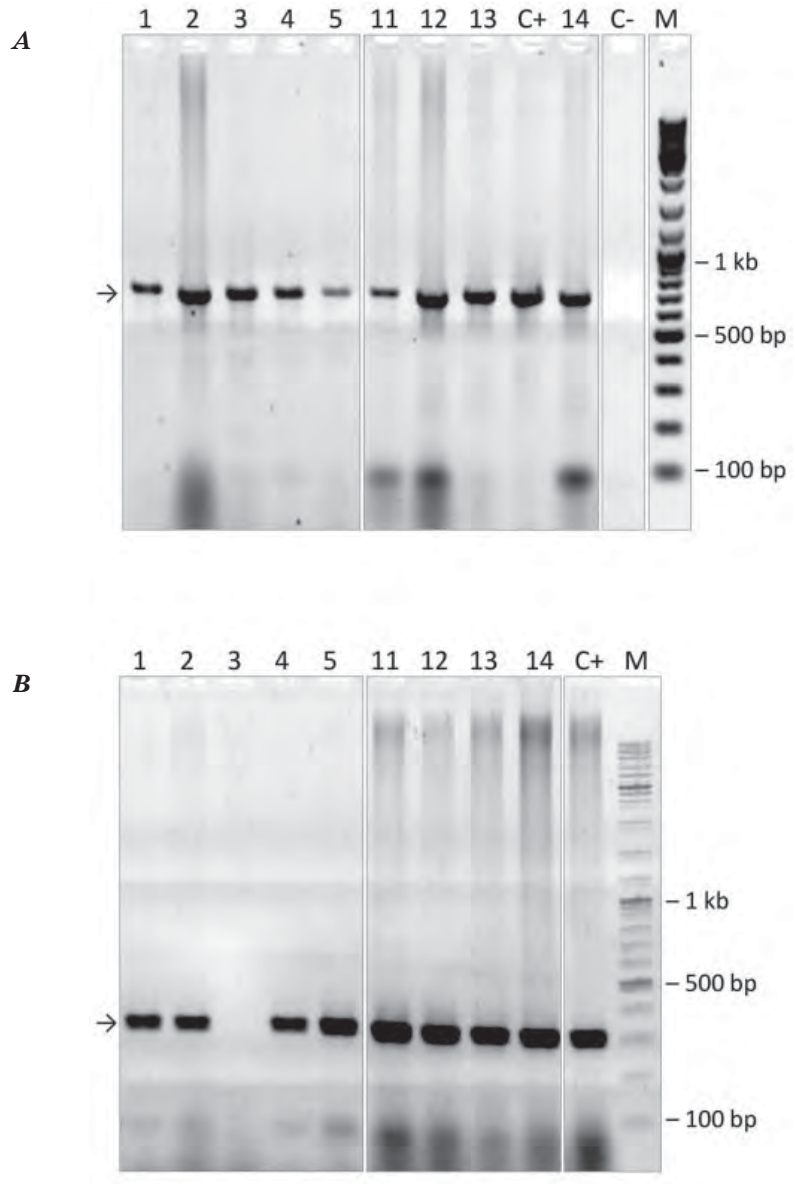

Fig. 5. **Electrophoregrams of the PCR analysis of tobacco regenerants obtained after transformation of *A. tumefaciens* containing pCB271 vector on the presence of *npt*II (*A*) and *gfp* (*B*) genes:**
Tracks 1–5 — DNA samples of the sensitive to the antibiotic tobacco regenerants; 11–14 — DNA samples of the resistant to the antibiotic tobacco regenerants; C — negative control; C+ — positive control; M — the Ladder Mix DNA molecular weight marker. The lengths of the PCR fragments were 311 bp for the *gfp* gene and 700 bp — for *npt*II

transformants and was at the fluorescence level of the leaf of a non-transformed plant (Fig. 6). High peaks of GFP fluorescence were observed for some transgenic lines due to fluorimetry of extracts. The intensity of the fluorescence of GFP in the extracts was up to 25 times larger than those measured from the leaf surface (Fig. 6). We analyzed leaf extracts of 22 lines of transgenic plants obtained as a result of two experiments on the *Agrobacterium*-mediated transformation by the vector pCB271 and the GFP fluorescence was observed in extracts of 7 lines. The lines of transformants differed in the intensity of the GFP fluorescence. The detecting GFP fluorescence in the extracts unlike leaf surface may be associated with a higher content of GFP protein in the extracts compared with a small leaf area taken for measurement in the surface study.

Thus, as a result of the *Agrobacterium*-mediated transformation by the pCB271 vector containing monocot introns between the promoter and the coding region of the genes, we showed that their expression occurs in dicotyledons of *N. benthamiana* and *N. tabacum*. However, there is a negative effect of the introns on transgenic expression exists. It was manifested in lower levels of GFP fluorescence than expected and the presence of a significant amount of kanamycin-sensitive tobacco regenerants containing the *npt*II gene in their DNA that provides resistance to the antibiotic.

So, the transgenes, which after the promoter contain an intron of the corn *hsp70* gene or the rice *OsAct1* gene, are expressed in *Nicotiana* plants. Consequently, *N. benthamiana* and *N. tabacum* plants can be used to test vectors carrying the mentioned regulatory elements for the gene expression in monocotyledonous plants. However, it has been shown that monocot introns can have a negative effect on the expression of transgenes in dicot *Nicotina* plants, which was found to be lower than expected the fluorescence level of GFP, and the presence of a significant amount of sensitive to kanamycin tobacco regenerants containing *npt*II in their DNA.

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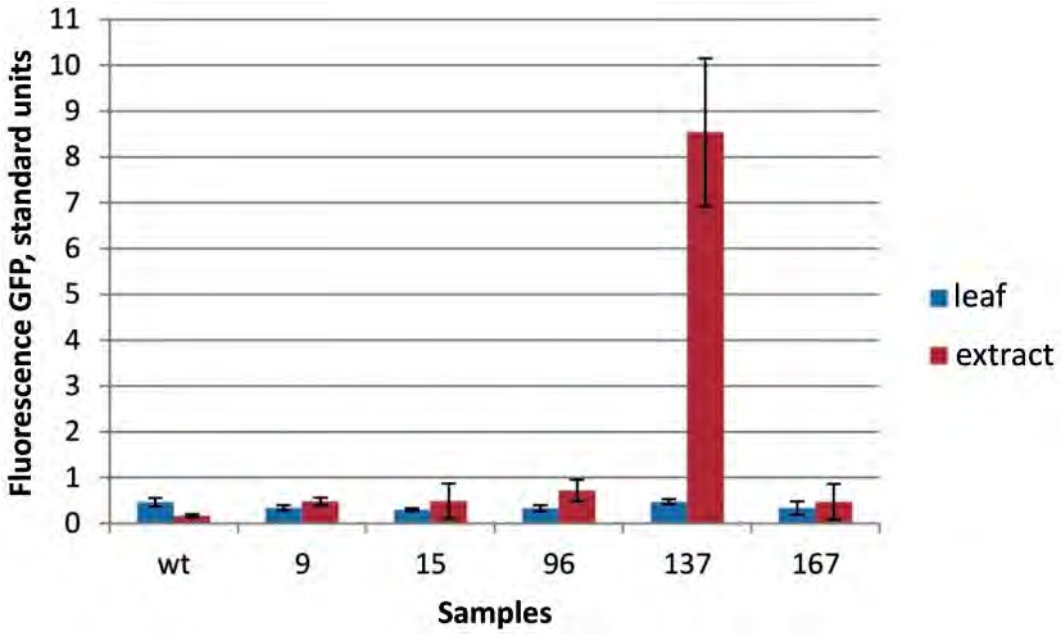

Fig. 6. **Flourimetry of GFP in the leaves of transgenic tobacco lines obtained after *Agrobacterium*-mediated transformation by the vector pCB271:**
wt — non-transformed plants; 9, 15, 96, 137, 167 — lines of transgenic plants

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ВПЛИВ ІНТРОНІВ ОДНОДОЛЬНИХ НА ЕКСПРЕСІЮ ТРАНСГЕНІВ У РОСЛИНАХ *Nicotiana*

І. О. Нітовська
М. Ю. Василенко
Б. В. Моргун

Інститут клітинної біології та генетичної інженерії НАН України, Київ

E-mail: molgen@icbge.org.ua

Метою роботи було дослідити вплив інтронів генів рису *OsAct1* та кукурудзи *hsp70* на експресію трансгенів у рослинах *Nicotiana* для їх застосування при тестуванні векторів, що містять зазначені інтрони однодольних. Використовували методи *Agrobacterium*-опосередкованої трансформації листків тепличних рослин *N. benthamiana* та асептичних рослин *N. tabacum* вектором pCB271, що містить інтрони однодольних, світлову флуоресцентну мікроскопію і флуорометрію зеленого флуоресцентного протеїну (GFP), детекцію трансгенів за допомогою полімеразної ланцюгової реакції. Спостерігали транзієнтну експресію GFP в інфільтрованих тканинах *N. benthamiana*. Отримали трансгенні рослини *N. tabacum*, що є стійкими до канаміцину. Показано флуоресценцію GFP в екстрактах трансгенних ліній тютюну. У частини трансформантів *N. tabacum* спостерігали припинення експресії трансгенів. Таким чином, трансгени, що містять інтрони генів кукурудзи *hsp70* або рису *OsAct1* після промотору, експресуються в рослинах *Nicotiana*. Отже, рослини *N. benthamiana* та *N. tabacum* можна використовувати для тестування векторів, сконструйованих для трансформації злакових. Виявлено, що зазначені інтрони однодольних можуть справляти негативний вплив на експресію трансгенів у рослинах *Nicotiana*.

Ключові слова: інтрони однодольних, *Nicotiana*, *Agrobacterium*-опосередкована трансформація, GFP.

ВЛИЯНИЕ ИНТРОНОВ ОДНОДОЛЬНЫХ НА ЭКСПРЕССИЮ ТРАНСГЕНОВ В РАСТЕНИЯХ *Nicotiana*

И. А. Нитовская
М. Ю. Василенко
Б. В. Моргун

Институт клеточной биологии и генетической инженерии НАН Украины, Киев

E-mail: molgen@icbge.org.ua

Целью работы было изучить влияние интронов генов риса *OsAct1* и кукурузы *hsp70* на экспрессию трансгенов в растениях *Nicotiana* для их применения при тестировании векторов, содержащих указанные интроны однодольных. Использовали методы *Agrobacterium*-опосредованной трансформации листьев тепличных растений *N. benthamiana* и асептических растений *N. tabacum* вектором pCB271, который содержит интроны однодольных, световую флуоресцентную микроскопию и флуориметрию зеленого флуоресцентного протеина (GFP), детекцию трансгенов с помощью полимеразной цепной реакции. Наблюдали транзиентную экспрессию GFP в инфильтрированных тканях *N. benthamiana*. Получены трансгенные растения *N. tabacum*, устойчивые к канамицину. Показана флуоресценция GFP в экстрактах некоторых трансгенных линий табака. Среди трансформантов *N. tabacum* наблюдалось прекращение экспрессии трансгенов. Таким образом, трансгены, содержащие после промотора интрон гена кукурузы *hsp70* или риса *OsAct1*, экспрессируются в растениях *Nicotiana*. Следовательно, растения *N. benthamiana* и *N. tabacum* можно использовать для тестирования векторов, сконструированных для трансформации злаковых. Установлено что указанные интроны однодольных могут оказывать негативное влияние на экспрессию трансгенов в растениях *Nicotiana*.

Ключевые слова: интроны однодольных, *Nicotiana*, *Agrobacterium*-опосредованная трансформация, GFP.