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Original Paper

EFFECTS OF BIOTEXTILE-RELATED NANOPARTICLES AND MICROPARTICLES (SUCCINITE, Ag, SiO_2, Al_2O_3) ON BARLEY (*HORDEUM VULGARE*) IMMATURE POLLEN CELLS

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With the rapid progress of nanotechnology, nanoparticles and microparticles are extensively studied for their diverse applications, alongside growing concerns regarding their environmental impact and potential effects on living organisms. Nanoparticles, due to their size, can penetrate cell membranes, accumulate intracellularly, and influence molecular mechanisms, while microparticles tend to adhere to cell surfaces, affecting cellular transport pathways. This study investigates the short-term impact of four types of nano- and microparticles (SiO_2 (0.2–0.3 μm), Al_2O_3 (2.5 μm), Ag (0.5–1 μm), and succinite (5 nm – 3 μm)), specifically those explored for developing novel biotextile materials with specialised protective capabilities against harsh environments. As a model we used Hordeum vulgare *immature pollen cells (microspores). Microspores were incubated* in vitro *for 1.5 hours in media containing these particles. Relative cell autofluorescence was measured, and DNA changes were assessed using the inter-primer binding site (iPBS) fingerprinting method. Our results indicate that Al_2O_3 and SiO_2 microparticles had a suppressing effect on microspore autofluorescence, whereas succinite (amber) particles had an increasing effect. Ag particles showed no significant effect on microspore fluorescence under the studied conditions. Crucially, the iPBS method revealed visible changes in the amplified barley microspore DNA spectrum in samples exposed to SiO_2, Al_2O_3, or succinite nano- and microparticles, suggesting potential genotoxic effects. These findings underscore the importance of evaluating the diverse physiological and genetic responses of plant reproductive cells to various types of engineered nanoparticles and microparticles.*

Keywords: *bio-textile, succinite nanoparticles, SiO_2 nanoparticles, Al_2O_3 nanoparticles, Ag nanoparticles,* Hordeum vulgare *microspores.*

INTRODUCTION

Nanoparticles (NPs) are defined as particles ranging from 1 nm to 100 nm, while microparticles (MPs) typically measure between 100 nm and 1000 nm (0.1 μm to 1 μm) (Dobson *et al*., 2019). Both of them can be naturally occurring or synthetically produced (Mei and Yang, 2007; Ding *et al*., 2019). The diverse impacts of these particles have been extensively investigated in both animal and plant cells (Grauda *et al*., 2015; Jiravova *et al*., 2016). On the one hand, NPs and MPs offer numerous biotechnological applications, such as enhancing plant productivity (Kokina *et al*., 2017) or serving as medication carriers and imaging systems for cancer treatment (Hassanpour *et al*., 2018; Pour-

madadi *et al*., 2023). On the other hand, particles of various sizes also pose a potential hazard and require further investigation (Oukarroum *et al*., 2013). NPs can penetrate both plant and animal cells through various mechanisms. In animal cells, this occurs via endocytosis, pinocytosis, or phagocytosis (Jiravova *et al*., 2016). In plant cells, NPs enter through the cell wall or pores and can accumulate in different plant parts. Research on plant-nanoparticle interactions has revealed cytotoxicity, changes in photosynthetic efficiency, and the production of reactive oxygen species (ROS) (Liwarska-Bizukojc and Olejnik, 2020). Research on MPs is less abundant; however, their frequent use in water purification systems (Veremchuk *et al*., 2016) highlights the need for more comprehensive studies on their interactions with living organisms. Although MPs generally do not penetrate cells due to their size, they can significantly affect cellular processes by blocking cell pores and altering membrane potential (Grauda *et al*., 2024).

Given the increasing integration of micro- and nano-sized particles into various textile and biotextile materials (Lasenko *et al*., 2016; 2022), it is becoming crucial to develop robust methods for assessing their impact on living cells (Grauda *et al*., 2023). In this study, we investigate the impact of NPs and MPs on immature *Hordeum vulgare* pollen cells. These cells offer a promising model system for nanoparticle research due to their developing cell wall, which is more permeable than a mature plant cell wall and lacks cellulose, making them a highly sensitive test system (Grauda *et al*., 2015).

Beyond altering physical properties, NPs and MPs can also affect cellular DNA. DNA damage response (DDR) signalling pathways are crucial in plants. DNA damage can be induced by abiotic factors, including heavy metals or metal ions (e.g., aluminium). The toxic effects of these metals range from impaired photosynthesis to inhibited uptake of essential metal ions, with many directly causing DNA damage or inducing ROS formation (Nisa *et al*., 2019). DNA methylation is a conserved epigenetic trait that regulates genome stability (Gallego-Bartolomé, 2020). Transposable elements (TEs) are mobile genomic units that drive genome diversity. Retrotransposons, resembling retroviruses, are ubiquitous and can significantly increase genome size (Kalendar *et al*., 2010; Papolu *et al*., 2022). Long terminal repeat retrotransposons (LTR-RTs) are the most prevalent TE sequences in plants. Retrotransposon genome fragment analysis (fingerprinting) systems leverage retrotransposon integration sites. The iPBS (inter-primer binding site) method, which uses cellular tRNAs as primers for reverse transcription to isolate LTR-RTs, is widely employed not only for polymorphism detection in populations but also for plant stress-related gene research (Hossein Pour *et al*., 2023; Huang *et al*., 2023).

The aim of this study was to investigate the short-term (1.5 h) effects of two different concentrations (0.1% and 0.01%) of four particle types (Ag, SiO_2, Al_2O_3, and succinite) on *Hordeum vulgare* microspores using *in vitro* cultures. To determine cellular changes, three methods were chosen: light microscopy to examine morphological changes, flow cytometry, which detects approximately 20 different cellular parameter changes in total, and DNA alteration identification, based on detecting the movement of mobile genetic elements. Changes in plant cell fluorescence can indicate phenomena such as alterations in fluorescent pigments, the phase of the cell life cycle, various stresses, or entry into an apoptotic pathway, where relative fluorescence typically decreases (Grauda *et al*., 2015).

MATERIALS AND METHODS

Plant material and immature pollen cell culture preparation and nanoparticle solution preparation. Standard Murashige and Skoog (MS) media (Murashige and Skoog, 1962) were prepared for cell incubation; pH was adjusted to 5.8. 0.3 M D-mannitol solution was also prepared by dissolving mannitol in deionised water for cell grinding. Both were stored in a refrigerator.

The particles used in this experiment were spherical Ag (silver) microparticles (Enola, Latvia) with dimensions of 0.5–1 μm, Al_2O_3 (alumina) microparticles (Alfa Aesar, USA) with dimensions of 2.5 μm, SiO_2 (silica) microparticles (Sigma-Aldrich, USA) with dimensions of 0.2–0.3 μm, and amber (succinite) composite particles (JLU Technologies, Latvia) with dimensions of 5 nm–3 μm (Lasenko *et al*., 2016). Tubes with two different particle concentrations, 0.1% or 1 mg/ml and 0.01% or 0.1 mg/ml, were prepared by diluting the nanoparticles with distilled water and adding 0.08% Polyoxyethylenesorbitan monopalmitate emulsifier Tween® 40, which prevents particle clumping (Grauda *et al*., 2015), per total volume. The resulting suspensions were mixed, labelled, and stored in a refrigerator. The suspensions were placed in an ultrasonic bath for 15 min before addition to the plant cell cultures.

Barley leaves and stamens were removed, leaving only the ear. The method of cell culture preparation was based on Maluszynski *et al*. (2003). Barley ears were placed in a blender with 0.3 M D-mannitol solution. Grinding was carried out in three stages with 30 s grinding and 30 s pause intervals, then the suspension was visually evaluated and filtered through a 50 μm filter. The filtrate was then poured into 15 ml centrifuge tubes. The tubes were then placed in a centrifuge set at 900 rpm, temperature 4 °C and time 15 minutes.

Once the centrifugation process was complete, the tubes were removed from the centrifuge and the liquid phase was removed, leaving only the pellet. MS medium was added immediately to maintain cell viability. The diluted pellet from all four tubes was collected in one and then transferred to a larger container to be diluted with more MS medium. Then 15-ml tubes were prepared and labelled for incubation of cells in particles and control, which were placed in a shaker. A shaker was used to prevent the cells from settling to the bottom of the tube and for aeration. The tubes were rotated by shaking every 30 seconds. Control tubes con-

sisted of 5 ml cell culture, and experimental tubes consisted of 4.5 ml of cell culture and 0.5 ml particle solution (0.1 and 0.01%).

The incubation time with particles was chosen to be 1.5 h because for flow cytometry analysis cells cannot be left in MS medium with or without particles for a long time as they start to grow and may either be too large or start to move to the next stage of development. Also, the time slot for adding nanoparticle solution was 5 min, leaving this time for examination of each tube in a flow cytometer; thus the incubation time for every tube was exactly 1.5 h.

Cell phenotyping using light microscopy. Prior to the flow cytometry measurement, the pollen cells were evaluated microscopically. After one hour from the start of incubation, a small amount of cell culture was poured onto a slide, covered with a coverslip, and viewed with a 40× and a 100× objective. The number of cells in the field of view and the stages of pollen development were assessed at 40× magnification, while cell morphology and changes in morphology were assessed at 100× magnification.

Determination of relative fluorescence changes using flow cytometry. A BD FACSJazz cell sorter (BD Bioscience, USA) flow cytometer was used for the measurements. Prior to flow cytometry measurements, the cytometer was calibrated with 8-peak fluorescent beads (3- 3.4 µm, BioLegend, USA).

After 1.5 h incubation, the samples were analysed one by one in the flow cytometer. The cell culture was filtered through a cytometric filter and transferred from the incubation tube into the flow cytometer tube and then analysed.

The cell populations on the cytometer screen were separated and gated according to different parameters (Fig. 1). For each measurement, the flow cytometer worksheet was obtained in PDF format and the mean fluorescence values for each channel were read from a table. For each of the plant cells, six replicates were performed, if measurements were performed on different days, each measurement had its own control from which the relative differences in autofluorescence were derived, the overall results were summarised in an Excel table. The autofluorescence was assessed on BD FACSJazz™ flow cytometry cell sorter (BD Biosciences, USA) channel 585, where the information obtained was based on forward scatter and information on changes in cell size was obtained.

Detection of changes in retrotransposon localisation using the iPBS method. Cell culture preparation was the same as for flow cytometry. 15–20 barley ears were needed for the experiment, depending on their size. After 1.5 h of incubation with particles, the samples were centrifuged again at 4 °C, 900 rpm, for 15 min. The liquid phase was discarded, leaving only the pellet as far as possible; then tap water was added to the tube (to provide ions) and centrifugation was performed again for 15 min at the same settings. The liquid phase was again removed, leaving only the precipitate, which was used for DNA extraction (Krasņevska *et al*., 2022).

After DNA isolation, its concentration and quality were assessed by agarose gel electrophoresis. Subsequently, PCR

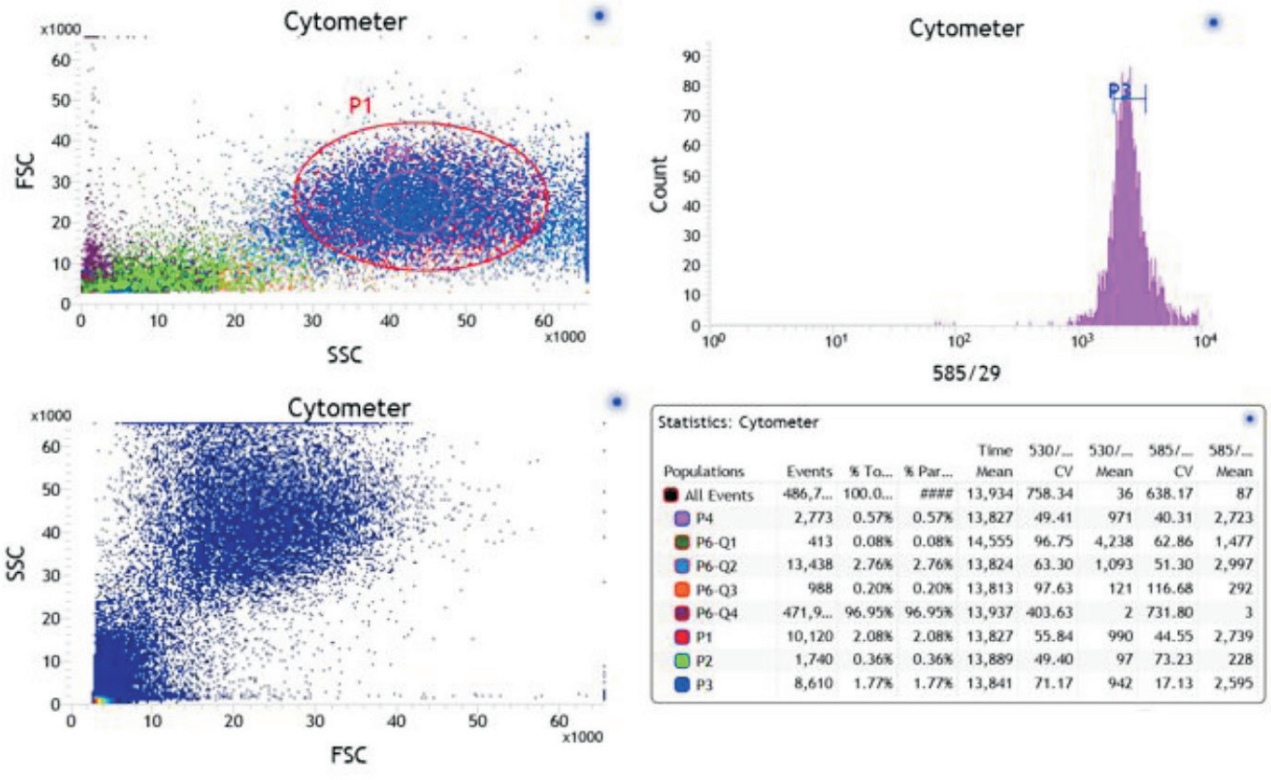

Fig. 1. Cell population separation in a flow cytometer. P1: group of living microspores with various relative fluorescence values; P3: precisely separated cell group in the uninucleate stage, particularly sensitive to a changing environment.

Table 1. iPBS primers and primer combinations used in the study

No.	Primer or combination	Sequence of primer
1	2272	GGCTCAGATGCCA
2	2239	ACCTAGGCTCGGATGCCA
3	2077	CTCACGATGCCA
4	2222	ACTTGGATGCCGATACCA
5	2230	TCTAGGCGTCTGATACCA
6	2074-2381-2384	GCTCTGATACCA-GTCCATCTTCCA-GTAATGGGTCCA
7	2083-2375-2385	CTTCTAGCGCCA-TCGCATCAACCA-CCATTGGGTCCA
8	2095-2374-2251	GCTCGGATACCA-CCCAGCAAACCA-GAACAGGCGATGATACCA

was performed using both positive and negative controls. PCR amplification was carried out with various primers (Table 1), selected based on a previous publication detailing barley primers and their efficiency. PCR reactions were performed either with a single primer per mixture or by combining three primers into one mixture, using optimised annealing temperatures. Only primers demonstrating an efficiency score of 5, as described by Kalendar *et al*. (2021), were selected for this experiment.

Electrophoresis (4 hours at 120 V) was used to evaluate the PCR products. Following electrophoresis, gels were stained with ethidium bromide and photographed under UV light. Images were processed using IrfanView64 software.

Statistical analysis. Data collection and processing was done using Microsoft Excel and RStudio. Graphs were created with Python Matplotlib.

RESULTS

Cell phenotyping using light microscopy. Light microscopic evaluation of gametic cell phenotype after incubation in various media with different types and concentrations of nanoparticles revealed observable cell damage and cytoplasmic alterations associated with the treatments. Over 80% of the cells in microscopic samples exhibited a nanoparticle-induced phenotype, which was further corroborated by changes in relative cell fluorescence compared to the control, observed during subsequent flow cytometry analysis. Figure 2 specifically presents images of intact (control group) and affected or damaged pollen cells incubated with nano- and microparticles. Affected cell groups showed an increased percentage of cells with unusual morphological features, including altered cell shape, modified cytoplasmic structure, or damaged cell walls. For example, cells incubated in 0.1% SiO_2 exhibited visible cytoplasmic changes and the presence of apoptotic cells. While Al_2O_3-treated cells also displayed cytoplasmic changes, those incubated in succinite tended to develop faster, with most reaching the division stage. Conversely, cells incubated in media with Ag particles showed no discernible changes in cell appearance compared to the control group.

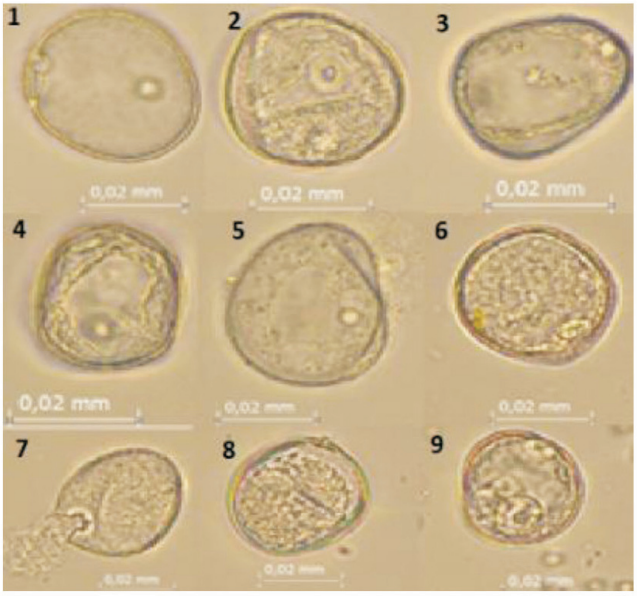

Fig. 2. Immature barley pollen cell morphological differences in different particles and concentration. 1 – control, 2 – Ag 0.01%, 3 – Ag 0.1%, 4 – Al_2O_3 0.01%, 5 – Al_2O_3 0.1%, 6 – SiO_2 0.1%, 7 – SiO_2 00.1%, 8 – succinite 0.01%, 9 – succinite 0.1%.

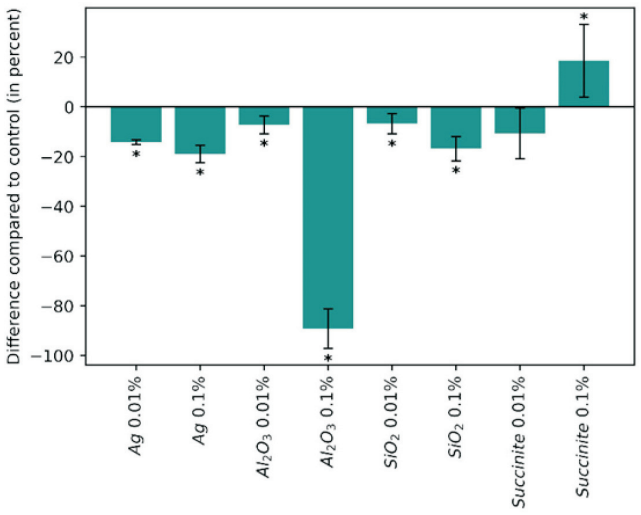

Fig. 3. Changes in relative autofluorescence of immature barley pollen cells at different particle concentrations, expressed as a percentage relative to the control group. (* = p 0.05)

Effect of nanoparticles and microparticles on the autofluorescence of barley gamete cells. The overall autofluorescence results for barley pollen cells are presented in Figure 3. A statistically significant decrease in relative autofluorescence compared to the control was observed for most particle groups at both tested concentrations ($p < 0.05$). The

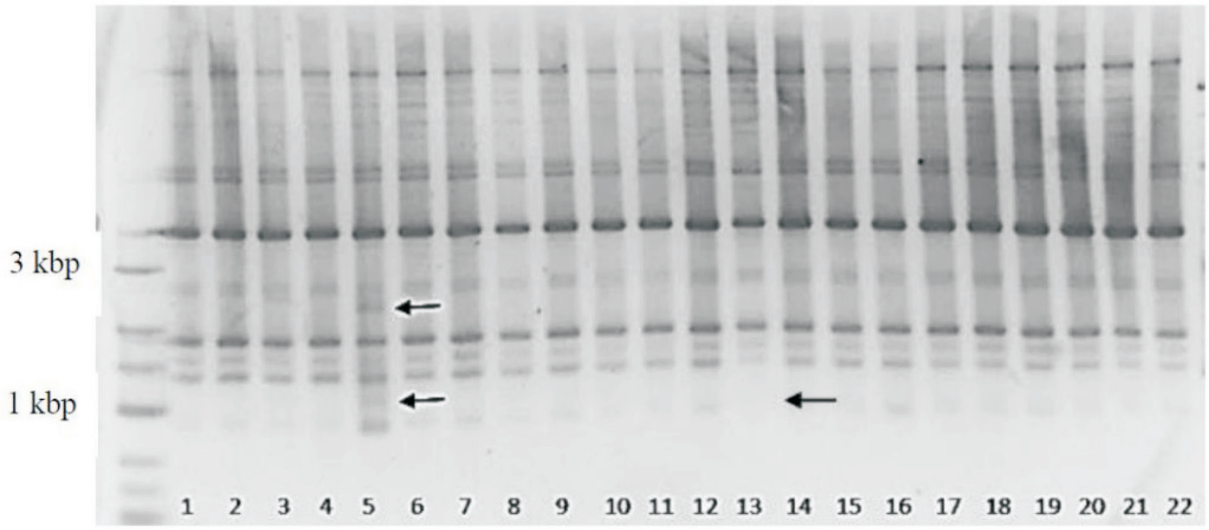

Fig. 4. Image of an electropherogram with visualised spectrum of retrotransposon fragments detected in groups of barley cells amplified using primer 2272: vertical lines 1, 2, 9, 10, 17, and 18 correspond to control group, lines 3 and 4 – succinite 0.1 %, lines 5 and 6 – SiO_2 0.01 %, lines 7 and 8 – SiO_2 0.1 %, 11. Lines 7 and 12 – Al_2O_3 0.01%, lines 13 and 14 – Al_2O_3 0.1%, lines 5 and 16 – succinite 0.1%, lines 19 and 20 – Ag 0.01%, and lines 21 and 22 – Ag 0.1%.

only exception was amber (succinite), where the 0.01% concentration showed no effect on autofluorescence, while the 0.1% concentration led to a significant increase. The most pronounced reduction in autofluorescence (an average decrease of up to 90%) was observed with Al_2O_3 at 0.1% concentration.

Retrotransposon localisation changes in response to particle exposure. The DNA extracted from immature barley gametic cells had suitable quality for detecting changes in retrotransposon localisation. Several individual primers and primer combinations (Table 1) were tested to identify alterations in retrotransposon banding patterns.

For the primer combination 2074-2381-2384, some overlapping fragments were observed, yet specific changes were detectable. Specifically, a loss of bands was noted in samples treated with 0.1% SiO_2 and 0.01% Al_2O_3. Conversely, additional bands appeared in samples treated with 0.1% Al_2O_3 and both concentrations of Ag nanoparticles. The primer combination 2083-2375-2385 produced less complex banding patterns. However, a loss of bands was observed in samples treated with both succinite concentrations, 0.1% SiO_2, and 0.01% Al_2O_3. The primer combination 2095-2374-2251 showed overlapping regions but no detectable differences in banding sites across treatments.

Individual primers 2222, 2230, 2077, 2272, and 2239 were also tested. Primer 2230 showed no discernible changes in banding patterns across treatments. However, primers 2222, 2077, 2272, and 2239 revealed specific alterations. Analysis with primer 2222 demonstrated a loss of bands in samples treated with 0.1% SiO_2 and 0.01% Al_2O_3, while additional bands were observed in samples treated with 0.1% Al_2O_3.

Figure 4 illustrates the results obtained using primer 2272. For example, in samples treated with 0.01% SiO_2, lane 5 showed an appearance of novel fragments at two specific loci. However, lane 6, representing a replicate of the same sample, did not exhibit these changes compared to the control, highlighting potential variability. A loss of a small fragment was also observed in samples treated with 0.1% Al_2O_3 (e.g., lane 13). No discernible changes were detected in samples treated with succinite or Ag nanoparticles for this primer.

Table 2. Number of amplified DNA fragments and changes per count of loci detected using iPBS primers and primer combinations

Primer or combination	Number of loci per sample	Loci changes per count of loci (%)
2272	15	0.009
2239	20	0.002
2077	18	0.005
2222	22	0.01
2230	17	0
2074-2381-2384	18	0.02
2083-2375-2385	17	0.02
2095-2374-2251	15	0.006

With primer 2239, no changes in loci were detected for the 0.01% SiO_2 treatment. However, the 0.1% SiO_2 treatment showed a difference at one locus (indicated by an arrow), suggesting a specific change in retrotransposon localisation. Analysis with primer 2077 did not reveal significant changes in the spectrum of amplified fragments.

Table 2 summarises the percentage change in the total number of loci (primer binding sites) for each individual primer or primer combination. As shown in the table, retrotransposon changes were successfully detected with all iPBS primers used, except for primer 2230. However, the observed changes varied significantly, with the most effective being the three-primer retrotransposon combinations 2074-2381-2384 and 2083-2375-2385.

DISCUSSION

In summary, our findings demonstrated that plant gamete cells respond distinctly to various particle additives. All methods employed in this study — microscopy, flow cytometry, and iPBS analysis — proved suitable for assessing the effects of micro- and nanoparticles on cells. While microscopy revealed visual changes in cell morphology between treated and control groups, flow cytometry results showed negative effects (decreased autofluorescence) in cells exposed to Al_2O_3 and SiO_2. Conversely, succinite exposure led to positive effects, indicated by an increase in relative autofluorescence. Silver particles did not significantly affect plant gamete autofluorescence.

Changes in plant cell fluorescence are known to indicate various phenomena, including alterations in fluorescent pigments, shifts in cell cycle phase, responses to stress (e.g., peroxidase formation), or entry into an apoptotic pathway characterised by decreased relative fluorescence (Grauda *et al*., 2015). The high homozygosity of barley (Pérez-de-Luque, 2017) further enhances its suitability for flow cytometry studies, ensuring more consistent cellular responses.

Most of the particle types used in this study, particularly the larger microparticles, are unlikely to enter the cell directly due to their size. Only the succinite particles, given their wide size range encompassing both nano- and micro-dimensions, possess the potential for cellular internalisation. However, it is important to acknowledge that micro- and nanoparticles can exert toxicity even without direct cellular entry, by altering membrane physiology (e.g., membrane potential, pore blockage) or by dissolving in water and inducing oxidative stress (Grauda *et al*., 2024). Such manifestations can be simultaneously detected through fluorescence analysis using flow cytometry, which captures overall changes in various cellular parameters (Nisa *et al*., 2019). A notable observation during this study was the formation of a gel-like consistency in the succinite particle solution after cell incubation, necessitating significant dilution for flow cytometry analysis. This suggests that the high succinite concentrations (0.1%) used may not be optimal for future studies, as excessive dilution can lead to lower cell counts. Despite this, the flow cytometry results consistently showed a significant increase in plant gamete autofluorescence when incubated with succinite particles.

Information regarding the effects of amber or succinite particles on plant cells at the genetic level is scarce in the literature, making a direct discussion of our findings challenging. Existing research primarily speculates on the potential positive effects of succinic acid on cells (Lasenko *et al*., 2016). Given that succinic acid is water-soluble, it is plausible that its dissolution from the succinite particles into the MS medium influenced the gamete plant cells.

The apparent similarities between flow cytometry and iPBS analysis results suggest that combining these two methods is highly effective for studies involving plant gametic cells. SiO_2 microparticles, being the smallest particles in this study (excluding the broadly sized succinite particles), demonstrated an impact on both autofluorescence and DNA levels. This aligns with other nanoparticle studies indicating that particle size, rather than concentration, often plays a more significant role in observed effects (Guerriero *et al*., 2016). Although 200–300 nm SiO_2 microparticles are generally too large for direct cellular entry, typical entry mechanisms for nanoparticles that are around 40–50 nm include pores, plasmodesmata, endocytosis, or transfer proteins, and they can still affect cellular processes by adhering to the cell surface. Our DNA analyses revealed significant effects of SiO_2 on plant gamete cell DNA, observed with multiple primers. While other researchers' findings on SiO_2 DNA effects are varied, a study on SiO_2 nanoparticles in algal cells (Metzler *et al*., 2012) reported toxicity without evidence of cellular penetration. This further supports the hypothesis that particles can affect cells by mechanisms such as pore clogging, even without internalisation. Aggregation or reactivity with the solution is a common challenge in nanoparticle studies. We attempted to mitigate this by using Tween® 40 emulsifier and sonicating suspensions prior to addition, which helps prevent aggregation (Grauda *et al*., 2015). However, ensuring complete absence of aggregation might require verification through electron microscopy.

Plants are broadly categorised into silicon excluders, intermediates, and accumulators, with barley belonging to the latter. Biosilicification, the normal process of insoluble SiO_2 uptake by plant cells, involves diverse mechanisms, including interactions with membrane polysaccharides, lignin, or cell wall-associated proteins (Chiba *et al*., 2009). However, the precise mechanisms by which the gamete cells in this study might have taken up SiO_2 or been influenced through the cell wall are not yet fully understood. While existing studies describe SiO_2 entry through pores or specific transporters like HvLsi1 (Poborilova *et al*., 2013), these mechanisms are primarily root-specific. Overall, there remains a significant gap in the literature regarding the penetration and effects of micro- and nanoparticles in plant gamete cells. Further sequencing studies are crucial to precisely identify the genes directly affected by various particle treatments.

Numerous studies in the literature report negative effects of Al_2O_3 on both plant and animal cells. For instance, research on *Nicotiana tabacum* BY-2 cell cultures investigated the effects of both Al_2O_3 nanoparticles (< 50 nm) and microparticles (5 μm). The authors concluded that while microparticles had no significant effect on cellular ROS production, lipid peroxidation, or dehydrogenase activity, nanoparticles exerted various negative impacts. Their fluorescence microscopy detected dissolved aluminium or free aluminium ions within the cell envelope, cytoplasm, and nucleus (Rodrigues *et al*., 2024). In our study, the relatively short incubation time with Al_2O_3 particles and limited changes observed with some primers suggest that longer exposure times or investigation of both nano- and micro-sized forms of all particles might be beneficial for a more comprehensive understanding.

The effect of succinite particles on plant gamete DNA was detected with fewer primers, although primer 2239 revealed a loss of bands in both concentrations. The scarcity of similar studies in the literature on succinite's effects on plant DNA highlights the need for further investigation, specifically through DNA sequencing, to pinpoint the affected genes.

While numerous studies exist on the diverse (both positive and negative) effects of silver nanoparticles (Yuan *et al*., 2018; Rodrigues *et al*., 2024), literature on the negative impact of silver microparticles on plant cell DNA is limited. This scarcity might suggest that silver particles at the tested sizes and concentrations may not significantly affect plant gamete cells or pose a major hazard for applications like coatings. Despite this, our research detected some changes in primer binding sites in samples treated with Ag particles, warranting further investigation to confirm and identify affected genes.

Further studies, including additional replications, are essential to more definitively characterise the effects of nanoparticles and microparticles on plant gametic cells. Nevertheless, our results affirm that combining flow cytometry with iPBS analysis is a useful and promising methodological approach for such investigations. It is noteworthy that iPBS-based studies of plant stress responses, particularly in gametic cells, are still relatively underexplored. Future research integrating these methods with gene sequencing holds significant potential for revealing deeper insights into particle-induced genetic alterations.

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BIOTEKSTILA IZVEIDĒ IZMANTOTO NANO- UN MIKRODAĻIŅAS (SUKCINĪTS, Ag, SiO_2, Al_2O_3) IETEKMES UZ MIEŽU (*HORDEUM VULGARE*) NENOBRIEDUŠU PUTEKŠŅLAPU ŠŪNU TESTA SISTĒMU NOTEIKŠANA

Straujais nanotehnoloģiju progress ir iniciējis plašus nano- un mikrodaļiņu pētījumus, kas saistīti ar to daudzveidīgajām pielietojuma iespējām, vienlaikus radot pieaugošas bažas, par to ietekmi uz vidi un potenciālajām sekām dzīvajiem organismiem. Nanodaļiņas, to izmēra dēļ, spēj iekļūt šūnu membrānās, uzkrāties šūnu iekšienē un ietekmēt molekulāros mehānismus, savukārt mikrodaļiņas mēdz pielipt pie šūnu virsmām, ietekmējot šūnu transporta ceļus. Šajā pētījumā tiek analizēta četru veidu nano- un mikrodaļiņu (SiO_2 (0,2-0,3 μm), Al_2O_3 (2,5 μm), Ag (0,5–1 μm) un sukcinīts (5 nm – 3 μm)), kuras tiek pētītas jaunu biotekstila materiālu izstrādei ar specializētām aizsardzības spējām, īstermiņa ietekme. Pētījumā kā modelis izmantotas miežu (*Hordeum vulgare*) nenobriedušas putekšņu šūnas (mikrosporas). Mikrosporas 1,5 stundas tika inkubētas *in vitro* MS barotnē, kas saturēja šīs daļiņas. Tika mērīta relatīvā šūnu autofluorescence, DNS izmaiņas tika novērtētas, izmantojot iPBS (inter-primer binding site) praimeru sistēmu. Iegūtie rezultāti liecina, ka Al_2O_3 un SiO_2 mikrodaļiņām bija negatīva ietekme uz mikrosporām, savukārt sukcinīta (dzintara) daļiņām pozitīva. Ag daļiņas pētītajos apstākļos neuzrādīja būtisku ietekmi. Ar iPBS metodi tika konstatētas DNS izmaiņas, ko izraisījusi retrotranspozonu pārvietošanas, paraugos, kas bija pakļauti SiO_2, Al_2O_3 vai sukcinīta nano- un mikrodaļiņām, kas liecina par iespējamiem genotoksiskiem efektiem. Pētījums parādīja, ka ir svarīgi ir novērtēt dažāda veida inženierijas nano- un mikrodaļiņu izraisītās daudzveidīgās fizioloģiskās un ģenētiskās šūnu reakcijas, respektīvi, to genotoksitāti, pirms jaunu produktu izstrādes.