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Research Article

Target Transportation of Auxin on Mesoporous Au/SiO_2 Nanoparticles as a Method for Somaclonal Variation Increasing in Flax (*L. usitatissimum* L.)

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Development of methods for direct delivery of different bioactive substances into the cell is a promising and intensively approached area of research. It has become a subject of serious research for multidisciplinary team of scientists working in such areas as physics, biology, and biotechnology. Plant calluses were grown on medium supplemented with different nanoparticles to be used as a model for biotechnological research. Gold nanoparticles with mesoporous silica coating were used as hormone carriers, since they possess many of critical properties required for cellular transportation instrument. Some of those properties are great biocompatibility and controlled release of carried molecules. Significant changes in hormones common impact were detected. The great increase in ploidy numbers, embryogenesis, induction, and methylation level was observed when compared to the "conventional" methods of targeted hormones delivery that embrace usage of Au nanoparticles as a main hormone carrier. The authors suppose the research under consideration can provide a new pathway to the design of a novel targeted plant hormone and bioactive substances carrier.

1. Introduction

Cellular delivery of drugs and bioactive molecules through the cell membrane attracts unabated interest of specialists working in different fields, due to its importance and high potential for medicine and microbiology [1]. Though this subject is extensively approached and reviewed, direct delivery of bioactive substances still faces asperities and is not efficient in general [2, 3]. That is why the effective methods of reagent delivery are considered to be a promising area of research. One of the most significant classes of carriers is inorganic nanoparticles (NPs). Gold nanoparticles were used in this research due to their special properties: it is easy to track their trajectory in cells, they can be tailored to a desirable size, and their surfaces might be modified to possess good biocompatibility [1, 4].

Nanotechnology is usually considered to be one of the most important and promising technological developments of the 21st century since it offers enormous potential for advancement across a wide range of industries [5]. Many fields of science like engineering, biotechnology, analytical chemistry, and agriculture have evolved with tremendous advancements due to nanotechnology employment [6]. According to Wong [7] nanotechnology is the science investigating the manipulation with substance of nanoscopic scale having a size range from 1 to 100 nanometres (nm). The uniqueness lies in the fact that nanotechnologies applications are multidisciplinary, and researches in the fields of electronics, energy, and medicine contribute greatly to the biotechnology. Consequently, the research under consideration studies the nanoparticles and the results can contribute to increasing productivity of agricultural industry, to the cell biology, to medicine, and so forth [6, 8].

The research has a solid basis since there are many technologies providing the potential for increasing farm productivity and also reducing the environmental and resource

costs related to agricultural production [6]. The use of nanoparticles as nutrient elements for increased germination could enhance the agricultural productivity [6, 9]. One of the most promising components used for this purpose is nanogold (AuNPs), which is becoming more and more important in nanotechnology within the last years [10]. There are some key aspects in relation to nanoparticles and the environment, which are especially significant for plant science. Nanonutrition is the application of nanotechnology for the provision of nanosized nutrients for the crop production. Such factors as nutrition and nanonutrition present the priority for the researchers in such sphere as agriculture since they are capable of affecting the sustainable crop production growth and productivity. Several studies have been made in this field and numerous investigations are expected in the nearest future. It is possible to increase the efficiency of micro- as well as macronutrients of the plants by using nanonutrition [6].

However, there are researches proving the negative impact of nanotechnology on the environment. According to Gokak and Taranath [11] increased application and use of nanoparticles are directly related to their release in the environment. These interactions of nanoparticles with plants and other organisms are studied insufficiently. Nevertheless, some reports have shown that nanoparticles (NPs) have adverse effects on seed germination and plant growth. For example, Siddiqi and Husen broadly discuss the cytotoxicity of gold nanoparticles in plants, reporting the negative impact on roots length and biomass while using medium with high concentration of AuNPs [12].

The partial solution for this problem can be found by implementing silica coating. For instance, there are researches showing that nanoparticles prevent the inflammatory processes in the living cell. So, Demokritou et al. [13] suppose that coating the nanosized cerium dioxide with amorphous silica does not allow the particle to cause lung inflammation after short-term inhalation. Also it was shown that crystalline silica induces wide and aggressive pulmonary inflammatory responses, whereas amorphous SiO_2 induced a poor and temporal pulmonary inflammatory response in rats [5, 13]. It was also recently shown that mesoporous coatings such as silica are biocompatible and therefore have been widely proposed for drug delivery applications [14–16].

Previous research reports indicate an important advantage of silica shell covering the metal particles; it prevents direct contact of the adsorbed molecules with the metallic surface [15, 17]. Silica-based coatings are environmentally stable with different materials. It also possesses simplicity of surface modification, reduces the potential for photocatalysis, and formation of free radicals [18–20]. According to Sanz-Ortiz et al. [15], coating of gold nanoparticles with silica is a popular technique for developing the nanomaterials with plasmonic applications for improved detection, drug delivery, and catalysis.

There is a study [21] demonstrating that gold nanoparticles (AuNPs) penetrate in calli and in plant regenerants. The obtained results also reveal that silica-coated AuNPs overpass the plant cellular membrane and then they accumulate in the plant cells. The penetration of silica-coated AuNPs was confirmed by optical absorption spectroscopy and scanning electron microscopy. However, whether silica-coated AuNPs with hormone 2,4-dichlorophenoxyacetic acid (2,4D) penetrate plant cell membrane and if 2,4D is released inside the cell are actually not researched enough.

Multiple plant hormones play significant roles in many regulating processes in plants, such as growth and plant responses to stresses [22, 23]. 2,4-Dichlorophenoxyacetic acid is the most extensively used growth regulator in all cereal crop species [24]. 2,4D belongs to auxin group of plant hormones. It has become common to induce somatic embryogenesis in seed cultures using 2,4D alone or in combination with other hormones [25]. Cytokinin BAP (6-benzyl-aminopurine) modulates the auxin pathway or polar auxin transport, but auxin initiates, organizes, and supports the root stem cells [20].

The goal of this paper is to investigate the technology of penetration of silica-coated AuNPs with hormone 2,4D additive and efficiency of uptaking this molecule into the flax *Linum usitatissimum* L. organism; there is also aim of presenting an innovative method of targeted hormones transportation into the plant cells with employment of Au nanoparticles coated with mesoporous silicon dioxide (SiO_2). This method is based on the idea that Au core can absorb light on a specific wavelength and then it starts to fluctuate, shaking out hormone molecules from the pores of SiO_2 directly into the cell.

Plant callus is usually considered to be a favourable model for biotechnological researches, since all the uncontrollable variables associated with the whole plant could be eliminated and NPs interactions with plant cells can be observed. Besides, callus culture growing is a widely used method for obtaining the new breeding material, due to the high somaclonal variation of cells [26].

The authors suppose the research under consideration can provide a new pathway to the design of a novel targeted plant hormone and bioactive substances carrier.

2. Materials and Methods

2.1. Nanoparticles Synthesis. Au nanoparticles coated with SiO_2 were synthesized in Institute of Solid State Physics, University of Latvia, using the original method described by Chen et al. [27]. 0.05 g of CTAB was dissolved in a solution containing 24 mL of deionized water and 0.6 mL of 0.5 M NaOH. After stirring at 80°C for 15 min, 1.0 mL of 3.7 wt.% formaldehyde solution was added, followed by 0.8 mL of 0.05 M $HAuCl_4$ aqueous solution. After stirring for additional 10 min, solution containing 0.25 g of TEOS and 0.50 g of ethanol was added. The products were centrifuged after reaction for 1 h, washed four times with water and once with absolute ethanol, and then dried at 60°C overnight. Removal of the surfactant template was achieved by calcination in air from room temperature to 550°C for 6 h at a rate of $1^\circ C \cdot min^{-1}$. To investigate the thermal stability, the silica nanospheres were calcined at 950°C in air for 1 h. The diameter of the gold core was regulated by changing the amount of $HAuCl_4$.

The photograph of particles received can be seen below (Figure 1). Gold nanoparticles were synthesized in G.Liberts'

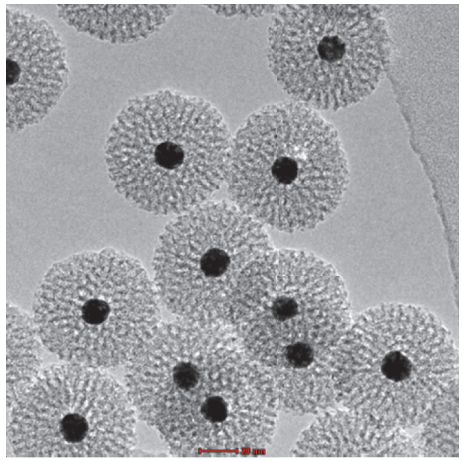

Figure 1: Au/SiO_2 nanoparticles obtained in the Institute of Solid State Physics, University of Latvia. The size of mesoporous nanostructures ranges from 40 to 60 nm, while the Au core of these particles is about 10–15 nm.

Innovative Microscopy Centre of Daugavpils University, using the methods described by Xirouchaki and Palmer [28] and Kelly and Arnell [29]. The control over the film thickness was done by the method, described in Mecea 2005 [30].

2.2. Plant Calluses and Regenerants Obtaining. Latvian origin flax (*L. usitatissimum* L.) known as "Lirina" was used for calli formation. Flax seeds were immersed in soap solution for 3 min and rinsed 5 times with deionized autoclave-sterilized (+120°C, 1 atm) water. After that seed surface sterilization was done in 0.007% potassium permanganate solution for 30 min with further rinsing in deionized sterile water as well. Than seeds were immersed in 3% sodium hypochlorite for 20 min and finally rinsed at least 5 times in deionized sterile water.

Seeds were placed on the basal Murashige and Skoog (MS) medium [31] containing 3% sucrose and solidified with 0.7% agar at pH 5.7. Then seeds were cultured in a growth chamber with diurnal regime of 16 h light/8 h dark and temperature of +22°C for 3 weeks with 80% humidity. Stem segments (3-4 mm) of the in vitro grown seedlings were used as a source of primary explants, which were placed horizontally onto MS medium and supplemented with 1 mg/l of 2,4D (Alfa Aesar, Haverhill, Massachusetts, USA) and 1 mg/l of BAP (SERVA Feinbiochemica, Heidelberg, Germany). Explants were left on medium in same conditions as stem segments for 5 weeks to accumulate callus biomass. For the experimental material, AuNPs (50 nm) and AuNPs (10 nm) coated with mesoporous SiO_2 till 20 nm thickness, with pore diameter of about 2 nm, were kept in solution of 2,4D hormone, separated from fluid, and added to the medium. Irradiation of calluses with Au/SiO_2 was carried out with a laser Verdi V6 Coherent at a wavelength of 532 nm with a low, cell safe intensity of 1 mW/cm^2 [32] to push the hormone molecules out from SiO_2 coating. The first treating with laser was done after 2 weeks of callus growth. The irradiation time was 5 min and the laser beam illuminated the entire area of callus. The irradiation was carried out 4 times every 5 days.

SiO_2 shells are stable and require externally triggered release systems, distally controlled by light irradiation or magnetic or electric effects [17, 33, 34]. Nanoparticles of gold absorb the radiation which is not absorbed by the callus tissue; that increases the vibrational motion, which leads to the release of hormone molecules from the pores of SiO_2 [35].

The calluses cultivation process was repeated for three times on each medium and took place in growth chambers set on +24°C, 2 Lx, 16/8 h (day/night) photoperiod, and 80% humidity during four weeks.

After that, all calluses were placed into the regeneration medium (MS medium with 1 mg/l of BAP). After two months of calluses cultivation, regeneration zones and regeneration type were detected. Regenerants were cultivated in growth chambers under the same conditions as those used for calli incubation.

Morphological parameters of calli were detected under the microscope Eclipse 90i (Nikon, Japan) and stereomicroscope AZ 100 (Nikon, Japan). Confocal images of samples treated with AuNPs were done using the microscope Eclipse Ti-E (Nikon, Japan), with an employment of 488 nm laser and region of interest equal to 3.077.

2.3. Detection of Cell Ploidy. The ploidy level of calluses cells had been determined six weeks after cultivation beginning. The analysis was done on Partec CYFlow® (Partec, Germany) space cytometer using UV excitation and blue emission measurements. To prepare samples, approximately 50 mg of calli tissue were chopped with a sharp razor directly into the Petri dish with 0.5 mL of CyStain UV Ploidy (Partec, Germany) added to release nuclei from the cells. After that, 5 mL of CyStain UV Ploidy was added and samples were incubated at room temperature for five minutes. Samples were filtered through a Partec 50 μm CellTrics disposable filter and analysed. Each sample was measured for three times and a minimum of 10 000 nuclei per sample were analysed.

2.4. DNA Extraction. Genomic DNA from calli cells cultivated on medium with MS+2,4D+BAP (control), MS+2,4D (AuNPs)+BAP (AuNPs carrying hormones), and MS+2,4D (Au/SiO_2 NPs)+BAP (Au/SiO_2 NPs carrying hormones) was extracted with the NucliSens easy-MAG reagents (BioMérieux, France) according to the manufacturers protocol. Before the DNA extraction, calluses were dried in silica gel for 3-4 days. In this study, the NucliSens easy-MAG (BioMérieux, France) nucleic acid extraction system was used.

2.5. Methylation Analysis. The 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) were used for determination of DNA methylation in calli cells of *Linum usitatissimum* L. PCR primers and the sequencing primer were designed using PyroMark® Assay Design SW 2.0 (Qiagen, Germany) presented in Table 1.

Table 1: PCR primer and sequencing primer sequences for PCR and pyrosequencing.

Primer	Sequence (5′-3′)
Forward PCR primer	AGGAGGGGTATTGTTTTTTTTTTTGGATT
Reverse PCR primer (biotinylated)	CAACCAAACTCCCCACCTAA
Sequencing primer	GGTATTGTTTTTTTTTTTGGATTAA
The PCR product was 246 bp	

Sodium bisulfite conversion of unmethylated cytosine in DNA was carried out using a EpiTect® Bisulfite Kit (Qiagen, Germany) according to the EpiTect Bisulfite Protocol. The bisulfite-treated DNA was amplified by PCR technique in a Veriti Thermal Cycler GeneAmp© PCR System (Applied Biosystems, CA, USA) in accordance with the protocol of the PyroMark PCR Master Mix (Qiagen, Hilden, Germany). The reaction mixture was incubated in thermocycler 95°C for 15 min, followed by 45 cycles of 95°C (denaturation) for 20 s, 55°C for 30 s (annealing), and 72°C for 30 s (extension), and then a final extension at 72°C for 10 min. A negative control was included in each PCR reaction.

The biotinylated PCR products were prepared for pyrosequencing in Pyromark Q24 (Qiagen, Germany) according to instruction supplied by the manufacturer. Data analysis was performed using PyroMark Q24 Software (Qiagen, Hilden, Germany). The methylation level in each CpG site in a percentage was calculated by the software as the peak height of cytosine divided by the sum of cytosine and thymine peak heights multiplied by 100. The acceptance criterion with regard to peak height for single nucleotides was 20 Relative Light Units (RLU). The Mann–Whitney test was used to detect significant ($p \leq 0.05$) differences in methylation levels between treated flax calli and control.

Statistical analysis was implemented on the basis of software Statistica-8.

3. Results and Discussion

This research is devoted to indirect modification of flax calluses with employment of silica-coated AuNPs. A strong relationship between plant tissues development and the type of growth medium used has been observed. The properties of silica-coated AuNPs as a possible target hormone carrier have been demonstrated on practice.

The microscopic confocal image of gold nanoparticles entering the flax cells can be seen on Figure 2.

Data on size, number of regeneration zones, and regeneration type of calluses growing on hormone reach medium and medium with nanoparticles is presented in Table 2.

300 samples were randomly selected from the grown callus with a random number calculator. The statistical analysis supported the authors' idea. The obtained results are statistically different with $p = 0.0000$. The only case where p is 0.0146 is between the induction frequency of control group and group grown with AuNPs. Nevertheless, p is less than 0.05 and we can ignore this result, which corresponds to not only statistical logics but also the logic of the research.

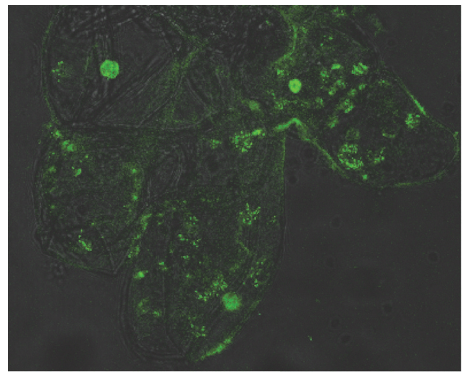

Figure 2: Confocal image of gold nanoparticles entering flax cells. The figure shows thin section of callus tissue under a confocal microscope. The cells were irradiated with a green laser with a wavelength of 488 nm at which light is actively absorbed by the gold nanoparticles clusters, so it is possible to record the fluorescence inside cells.

The frequency of calluses induction varied largely among different approaches; in particular, induction frequency in control samples was 93%; in samples treated with AuNPs carrying hormones (MS+2,4D (AuNPs)+BAP) and in samples with Au/SiO_2 NPs carrying hormones (MS+2,4D (Au/SiO_2NPs) +BAP) it was 87% and 99% respectively.

Calluses growing on medium with mesoporous Au/SiO_2 showed the largest mean size (average callus width was 6.80 ± 0.36 mm and length 9.90 ± 0.40 mm), while control samples were smallest among tested groups (width 4.31 ± 0.41 mm, length 7.54 ± 0.39 mm).

Analysed samples showed different regeneration ability and the number of regeneration zones varied between different samples (Figure 3.). Two types of regeneration were found: rhizogenesis and embryogenesis. Among all the samples tested, the highest percentage (74%) of shoot formation was detected in control sample. The highest level of embryogenesis (91%) was found in the Au/SiO_2NPs sample. In AuNPs sample, embryogenesis level was 81%, while in control samples embryogenesis level was only 26%.

Both groups of calluses that grew on medium with presence of NPs showed massive increase in size in contrast to the control group (Table 2). Those affected with AuNPs were 1 cm larger, and those cultivated on Au/SiO_2 NPs were 2 cm larger both in length and in width than calluses from control group. These observations signify a great increase in biomass accumulation.

Table 2: Collected information on calluses size, number of regeneration zones, and their regeneration type.

Sample	Calli induction frequency, %	Number of measured calli	Calli width, mm	Calli length, mm	Number of regeneration zones	Regeneration type
Control MS+2,4D+BAP[1]	93	300	4.31 (±0.41)	7.54 (±0.39)	1.75 (±0.26)	Rhizogenesis (74%) Embryogenesis (26%)
MS+2,4D (AuNPs)+BAP[2]	87	300	5.40 (±0.35)	8.40 (±0.41)	3.40 (±0.34)	Rhizogenesis (19%) Embryogenesis (81%)
MS+2,4D (Au/SiO_2 NPs)+BAP[3]	99	300	6.80 (±0.36)	9.90 (±0.40)	6.4 (±0.33)	Rhizogenesis (9%) Embryogenesis (91%)

[1] Hormone reach medium (control).
[2] AuNPs carrying hormones.
[3] Au/SiO_2 NPs carrying hormones.

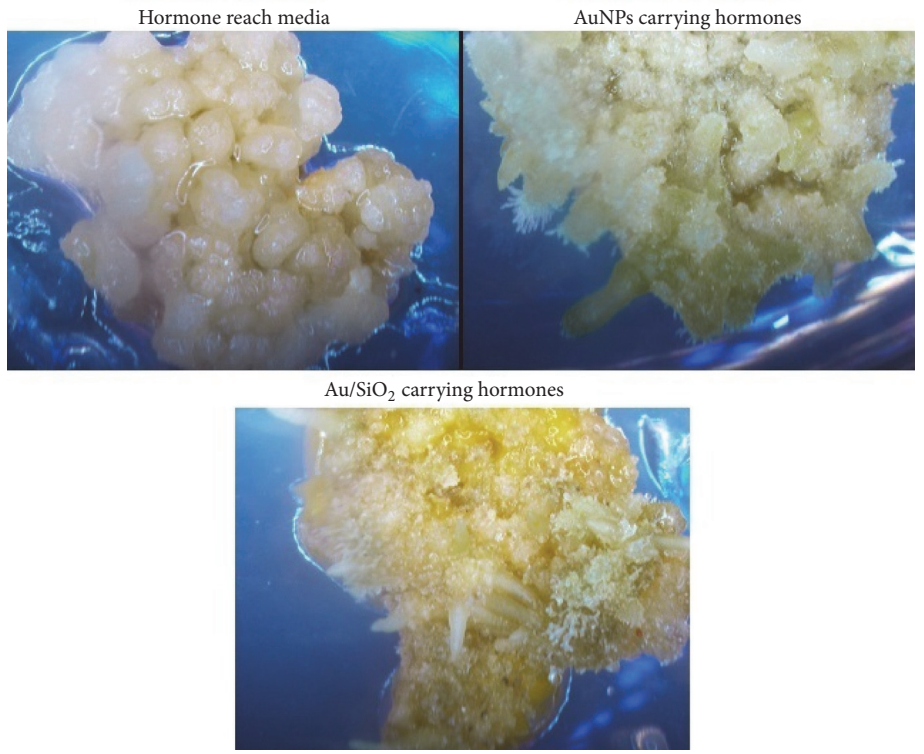

Figure 3: Three groups of calluses grown on different medium with clearly seen regeneration zones. The morphological differences of calluses can be clearly seen: calluses grown on medium with AuNPs have both zones of rhizogenesis and embryogenesis seen, while callus grown on mesoporous Au/SiO_2 with hormones shows massive zone of embryogenesis with no rhizoids seen.

The number of regeneration zones varies significantly in all test groups. It can be clearly seen that NPs of different types affect the abundance of the regeneration zones appearance, which supports the results obtained in the previous studies conducted in this field [36]. The smallest mean number of regeneration zones was 1.75 in control group, while Au and silica-coated Au treated groups showed mean values of 3.40 and 6.40, respectively. The regeneration effect changed dramatically, while the reference calluses showed only 26% of cells divided by embryogenesis; calluses treated with Au/SiO_2 NPs had 91%, yielding to difference in 65% total. It has serious importance for bioengineering, since all the plants growing from cells divided by embryogenesis will inherit all the features of mother-plant.

It is important to mention the differences in the induction numbers observed. The slightly negative influence of AuNPs carrying hormones on calluses vitality can be seen, referring to the numbers in control group. Though the numbers acquired do not vary strongly, they tend to appear near to 90% mark; it might be a statistical error only since this subject is not studied well and needs further investigation. In contrary, the percent of inducted calluses that grew in medium with silica-coated AuNPs was unusually high (99%), allowing drawing a conclusion that these NPs have a positive effect on calluses induction.

Data on calluses ploidy after 6 months of cultivation on different medium is presented in Table 3. 95% control calluses were diploid. In samples growing on AuNPs and Au/SiO_2 NPs

Table 3: Calli ploidy after 6 months of cultivation in control and after treatment with different nanoparticles.

Sample	Cell ploidy, %				
	$2n$	$4n$	$6n$	$2n + 4n$	$2n + 6n$
Control MS+2,4D+BAP	95	5	—	—	—
MS+2,4D (AuNPs)+BAP	68	28	1	2	1
MS+2,4D (Au/SiO_2 NPs)+BAP	56	35	2	6	1

Table 4: Methylation level (%) of six CpG sites in flax calli on control and on medium with nanoparticles.

CpG sites	Sample		
	Control MS+2,4D+BAP	MS+2,4D (AuNPs)+BAP	MS+2,4D (Au/SiO_2 NPs)+BAP
26S ribosomal RNA gene (Pos 1)	38	45	64
26S-18S ribosomal RNA intergenic spacer (Pos 2)	65	81	91
18S ribosomal RNA gene (Pos 3)	55	69	82
Internal transcribed spacer 1 (Pos 4)	70	87	94
5.8S ribosomal RNA gene (Pos 5)	74	91	100
Internal transcribed spacer 2 (Pos 6)	45	60	83

medium proportion of diploid cells decreased by 27% and 40%, respectively. Tetraploid cells were found in all samples, though the proportion of them was much higher in AuNPs (28%) and Au/SiO_2 NPs (35%) medium. Mixoploid cells with low frequencies were detected in samples which were grown on medium supplemented by nanoparticles only.

Nowadays it is known that NPs of some metals affect cells ploidy dramatically [26]. Plants ploidy examination is considered to be an important and common direction of genetic researches for plant breeding, since it may affect economically valued plant attributes. For example, increases in clover cells ploidy tend to enhance biomass accumulation and stress tolerance [37]. Diploid flax was used in this research; it is clearly represented by control calluses that had 95% of diploid cells. AuNPs carrying hormones had a predictable effect on ploidy: the number of tetraploid cells increased by 23% and some mixoploid cells appeared. Calluses cultivated with employment of silica-coated AuNPs showed even greater increase in tetraploid cell numbers, yielding to 30% increase compared to control ones. Considering the calluses measurements results, it could be confidently concluded that ploidy changes in relation to *Linum usitatissimum* have a truly large effect on plant characteristics. This is especially important, since flax is widely used around the world agronomic culture; it also has various uses in manufacturing industries and machinery. Nevertheless, significant increase in mixoploid cells numbers has been observed in all calluses grown on NPs, which is usually considered to be an inferior feature, leading to chimaeric plants formation. Unfortunately, it is still not known how to control the chimerisation process; this is a subject of intensive modern researches.

Results of analysis of methylation level in six CpG sites (26S ribosomal RNA gene, 26S-18S ribosomal RNA intergenic spacer, 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2) are presented in Table 4. The lowest level of methylation was detected in control sample. The highest level of methylation in all sites was detected in the sample with mesoporous Au/SiO_2 NPs nanoparticles; it ranged from 64% in position 1 up to 100% in position 5.

The level of CpG sites methylation in some ribosomal RNA genes has been assessed at last. Pyrosequencing had been used as a powerful tool for methylation detection. This method is based on quantitative monitoring of real-time incorporation of nucleotides through the enzymatic conversion of released pyrophosphate into a proportional light signal [38]. The uncommon tendency in genes conversion has been observed. As it can be seen from Table 4, significant increase of genes methylation was registered in both groups treated with nanoparticles carrying hormones (AuNPs and Au/SiO_2 NPs). Furthermore, it could be witnessed that methylated genes number in calluses cultivated on silica-coated NPs exceled over those obtained from calluses treated with AuNPs. A possible explanation for this is that the penetration of auxin into cell is more active using mesoporous Au/SiO_2 NPs. According to the LoSchiavo et al. [39] report, auxin has an effect on methylation level in embryogenic carrot cell cultures and at the same time cytokine does not induce an increase of methylation. Furthermore, it is well known that gold nanoparticles generally are "nontoxic" in low concentrations [40, 41], which is reflected in many investigations [42–46], though some researchers mention reverse effects of AuNPs treatments [10]. Those facts allow us to conclude that observed changes of methylation level in different CpG sites were not caused by nanoparticles.

The statistical analysis demonstrates that the percent of methylation in the control sample is 57.83 with the standard deviation of 14.33, which is less than the level of methylation in the presence of Au/SiO_2 NPs, 85.67%, and standard deviation is 12.60 ($F(2, 15) = 5.175$, $p = 0.02$). At the same time, the level of methylation in the presence of AuNPs is 72.17% and standard deviation is 17.60; it is not significantly different from the methylation percent in groups 1 and 3 (Figure 4.).

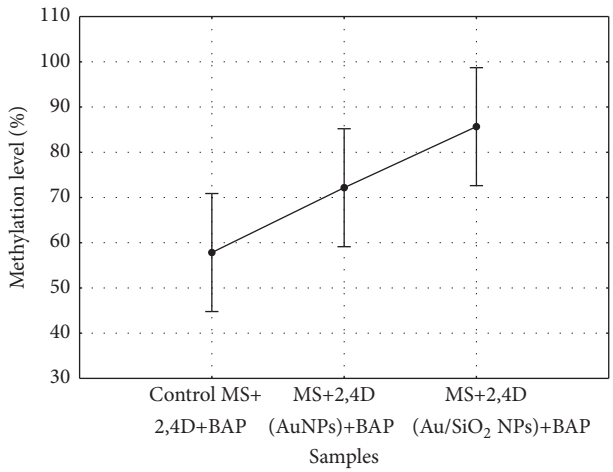

Figure 4: Graphical representation of ANOVA analysis.

The obtained results support the theory and assumptions of the authors; therefore, the authors expect that statistical analysis of big amount of data will show that all values are definitely different.

In accordance with the obtained results it is possible to conclude that the rough control on genes is possible; the genes control the synthesis of ribosomes, and they allow changing the intensity of protein synthesis and also controlling the supply of vitamins and inorganic salts to the cells.

Nanoparticles have a great significance for contemporary science and are used mostly to develop new methods of interaction with animal cells and drugs. The objective of the research group, in turn, is radically different: to find a use of recent inventions on plant cells and to develop novel methods of nanoparticles application. This paper puts an accent on a completely innovative method of hormones transportation into the cell. The type of impact of the new particles created might be described as a "nanobomb," which penetrates the cell walls and explodes inside after it has been triggered by the laser beam of a particular wavelength. It results in much more effective hormones absorption, as it was proved by this research. It is obvious that hormones concentration inside cell has importance to the specific effects obtaining and this method is considered to be a real breakthrough in NPs effect exploration, since it is the first real possibility of controlling hormones concentration with employment of NPs in plants.

The methods described in this paper open numerous opportunities for the hormones delivery methods development and allow conducting more effective researches in future.

4. Conclusions

The paper under consideration reviews the results of a long cross-disciplinary experimental work. The innovative method of targeted hormones delivery has been introduced and its capabilities of effective hormones control have been discussed. It has been experimentally confirmed that specially designed nanostructures with Au core and mesoporous outer envelope are able to carry hormones of specific size and penetrate cell walls. Their ability to "trigger" and detach appendant hormones after being exposed to the light of green spectra has been affirmed. As a result, significant changes in hormones common impact have been detected. The great increase in ploidy numbers, embryogenesis, induction, and methylation level has been observed when compared to the "conventional" methods of targeted hormones delivery that embrace usage of AuNPs as a main hormone carrier.

It is possible that the developed method might be used in future as a main approach to hormones transportation into the plant cells, since it is a great way to manage hormones concentration in cells and therefore is a probable entry point for the management of methylation, epigenetics, and somaclonal changes in plant cells. However, despite the fact that it opens a wide range of possible implementation ways, this subject is poorly studied and has broad prospects for further investigations.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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