INTRODUCTION

Linum usitatissimum L. (flax/linseed) is an important crop as a source of oil and fibre in Europe and North America (Millam et al., 1992; Bartoðová, 2005). Flax has been widely used in biotechnology studies, including plant cell, tissue and organ culture, as it has a relatively short life-cycle, high regeneration capacity and small genome size (Bennet and Smith, 1976). Flax tissue culture has been used in studies of in vitro morphogenesis, embryo cultures, growth regulator influence on explant culture systems, environmental and genotypic effects on regeneration, stress tolerance lines obtaining and in many other fundamental studies (McHughen and Swartz, 1984; Pretova, 1990; O’Connor et al., 1991; Jain and Rashid, 2001; Bonell and Lassaga, 2002; Obert et al., 2004, Millam et al., 2005).

The cultivation of tissue and cells in vitro causes high variation, called somaclonal variation. Somaclonal variation has been described for many plant species (Larkin and Scowcroft, 1981; Leike, 1985; Kaeppler et al., 2000, Rutkowska-Krause et al., 2003), including flax. This variation depends on the duration of cultivation and usually occurs during the callus stage (D’Amato, 1995). Somaclonal variation has been used as a source of genetic variability in breeding programmes for many crops (Rutkowska-Krause et al., 2003). Due to somaclonal variation, new flax genotypes have been derived with resistance to biotic and abiotic stress, plant height, a number of seeds in a vessel, a number of seeds (McHughen and Swartz, 1984, Poliakov, 2000; Rutkowska-Krause et al., 2003) and new oil flax varieties have been produced (Rowland et al., 2002). Therefore, this method is useful for obtaining source material for flax breeding (Grauda et al., 2006). The problem in use of this method in breeding programmes is the high genotype dependence of calli regeneration capacity and unclear causes of somaclonal variation.

There are many possible mechanisms of somaclonal variation, including changes in chromosome number, chromosome breakage and rearrangement, DNA amplification, point mutations, activation of transposons, changes in DNA
of organelles and changes in DNA methylation (Grandbastien, 1998; Kalendar et al., 2000; Cassells and Curry, 2001). Polyploidy, aneuploidy and chromosome aberrations in somatic plant and animal cells cultivated in vitro have been observed (Sacristán, 1971). Ploidy variations have been reported for different plants, including both regenerated and transgenic tomato (Ellul et al., 2003), in vitro regenerated Arachis hybrids (Singsit and Ozias-Akins, 1992), somatic hybrids and in vitro propagated potato (Uijtewaal, 1987; Chaput et al., 1990), sugar cane, tobacco (Larkin and Scowcroft, 1981), rice (Chen, 1977), Brassica (Keller et al., 1975), pear (Kadota and Niimi, 2002), strawberry (Hao et al., 2002) and many others. The use of flow cytometry for the measurement of nuclear DNA content, which is based on the use of DNA-specific fluorochromes and on the analysis of the relative fluorescence intensity of stained nuclei (Doležel, 1991), has been considered as a fast and reliable method (Doležel et al., 1989). Using this method, nuclear DNA amounts can be analysed with high precision and representative numbers of nuclei can be measured in a short time (Kubaláková et al., 1996; Doležel et al., 2007).

Variation in DNA methylation is an additional factor of inherited changes in tissue culture and has both mutagenic and epigenetic effect (Matthes et al., 2001; Li et al., 2002). It is one of the mechanisms of rapid modifications of genome and can control gene expression (Cullis, 2005). The cultivation of tissue cultures can induce changes in methylation on a global level and in specific sites in regenerated plants and their progeny (Philips et al., 1994; Lei et al., 2006).

Nanoparticles are defined as particles with dimensions less than 100 nm and play a central role in many natural processes, for example, they serve as nanomagnets in bacteria for reception of the earth magnetic field and have a role in transport of radionuclides in groundwater (Kan and Tomson, 1990; Matsumaga et al., 2000). In engineered nanoparticles, different features can be optimised and created according to the expecting result, which is why engineered nanoparticles are widely used in many materials (Colvin, 2003). Modern nanomaterials have new optical, mechanical and electrical properties, which are vastly different from their conventional-sized counterparts. New features of these new materials can lead to unpredictable outcomes when they interact with biological tissues (Wang et al., 2010). Very few studies have describe the biotransformation of nanoparticles in plant species, and the possible biomagnification of nanoparticles in the food chain is unknown (Rico et al., 2011). For this reason, investigations on effect of nanoparticles on live cells, including those of plants, are necessary.

Autofluorescence examination is considered as a standard for screening and diagnostic of neoplastic changes, and for screening of early stages of infection in plants and changes in reinitiated calli. In addition, fluorescence can be used to identify apoptotic cells (Vigneswaran et al., 2009; Martínez et al., 2010). There are many causes for autofluorescence of tissue. Some types can be found in mammalian tissue only, such as the fluorescent pigment lipofuscin, while others are specific to plants or are independent of the species (Neumann and Gabel, 2002). Cells have autofluorescence due to naturally fluorescent products within the cell. Combination of photochemical destruction of fluorophores and changes in the quantum efficiency of fluorophores cause the effect of photobleaching (Rost, 1991). In photobleaching experiments, a high intensity laser is used and fluorescence recovery after photobleaching can be observed, and furthermore, the photobleaching can affect plant cell dynamics (Sparkes et al., 2011). Fluorescence recovery after photobleaching (FRAP) has received increasing attention ever since it was first introduced into cell biological research (Houtsomuller, 2005). For example, FRAP revealed the mobility of many nuclear proteins including histones (Kimura, 2005). To our knowledge there is no papers published so far on autophuorescence and photobleaching effects in flax calli cultures.

The objectives of our study was to determine the effect of different C nanoparticle concentrations on morphological parameters, type and frequency of regeneration, change of ploidy level, features of histology, degree of genome methylation alteration, autophuorescence and photobleaching effects in flax calli cultures.

MATERIALS AND METHODS

Calli cultures. Latvian origin flax (Linum usitatissimum L.) accession ‘Blue di Riga’ was used for calli formation. Seeds were washed in a soap solution for 3 min, rinsed with deionised water and soaked in 0.007% potassium permanganate for 30 min, rinsed with deionised water and sterilised in a 3% sodium hypochlorite for 20 min, and then rinsed at least five times in deionised autoclave-sterilised (120 °C, 1 atm) water. Seeds for germination were placed on basal Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) with 3% sucrose and 0.7% agar, pH 5.8. Donor plants were grown in growth chambers at 24 °C, 2 Lx, 16/8 h (day/night) photoperiod and 80% humidity. Stem segments (5 mm) from seedlings (3–5 leaves stage) were used as explants for calli formation. For calli formation, explants were cultivated for 3 to 5 weeks on the basal MS medium and medium supplemented with 1 mg/l of 2,4-D (2,4-Dichlorophenoxyacetic acid) and 1 mg/l of BAP (6-Benzyl-aminopurine). For further cultivation, calli were transferred on the MS medium without nanoparticles (control) and MS medium supplemented with different C nanoparticles (carbon nanoparticles corresponded to multiwalled carbon nanotubes with the following parameters: purity more than 85wt%, diameter 5–20 nm, length ~10 µm, manufactured by Nanohub Co. Ltd, South Korea) concentrations: 10⁻³ (g/l), 2 x 10⁻³ (g/l) and 3 x 10⁻³ (g/l).

Calli cultivation on each medium treatment was repeated three times. Calli were maintained in growth chambers at 24 °C, 2 Lx, 16/8 h (day/night) photoperiod and 80% humidity. After four weeks of cultivation all calli were placed onto regeneration medium (MS medium with 1 mg/l of BAP). Af-
Morphological features of calli were examined using a microscope Nikon 90i and stereomicroscope Nikon AZ 100. Images were processed by NisElement AR and NisElement BR software. For extraction of specific features a laser scanning microscope Zeiss LSM 5 PASCAL was used.

Histology. Control calli, as well as calli grown on medium with different concentrations of C nanoparticles, were fixed by immersion in 10% neutral buffered formalin approximately for 48 h. Fixed calli were dehydrated in alcohol and xylene, embedded in paraffin wax, sectioned at 5 µm, stained with hematoxylin and eosin and observed with a AxioLab light microscope (Carl Zeiss, Jena), camera AxioCam MRc5. Images were processed by AxioVision Rel.4.7.1 software.

Detection of cell ploidy. The ploidy level was tested in somatic calli after four months of cultivation. For determination of ploidy, approximately 50 mg of calli tissue were placed onto a plastic Petri plate, 0.5 ml of CyStain UV Ploidy (Partec, Germany) was added, and the specimen was chopped with a sharp razor to release the nuclei from the cells. Additionally, 5 ml of CyStain UV Ploidy were added and tissues were incubated at room temperature for five minutes. Then, calli were filtered through a Partec 50 µm CellTrics disposable filter and analysed on Partec CyFlow® space Cytometer using UV excitation and measure blue emission. Each measurement was made three times and a minimum of 5 000 nuclei per sample were analysed. As a diploid control, young leaves of the laboratory-grown diploid plant from flax accession ‘Blue di Riga’ were used.

DNA extraction. Genomic DNA was extracted from calli cultivated on medium with and without nanoparticles. Before DNA isolation, calli were dried in silica gel for 3–4 days. DNA isolation was made using NucliSens® easy-MAG™ (bioMérieux, France) extractor and the Versatile and Flexible DNA/RNA extraction kit (bioMérieux, France) following the protocol of the manufacturer.

Methylation analysis. For determination of DNA methylation in calli cells of *Linum usitatissimum* L., genes rich with CpG sites were chosen: complete sequence of 26S ribosomal RNA gene, 26S-18S ribosomal RNA intergenic spacer, 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8 S ribosomal RNA gene, and internal transcribed spacer 2 (Gene bank accession number EU307117.1). PCR primers and the sequencing primer were described spacer 2 (Gene bank accession number EU307117.1). PCR primers and the sequencing primer were described spacer 2 (Gene bank accession number EU307117.1). PCR primers and the sequencing primer were described.

**Table 1**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’-3’)</th>
</tr>
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<tbody>
<tr>
<td>Forward PCR primer</td>
<td>AGAGAGGGAATTGGTTTTTTTTTTTTTGATT</td>
</tr>
<tr>
<td>Reverse PCR primer (biotinylated)</td>
<td>CAACAAACTCCCCACCTAA</td>
</tr>
<tr>
<td>Sequencing Primer</td>
<td>GTATTGTGTTTTTTTTTTGGATTAA</td>
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PCR product was 246 bp

USA) following the protocol of the PyroMark PCR Master Mix (Qiagen, Hilden, Germany). Two modifications of the protocol were used: with and without Q solution (Qiagen, Hilden, Germany) in the reaction mix. Each 25 µl PCR reaction without Q-solution contained 9.5 µl Milli-Q water, 12.5 µl 2 × PCR buffer, 0.5 µl 25 mM Mg2+ solution, 0.5 µl 10 µM mix of F and R primers (F primer was biotinylated) and 2 µl of DNA. Each 25 µl PCR reaction with Q-solution contained 4.5 µl Milli-Q water, 12.5 µl 2x PCR buffer, 0.5 µl 25 mM Mg2+ solution, 0.5 µl 10 µM mix of F and R primers (F primer was biotinylated), 5 µl of 5x Q-solution and 2 µl DNA. The reaction was performed using the following protocol: 95 °C for 15 min, followed by 45 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s with the final extinction at 72 °C for 10 min.

The sequence created by PyroMark® Assay Design SW was used for creation of the assay setup with PyroMark Q24 Application Software. Pyrosequencing was conducted using PyroMark Q24 and data analysis was performed using PyroMark Q24 Software (Qiagen, Hilden, Germany). The methylation level of each CpG site was calculated as a percentage using the software as the peak height of cytosine, divided by the sum of cytosine and thymine peak heights multiplied by 100. The Mann-Whitney test was used to detect significant (*P* < 0.05) differences between different calli and between calli and control.

Detection of autofluorescence and photobleaching effect of calli. The autofluorescence spectrum was measured using a confocal microscope LEICA TCS SP5. Calli were irradiated with an argon laser under 458 nm wavelength. The fluorescence spectrum was observed in the range 480 nm to 800 nm with step 3 nm. The laser star was focused till 2 µm, power was 0.3 mWt and scanning square was 24x24 µm. Autofluorescence intensity was detected in three spectral ranges: 530, 690, and 750 nm. For detection of photobleaching effect of calli, the excitation source was a 458 nm laser. Irradiation time was (i) 5 min under continuous excitation and (ii) 5 min under excitation source repeated with interval 1 minute.

RESULTS

Calli formation. Calli induction frequencies were high and varied from 65 to 98% (Table 2). Among the control and calli grown on different concentrations of C nanoparticles (10⁻³ mg/l, 2 × 10⁻³ mg/l and 3 × 10⁻³ mg/l) callusogenesis
frequency differed considerably: control explants placed on MS medium exhibited the highest (98%) percentage of calli formation. Explants grown on the medium with highest concentration (3 × 10⁻³ mg/l) of C nanoparticles had the lowest level of callusogenesis (65%). The range of callus diameter after four weeks of cultivation varied from 5.0 mm (calli on medium supplemented with 3 × 10⁻³ g/l C nanoparticles) to 8.5 mm (control). A similar tendency was observed in mean weight of calli, which varied from 0.2 g (calli on medium supplemented with 3 × 10⁻³ g/l C nanoparticles) to 0.6 g (control).

Cross sections of calli developed on MS medium with and without nanoparticles after six weeks of cultivation are shown in Figure 1. Somatic embryos with concentric tracheary elements were easily discernible in control calli. Somatic embryos were visible in calli developed on MS medium with 10⁻³ g/l C nanoparticles. Aggregates of vacuolated embryos were visible in calli developed on MS medium with 2 × 10⁻³ g/l C nanoparticles. Calli on MS medium with 3 × 10⁻³ g/l C nanoparticles were composed of large cells with dense cytoplasm, which formed vast and disorganised cell aggregates. Embryo-like structures were not visible; however on the outer side of calli a tuber-like structure was observed. Reticular-like network in intracellular spaces of calli tissue resulted in high calli induction frequencies in all variants: range 65–98% (Table 2). Morphological and histological analysis of induced flax calli showed that there were differences between control and calli grown on medium with different concentrations of nanoparticles were found. In calli grown on medium with nanoparticles the methylation level was significantly higher than in control calli (P < 0.05). Increased concentration of nanoparticles caused increased methylation level in all CpG sites, excepting in position 4 for which the methylation level in control calli and in calli grown on medium with 10⁻³ mg/l C nanoparticles was the same.

### Ploidy level in calli culture

Flax calli grown both on medium with and without C nanoparticles were tested by flow cytometry (Fig. 3). In the control 90% of calli were diploid. In calli with single and double concentration of nanoparticles, the proportion of diploid cells decreased by 25%, and by 10% in calli grown on triple concentration of nanoparticles. Tetraploid cells were found in all examined groups of calli, and their proportional occurrence varied from 10% in the control to 75% in calli grown on medium with 10⁻³ mg/l C nanoparticles. In calli cells grown on MS with 2 × 10⁻³ mg/l and 3 × 10⁻³ mg/l C nanoparticles, 4n calli were detected in 60% of cases. Mixoploid calli (2n+4n) also were observed in calli grown on medium with double and triple concentrations of nanoparticles at relative frequencies of 15% and 30%, respectively.

### Methylation analysis

Analysis of methylation level in the six CpG sites in the 26S ribosomal RNA gene, 26S-18S ribosomal RNA intergenic spacer, 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8 S ribosomal RNA gene, and internal transcribed spacer 2 in calli revealed significant differences between control calli and calli grown on medium supplemented with C nanoparticles. The results of methylation analysis are presented in Figure 4. The lowest level of methylation was detected in control calli, for which the methylation level varied from 36% in position 1 to 76% in position 5. The highest level of methylation in all sites investigated was detected in calli grown on medium with 3 × 10⁻³ mg/l C nanoparticles: range 61% in position 1 to 100% in position 5. Significant differences between control and calli grown on medium with different concentrations of nanoparticles were found. In calli grown on medium with nanoparticles the methylation level was significantly higher than in control calli (P < 0.05). Increased concentration of nanoparticles caused increased methylation level in all CpG sites, excepting in position 4 for which the methylation level in control calli and in calli grown on medium with 10⁻³ mg/l C nanoparticles was the same.

### Autofluorescence and photobleaching effect

Optical spectrums of flax calli in the control group and in calli grown on medium with 3 × 10⁻³ mg/l C nanoparticles are presented in Figure 5. Differences between groups were detected in peaks 530 nm and 750 nm, which were degraded within 2–5% in the case of presence of C nanoparticles in medium.

Intensity of photobleaching during continuous laser irradiation 458 nm of calli grown on medium with 3 × 10⁻³ mg/l C nanoparticles and for the control calli group during continuous laser irradiation 458 nm is presented in Figure 6. Calli demonstrated an exponential decrease of fluorescence intensity during irradiation, but control calli differed in fluorescence amplitude.

### DISCUSSION

In our experiment the earlier elaborated modification of the calli induction and cultivation method (Graudza et al., 2006) resulted in high calli induction frequencies in all variants: range 65–98% (Table 2). Morphological and histological analysis of induced flax calli showed that there were differences in development of calli structures, such as somatic embryos, tracheary elements and reticular network, caused

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**Table 2**

<table>
<thead>
<tr>
<th>Variant</th>
<th>Calli induction frequency, %</th>
<th>Number of measured calli</th>
<th>Calli diameter (mm) after 4 weeks cultivation</th>
<th>Calli weight (g) after 4 weeks cultivation</th>
<th>Direct embryogenesis, %</th>
<th>Indirect embryogenesis, %</th>
<th>Organogenesis, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control medium</td>
<td>98</td>
<td>40</td>
<td>8.5 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>10.0</td>
<td>40.0</td>
<td>0</td>
</tr>
<tr>
<td>Medium with 1×10⁻³ g/l C nanoparticles</td>
<td>83</td>
<td>40</td>
<td>8.5 ± 0.3</td>
<td>0.5 ± 0.1</td>
<td>0</td>
<td>25.0</td>
<td>0</td>
</tr>
<tr>
<td>Medium with 2×10⁻³ g/l C nanoparticles</td>
<td>79</td>
<td>40</td>
<td>6.6 ± 0.1</td>
<td>0.5 ± 0.4</td>
<td>0</td>
<td>25.0</td>
<td>0</td>
</tr>
<tr>
<td>Medium with 3×10⁻³ g/l C nanoparticles</td>
<td>65</td>
<td>40</td>
<td>5.0 ± 0.4</td>
<td>0.2 ± 0.4</td>
<td>0</td>
<td>0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

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by different concentrations of C nanoparticles in the cultivation medium. Several authors have shown that a reticular-like network mediates intercellular contacts among cells, and that a dense reticular network filling large intercellular “caves” forms during somatic embryogenesis (Ovečka and Bobak, 1999; Popielarska et al., 2006). The formation of the extracellular matrix surface network can be associated with a stress response in explants imposed by specific in vitro conditions (Bobák et al., 2004). We suppose that addition of C nanoparticles to cultivation medium influences intercellular interaction which, in turn, modifies differentiation of tissues and organs in calli cultures.

Calli grown both on medium with and without C nanoparticles were tested by flow cytometry to determine possible

Fig. 1. Cross section of calli developed on MS medium with and without nanoparticles after six weeks of cultivation. A, control sample developed on MS medium without C nanoparticles; somatic embryos (arrowheads) were visible, which contained tracheary elements (arrows). Magnification ×200. B, sample developed on MS medium with 10^{-3} g/l C nanoparticles; somatic embryos (arrows) were visible. Magnification ×50. C, D, sample developed on MS medium with 2×10^{-3} g/l C nanoparticles; masses of vacuolated cells (C) and embryo-like structures (D, arrows) were visible. Magnification ×50. E, sample developed on MS medium with 3×10^{-3} g/l C nanoparticles; embryo-like structures were not visible, tuber-like structure (arrows) on the outer side. Magnification ×50.
cause of differences in regeneration capacity of calli. Changes of ploidy are considered as one of the results of somaclonal changes (Millam, 2005). Many studies that nanoparticles can cause a decrease of mitotic index, cell activity, and chromosomal stickiness in metaphase and anaphase stages (Li et al., 2003; Kumari et al., 2009; Kumari et al., 2011). Our study showed that the ploidy variation in calli significantly depended from C nanoparticle concentration in medium. With increasing concentration of C nanoparticles, the numbers of cells containing a mixture

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**Fig. 2.** Intercellular spaces within callus tissue. Reticular structures (arrows) in calli developed on MS medium with $10^{-3}$ g/l C nanoparticles (A) and in calli developed on MS medium with $2\times10^{-3}$ g/l C nanoparticles (B).
eration. Regeneration occurred only in diploid calli. The ob-
ploidy was related with the frequency and the type of regen-
indirect) and organogenesis. The results showed that calli

grown on medium with C nanoparticles. Regeneration abil-
ity was reached 50%, including 10% direct embryo-
genesis of calli, which produced more embryos per explant.

calli for different plants (Chakrabarty et al., 2011). Our data
showed C nanoparticle concentration-dependent hypermethylation in flax calli cells. DNA methylation, sim-
lar to other epigenetic mechanisms, can be considered as a
key factor in the regulation of cell growth and differentia-
tion, and thereby, the nuclear reprogramming necessary for
dedifferentiation and calli formation (Berdasco et al., 2008).

Regarding calli cultures, methylation rates in genomic DNA
from embryogenic callus have been observed to be signifi-
cantly lower in comparison with those in non-embryogenic
calli for different plants (Chakrabarty et al., 2003). Our data
also demonstrated decreased somatic embryogenesis in calli
(indicated by hypermethylation) grown on medium with
nanoparticles, compared to that in the control (Table 2).

Significant differences in photobleaching after laser irradia-
tion 458 nm repeated with interval 1 min were found be-
tween control and calli grown on C nanoparticles (Fig. 7).

Fluorescence intensity exponential decreased during irradia-
tion. However, in the control group, after 1 min of relaxa-
tion between irradiations, autofluorescence intensity re-
turned to close to the initial level (lower only by 2–3% only).
In calli grown on the medium with C nanoparticles
autofluorescence after relaxation had returned to only
16–17% of the initial level. Thus, C nanoparticles decreased
the effect of photobleaching of calli. Untreated calli were
found to have decreased repair ability during laser irradia-
tion, but it is possible that the laser pulses caused more se-
vere damage to calli grown on medium with C nanoparti-
cles. It is known that changes in autofluorescent pattern in

globular stages were detected (Salaj et al., 2005; Vidoz et
al., 2006; Chakravarti et al., 2010). Calli grown on me-
dium with C nanoparticles, formed numerous abnormal em-
bryos with different variations like embryous fusion. There
are no previous studies on the influence of nanoparticles on
ploidy level of calli culture. However, optimal culture con-
ditions, medium, type and level of plant growth regulators
to induce somatic embryogenesis in different species, have
been widely discussed (Komamine et al., 1992; Jimenez,
2001; Jimenez et al., 2005; Park et al., 2011). Obtaining all
the developmental stages similar to those of zygotic embry-
ous are exclusive (Hofmann et al., 2004; Korbes and
Droste, 2005; Santos et al., 2006; Chitra Devi and Nar-
mathabai, 2011).

Our data showed C nanoparticle concentration-dependent
hypermethylation in flax calli cells. DNA methylation, sim-
lar to other epigenetic mechanisms, can be considered as a
key factor in the regulation of cell growth and differentia-
tion, and thereby, the nuclear reprogramming necessary for
dedifferentiation and calli formation (Berdasco et al., 2008).

A lack of direct embryogenesis was observed in all calli
grown on medium with C nanoparticles. Regeneration abil-
ity was the highest in control calli: 50% embryogenesis and
capacity was reached 50%, including 10% direct embryo-
genesis of calli, which produced more embryos per explant.

Calli grown on medium with 10⁻⁵ mg/l and 2 × 10⁻³ mg/l of
C nanoparticles were embryogenic as well, but the relative
frequency of embryogenesis was twice lower than in the
control. Regeneration through rhyzogenesis (percentage of
regeneration was 10%) was observed only in calli grown on
triple concentration of nanoparticles (Table 2). Calli grow-
ing on medium without C nanoparticles formed embryos
with better quality: higher number of normal embryos in

of ploidy levels increased. We observed two in vitro regen-
eration systems for flax: somatic embryogenesis (direct or
indirect) and organogenesis. The results showed that calli
ploidy was related with the frequency and the type of regen-
eration. Regeneration occurred only in diploid calli. The ob-
erved yellowish-white friable calli were noneembryogenic,
while embryogenic callus was green, round and compact.

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grown on medium with C nanoparticles. Regeneration abil-
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triple concentration of nanoparticles (Table 2). Calli grow-
ing on medium without C nanoparticles formed embryos
with better quality: higher number of normal embryos in

Fig. 5. Autofluorescence spectrum of calli grown on medium with 3×10⁻³
mg/l C nanoparticles and of calli grown on medium without nanoparticles
(control) (excitation source 458 nm).

Fig. 6. Intensity of photobleaching of calli grown on medium with 3×10⁻³
mg/l C nanoparticles and of calli grown on medium without nanoparticles
(control) under continuous excitation source 458 nm.


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plants occurs in early stages of infection, in calli reinitiated from cell suspension cultures, and in intact secretory cell of plants (Kwa et al., 1997; McEwan, 2000; Roschina, 2003). It is possible that the autofluorescence changes found in our study were related to peroxidation of fluorescing products under C nanoparticle-induced stress conditions. Our data suggested that calli autofluorescence could be a sensitive parameter for the analysis of nanoparticle-cell interaction and that spectral characteristics of calli cells measured in vitro could be a significant tool for identification of nanoparticles and for diagnostics of cellular damage. However, more detailed studies are necessary to determine the precise mechanisms of the observed effects.

This is the first study to report on the response of flax calli culture to medium supplemented with carbon nanoparticles. The results showed considerable differences in callousogenesis frequency between the treatments. Somatic embryos with concentric tracheary elements were only detected in calli grown on medium without C nanoparticles. Reticular-like network in intracellular spaces were typical for callus tissue developed on MS medium with 10−3 g/l and 2 × 10−5 g/l carbon nanoparticles. Ploidy variation in calli significantly depended on the carbon nanoparticle concentration in medium. Two in vitro regeneration systems were observed for flax calli (direct and indirect somatic embryogenesis and organogenesis), although only diploid calli were capable of regeneration. The level of DNA methylation in calli grown on medium with C nanoparticles was significantly higher than in control calli. Somatic embryogenesis was significantly lower in calli grown on medium with C nanoparticles compared to calli on control medium, estimated by hypermethylation in calli cell DNA. Decreasing effect of photo-bleaching was observed in calli grown on medium supplemented with carbon nanoparticles. To clarify mechanisms of interactions between cells and nanoparticles in vitro it is necessary to conduct a more detailed study on various plants with different nanoparticles in various doses. It is of interest to investigate this phenomenon in more detail and to determine whether nanoparticles could be used to increase somaclonal variation. The study was a good example of interdisciplinary research where bioscience, biotechnology and nanoscience can be combined successfully.

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LINU (Linum usitatissimum L.) KALLUSU KULTÜRÜ REAKCIYA UZ BAROTNES PAPILDINASANU AR OGLEKLÂ NANOĐALÎNAM


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