Усвідомлення і пересторога – два принципи усіх міжнародних нормативно-правових документів щодо біобезпеки при використанні ГМ-рослин. Принципова полеміка навколо трансгенних організмів корисна, оскільки примушує генних інженерів постійно поліпшувати конструкції, посилювати контроль за наслідками і, таким способом, працює на користь стратегії виживання людства в умовах стрімкого росту населення і виснаження біоресурсів. Але суспільство сьогодні має право робити вибір – споживати органічну чи генетично трансформовану їжу. Тому державі необхідно обов'язково забезпечити маркування ГМ-продуктів.

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EFFICIENT PLANTLET REGENERATION AND *AGROBACTERIUM* TRANSFORMATION OF FLAX BY CHIMERIC *GFP-TUA6* GENE

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Introduction

Flax (*Linum usitatissimum* L.) is one of the perspective agricultural culture in Europe and in the Ukraine, particularly. One of the aims of flax selection is a creation of new varieties with improved agro-technical characteristics that include the higher wind-resistance. Cell wall plays an essential role in formation of mechanical flax resistance to wind. It is well known that cellulose microfibrils is the main mechanical element of the cell wall, and it is generally considered that cortical microtubules control the direction of cellulose microfibril deposition [1, 2]. Thus, elucidation the correlation between microtubules organization peculiarities and their role in cellulose microfibril arrangement helps to understand the wind resistance in flax plants

on cell and tissue levels.

During last decades *in vitro* cultivation of flax (*L. usitatissimum*) has been successfully elaborated. Protocols have been established for *in vitro* shoot regeneration [4, 5, 6] and also for *Agrobacterium*- mediated transformation [3, 7, 8, 11, 13, 15].

In the present study, we have attempted to develop an efficient system for high frequency shoot regeneration of several *L. usitatissimum* varieties with different wind-resistance zoned on territories of Belarus and Ukraine by exploiting six combination of plant regulators 6-benzylaminopurine (BAP) and α-naphtalene acetic acid (NAA), and addition of supplements in order to optimize *Agrobacterium tumefaciens* transformation procedure of them and selected transgenic lines expressing chimeric GFP-TUA6 gene that could allow viasualize microtubules in flax cells.

Objects and methods of investigation

Culture medium. During experiments, MS [14] medium supplemented with 200 mg/l myo-inositol, 250 mg/l MES was used. Addition of 6 variants of plant regulators concentrations of BAP (1-3 mg/l) and NAA (0,05 – 0,1 mg/l) plus 20 g/l sucrose were added into the medium to test the plantlets regeneration. For shoots and roots development the hormone free MS medium with 10 mg/l sucrose was used. All media were gelled with 0,8 agar and adjusted to pH 5,8.

Plant material. L. usitatissimum seeds were kindly provided by the Institute of Genetics and Cytology NAS of Belarus (genotypes 'Dashkovskiy', 'K-65', 'Niva') and by the Institute of Fibre Cultures of Ukrainian Agricultural Academy of Sciences (genotypes 'Vruchiy', 'Zorya 87', 'Rushnichek', 'Svitanok', 'Ukrainskiy 3', 'Tomskiy 16'). Seeds sterilization was performed as described before [4].

Shoot regeneration. Hypocotyl explants from well germinated 6-days-old seedlings were cuted of 3-5 mm long and immediately placed onto plantlet regeneration medium. Green vigorous shoots were separated and placed on medium for shoots and roots development. The cultures were maintained at 22-24°C under 16-h light and 8-h dark photoperiods.

Agrobacterium-mediated transformation. Transformation was performed using *A.tumefaciens* strain LBA4404 harbouring a binary vector pBI121/GFP-TUA6 carrying chimeric tubulin gene TUA6 from *Arabidopsis thaliana* fused with GFP reporter gene from *Aequorea victoria* driven under cauliflower mosaic virus 35S promoter and *npt*II gene as selectable marker gene conferring resistanse to kanamycin [17].

Agrobacterium was cultured overnight at 28°C in liquid LB medium [16] containing 50 mg/l kanamycin and 100 mg/l rifampicin. An overnight culture of *A.tumefaciens* was centrifuged at 4000 *rpm* for 15 min and then resuspended in liquid MS medium without sucrose and phytohormones. The OD_{600} of bacteria was to 0,3-0,5 before inoculation.

The hypocotyl explants were immersed into *Agrobacterium* inoculum for 60 min and then placed onto MS medium supplemented with 200 mg/l myo-inositol, 250 mg/l MES, 20 g/l sucrose for co-cultivation period during 1, 2 or 3 days. Then co-cultivated hypocotyls were transferred onto medium for plantlets regeneration supplemented with 100 mg/l kanamycin for further selection of transformants and 400 mg/l carbenicillin for bacteria elimination. Explants were sub-cultured every 3 weeks on fresh medium. Regenerating shoots were picked up after 6-7 weeks and placed onto medium for shoots and roots development supplemented with 10 mg/l kanamycin.

DNA isolation and PCR analysis. Total genomic DNA was isolated from kanamycin-resistant shoots and untransformed control plant using Plant DNA Iolation Kit (Sigma, Germany). The primers used for amplification of a 622 base pair fragment of *npt*II gene were: 5`-CCTGAATGAACTCCAGGACGAGCA-3', 5'-GCTCTAGATCCAGAGTCCCGCTCAGAAG-3' and for amplification of a 840 base pair fragment of *npt*II gene were: 5'-GAGGCTATTCGGCTATGACT-3', 5'- AATCTCGTGATGGCAGGTTG - 3'. Amplification was

carried out under the following conditions: 5 min at 94°C (initial denaturation); followed by 25 cycles of 30 s at 94°C (denaturation), 45 s at 65°C (annealing) and 40 s at 72°C (extension); and a final stage of 7 min at 72°C (final extension) for primers which used for amplification of a 622 base pair fragment of the *npt*II gene and 5 min at 94°C (initial denaturation); followed by 25 cycles of 30 s at 94°C (denaturation), 60 s at 60°C (annealing) and 40 s – 72°C (extension); and a final stage of 7 min at 72°C (final extension) for 840 base pairs fragment of the *npt*II gene.

Microtubule visualization. Incorporation of tubulin labeled by GFP into native microtubules into cells of transformed flax lines was studieds by confocal laser scanning microscopy LSM 510 META (Carl Zeiss, Germany).

Results and discussion

Optimum concentrations of plant regulators for effective shoots regeneration were determined using six different combinations of BAP and NAA concentrations. Plantlets regeneration was observed using all tested variants BAP and NAA concentrations (Fig. 1). More large in size formation of calli was observed on plantlets regeneration medium supplemented with 3 mg/l BAP and 0,05 or 0,1 mg/l NAA but they practically did not produced abundant shoots on such media. A higher shoot regeneration frequency was observed on the medium with 1-2 mg/l BAP and 0,05-0,1 mg/l NAA (Fig. 1). On such media all tested flax varieties produced up 10-15 shoots per explant.

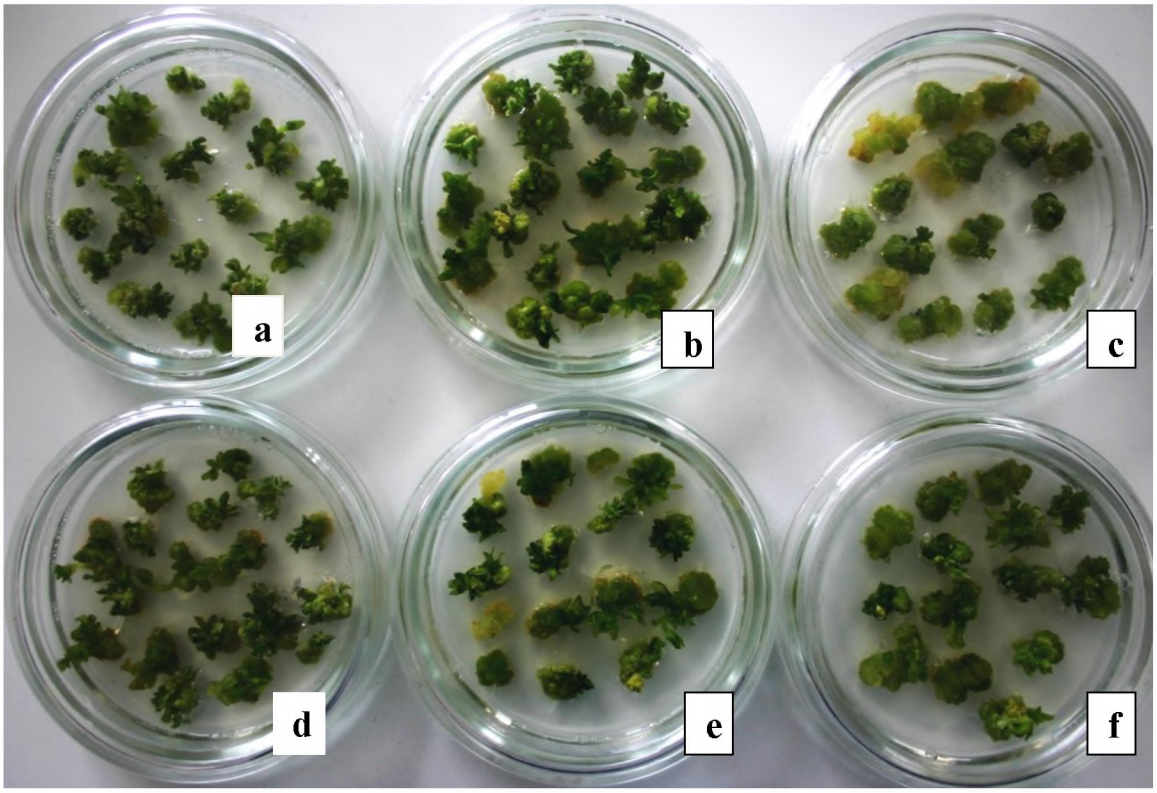

Fig. 1. Effects of growth regulators on *in vitro* shoot regeneration from hypocotyls explants of flax cultivar Ukrainskiy 3 after 5 weeks in culture: a – 1 mg/l BAP + 0,1 mg/l NAA; b – 2 mg/l BAP + 0,1 mg/l NAA; c – 3 BAP mg/l + 0,1 mg/l NAA; d – 1 BAP mg/l + 0,05 mg/l NAA; e – 2 BAP mg/l + NAA 0,05 mg/l; f – 3 BAP mg/l мг/л + NAA 0,05 mg/l, Bar = 1cm

It was shown earlier that flax transformation characterizes sometimes with a formation «shoot escapes» [9, 12]. Taking into account this fact a two steps selection was used in our experiments. The flax transformants were selected during shoots regeneration phase on the

respective medium containing 100 mg/l kanamycin according [18], and then during shoots and roots development phase on the corresponding medium containing 10 mg/l kanamycin (Fig. 2). From data obtained we can conclude that cultivar Svitanok has the highest transformation efficiency then others cultivars.

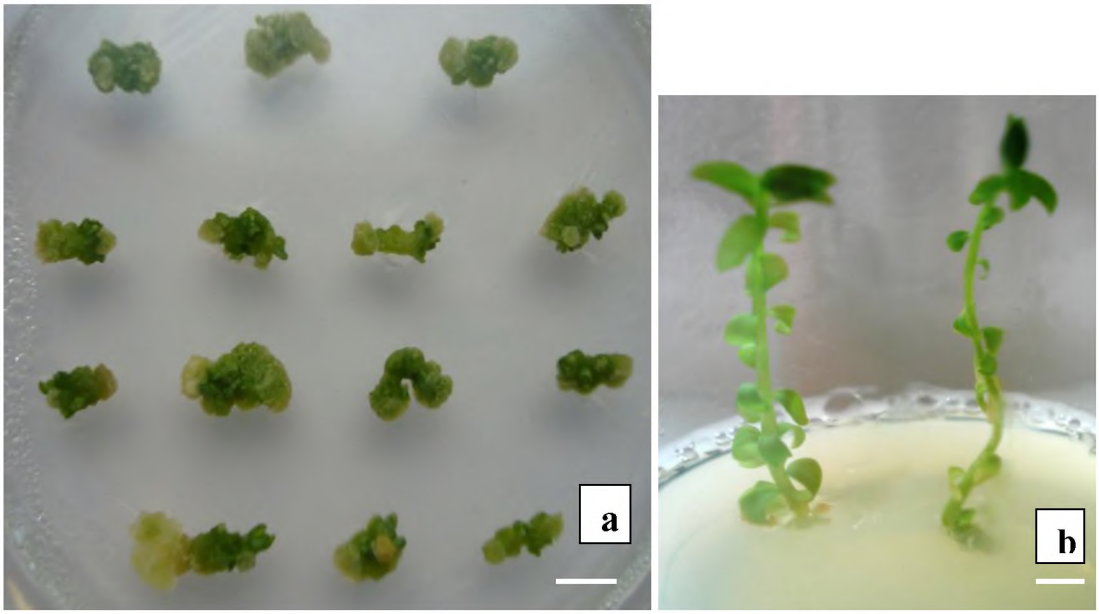

Fig. 2. Two selection phases: a – shoots regeneration phase (hypocotyls of germplasm 'Tomskiy 16' subcultured for three weeks on 100 mg/l kanamycin, Bar = 1,3 cm; b – shoots and roots development phase (putative transgenic shoots during selective shoot elongation (10 mg/l kanamycin), Bar = 0,7 cm

It was also found that optical density of *Agrobacterium* during transformation as well as co-cultivation time in our experiments were an important factors in increasing the transformation efficiency. The OD_{600}=0,4 of *Agro*-inoculum and 2 days of co-cultivation period were more suitable for flax transformation as compared to OD_{600}=0,3 or OD_{600}=0,5 and 1 or 3 days co-cultivation period because the transformation frequency in first case was more high for all tested genotypes. The OD_{600}=0,3 was enough low for successful integration of T-DNA into the plant cells but OD_{600}=0,5 was too high due to high level of *Agrobacterium* contamination. The same problem was observed when co-cultivation period was 1 or 3 days.

To confirm the transgenic nature of selected plants the molecular genetic analysis was carried out. The presence of transferred *npt*II gene in transgenic shoots was investigated by the PCR analysis using specific primers. As it was found the 621 and 840 bp length fragments of the *npt*II were amplified from most regenerated plants of all flax varieties used in our study (Fig. 3).

Also transgenic nature of obtained lines was confirmed by confocal laser scanning microscopy. It was found that exogenous GFP-labeled tubulin is capable to co-polymerize with endogenous cell tubulin and to participate in cortical microtubule network formation in transgenic flax cells. The obtained lines will used for further investigation of microtubule organization and cellulose deposition in flax cell wall for understanding the processes of mechanical resistance in flax plants.

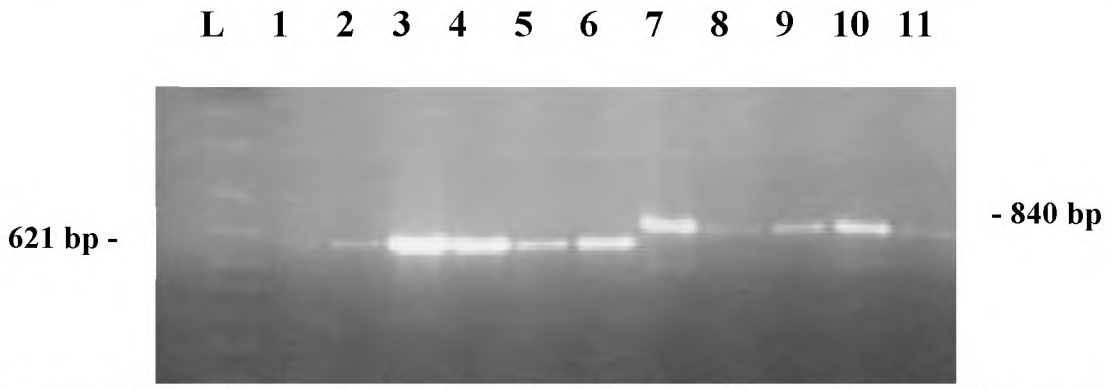

Fig. 3. PCR analysis of transformed flax lines with a probe to *nptII* (the primes used for amplification of a 621 base pair fragment of the *nptII* and 840): 1 – purified water; 2 – non-transformed control line; 3 – plasmid pBI121; 4 – cv. Ukrainskiy 3 – line 5; 5 – cv. Svitanok – line 7; 6 – cv. Svitanok – line 12; 7 – plasmid pBI121; 8 – cv. Vruchiy – line 1; 9 – cv. Svitanok - line 8; 10 – cv. Svitanok - line 10; 11 – cv. Ukrainskiy 3 – line 3

Conclusions

Our results have shown efficient plantlets regeneration of several *L. usitatissimum* cultivars with different wind-resistance. The highest shoot regeneration frequency was obtained from hypocotyls explants on MS medium containing 1-2 mg/l BAP and 0,05-0,1 mg/l NAA. Moreover, reported results show the successful *Agrobacterium*-mediated transformation of some flax cultivars by plasmid carrying chimeric GFP-TUA6 gene.

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РЕГЕНЕРАЦІЯ УКРАЇНСЬКИХ СОРТІВ КАРТОПЛІ ТА ЇХ ГЕНЕТИЧНА ТРАНСФОРМАЦІЯ СИНТЕТИЧНИМИ *CRY*-ГЕНАМИ

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Вступ

Картопля (*Solanum tuberosum* L.) є однією з основних продовольчих сільськогосподарських культур, які вирощують в Україні, і стійко займає перше місце в овочевому раціоні жителів нашої країни. Однією з основних задач селекціонерів на сьогоднішній день є підвищення споживчих якостей картоплі на фоні повного виключення хімічних засобів захисту рослин, використання екологічно безпечних методів та засобів пригнічення бур'янів і комах-шкідників, які забезпечують запобігання забруднення навколишнього середовища та врожаю картоплі токсичними речовинами. Найбільш злісними для картоплі є комахи-шкідники, які можуть повністю знищувати як наземну частину рослин, так і підземну, і призводити до повної або значної втрати врожаю данної культури. Широко застосовують для боротьби з такими шкідниками ефективні препарати на основі Bt-білків бактерії *Bacillus thuringiensis*, які є активними інсектицидними агентами [1, 7]. Перевагами біопестицидів на основі Bt-білків у порівнянні з хімічними інсектицидами є відсутність забруднених залишків, висока специфічність дії, що обумовлює їх нешкідливість для нецільових організмів і відносно низька ціна [1, 2, 6].

Необхідно відмітити, що протягом останніх десятиліть були успішно розвинені різні прийоми культивування та регенерації картоплі в умовах *in vitro*. Для цієї сільськогосподарської культури достатньо добре розроблені ефективні методи переносу