horticulturae

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The Impact of Genotype and Controlled Environment Cultivation Parameters on Tomato-Leaf-Derived Exosome-like Nanoparticle Yield and Properties

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Abstract: Horticultural plant material offers several advantages for isolating exosomes and other natural plant-derived exosome-like nanoparticles (PDENs) due to the accessibility and affordability of plant material for widespread applications. This study aims to explore the impacts of the tomato genotype ('Admiro', 'Roma', 'Brooklyn', 'Marmande' and 'Betalux') and the main cultivation parameters in controlled environment agriculture on the yield and properties of their PDENs for pharmaceutical and cosmeceutical applications. The PDEN yield, size distribution, and antioxidative properties of young tomato seedlings were evaluated. The 'Betalux' tomato was distinguished by a remarkably higher nanoparticle concentration and a uniform size distribution and was selected for further experiments. The impact of cultivation temperature (18, 22, and 26 °C), nitrogen nutrition (0, 250, and 500 mg L^{-1}), and the lighting photosynthetic photon flux density (PPFD; 150, 250, and 450 µmol m^{-2} s^{-1}) on nanoparticle properties was investigated. Optimal conditions consisting of a temperature of 22 °C, 250 mg L^{-1} nitrogen nutrition, and 250 µmol m^{-2} s^{-1} lighting PPFD were used as a reference. Optimal temperature, nitrogen nutrition, and lighting intensity resulted in the highest nanoparticle yield, the most uniform particle distribution, and the highest impact of PDEN preparations on keratinocyte metabolic activity. Deviation from optimal cultivation conditions reduced the tomato biomass and the PDEN protein and yield.

Keywords: antioxidative activity; exosomes; lighting intensity; nitrogen nutrition; size distribution; temperature; tomato cultivars

Citation: Viršilė, A.; Samuolienė, G.; Laužikė, K.; Mikalauskienė, E.; Balion, Z.; Jekabsone, A. The Impact of Genotype and Controlled Environment Cultivation Parameters on Tomato-Leaf-Derived Exosome-like Nanoparticle Yield and Properties. *Horticulturae* **2024**, *10*, 477. https://doi.org/10.3390/horticulturae10050477

Academic Editors: Pascual García-Pérez and Lucía Cassani

Received: 29 March 2024
Revised: 2 May 2024
Accepted: 4 May 2024
Published: 7 May 2024

1. Introduction

Using horticultural plant material to isolate plant-derived exosome-like nanoparticles (PDENs) represents a promising approach in nanobiotechnology and biomedicine. PDENs are nanosized extracellular vesicles with membrane structures originating from the endomembrane system, serving as protective compartments and the long-distance carriers of various bioactive ingredients such as proteins, nucleic acids, and secondary metabolites [1–3]. Abundant evidence confirms the implication of those nanoparticles in intercellular signaling, defense mechanisms, and interspecies communication [4], as well as their anti-tumor, anti-inflammatory, cardioprotective, and wound-healing properties [4,5]. Moreover, bringing to the fore their ability to efficiently transport bioactive molecules to target tissues [6] and their safety and immune tolerance compared with exosomes derived from animals [4], PDENs have a high potential for applications in functional foods, drug delivery, and therapeutics.

It was shown that nanoparticles can be isolated from various plants, such as lemon [7], ginger [8,9], broccoli [10], grapefruit [11], allium [12], grapes [13], lemon [14], celery [15], various medicinal plants [16,17] and others.

Horticultural plant material offers several advantages for isolating exosomes and other plant-derived nanoparticles, which is mainly due to the accessibility and affordability of plant material for widespread applications. Horticultural plants are highly productive and can be cultivated on a large scale, offering a renewable and cost-effective source of raw material. Moreover, large quantities of residual biomass are discarded during fruit and vegetable production; therefore, new value chains to utilize horticultural leaf biomass efficiently are important. Greenhouse tomato crops, in particular, create significant vegetative biomass of up to 49 t per ha per year [18]. The waste products include the lower leaves (~15 t per ha) and stems that are regularly removed from the vines to improve fruit production [19]. Increasing demand for sustainability in the food and agriculture sectors is driving the search for new ways to use these waste products beneficially while compensating for the costs of their processing. Tomato-leaf waste is an unexploited source of bioactive molecules, carotenoids, flavonoids, phylloquinone, solenasol, etc. [18–20]; therefore, together with tomato fruits, leaves could be used as the source material for PDEN isolation.

Despite these advantages, several challenges exist in utilizing horticultural plant material for nanoparticle isolation. One is the standardization of plant production processes to ensure consistency in the quality and characteristics of PDENs [21]. Variability in plant species, cultivation conditions, and tissue types can impact the quality, cargo content, yield, and functionality of the PDENs [22]. Controlled environment agriculture (CEA) can be highly beneficial for cultivating consistent plant material for PDEN isolation under constant conditions and for research. By manipulating controllable cultivation environmental parameters, such as lighting, temperature, mineral nutrition, and CO_2 [23], the level of the impact on the yield and quality of PDENs in plant material can be evaluated, leading to the development of optimized cultivation protocols. Moreover, CEA is promising for the high reproducibility and quality of plant-based products. CEA facilities can operate year-round regardless of seasonal variations in the weather conditions; consistent plant access to water, nutrients, and light promotes uniform growth and minimizes variability in plant characteristics [24]; and the enclosed environments in CEA provide protection against pests and diseases [25], minimizing the risk of crop damage and contamination of PDEN preparations.

Considering the above-discussed benefits of CEA, this study aims to explore the impacts of tomato genotype and the main controllable cultivation environmental parameters on the yield and properties of their leaf-derived exosome-like nanoparticles. In this study, CEA is employed for dual purposes. First, to foresee the possible impacts of varying cultivation conditions on the quality of tomato-leaf material cultivated in commercial systems, substantiating the idea of using the residual leaf biomass for PDEN isolation. Second, to propose reference values for the CEA cultivation method for tomato plant material for the highest yield and quality of leaf-derived nanoparticles.

2. Materials and Methods

2.1. Cultivation Conditions

Experiments were performed in controlled environment chambers under constant and controllable environmental conditions that imitated a vertical-farming cultivation system. Light-emitting diode (LED; Tungsram Agritech Research Toplight, Hungary) lighting modules with a spectrum consisting of 61% deep red, 20% blue, 15% white, and 4% far red light were used as the source of artificial lighting. If not indicated otherwise, a temperature of 22 °C, relative air humidity of ~55%, a 16 h photoperiod, and 250 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) were maintained at the top of the plant. Different tomato (*Solanum lycopersicum* L.) genotypes were cultivated from seeds: 'Admiro'; 'Brooklyn'—indeterminate hybrids; 'Marmande'—indeterminate variety; 'Roma'—determinate variety;

and 'Betalux'—dwarf variety (all obtained from Agrofirma Sėklos, Lithuania). Seeds were sown in seedling trays in peat substrate (Profi 1, Durpeta, Lithuania; 5.5–6.0 pH; EC 0.6–0.9 mS/cm; N 70–110 mg L^{-1}; P 80–120 mg L^{-1}; K 130–210 mg L^{-1}.), and after the development of the first pair of true leaves, the seedlings were transplanted into 450 mL volume plastic containers, watered to maintain equal humidity, and fertilized twice a week with ammonium nitrate solution (N 250 mg L^{-1}). Two experiments were performed. During the first experiment, the seedlings of 5 tomato genotypes were cultivated under similar and constant CEA cultivation conditions until 5–6 leaves had fully developed. In the second experiment, dwarf tomato 'Betalux' seedlings were cultivated under different temperature, nitrogen nutrition, and lighting intensity conditions (Table 1). Optimal condition parameter values (T 22 °C, N 250 mg L^{-1}, PPFD 250 µmol m^{-2} s^{-1}) served as the reference, and the impacts of lower and higher temperature, PPFD, and N nutrition were investigated.

Table 1. Experimental design. Values in bold represent the deviation of the parameter from the optimal conditions in each treatment.

	Temperature	Nitrogen Nutrition	Lighting PPFD
Low	T 18 °C N 250 mg L^{-1} PPFD 250 µmol m^{-2} s^{-1}	T 22 °C N 0 mg L^{-1} PPFD 250 µmol m^{-2} s^{-1}	T 22 °C N 250 mg L^{-1} PPFD 150 µmol m^{-2} s^{-1}
Optimal; reference	T 22 °C N 250 mg L^{-1} PPFD 250 µmol m^{-2} s^{-1}	T 22 °C N 250 mg L^{-1} PPFD 250 µmol m^{-2} s^{-1}	T 22 °C N 250 mg L^{-1} PPFD 250 µmol m^{-2} s^{-1}
High	T 26 °C N 250 mg L^{-1} PPFD 250 µmol m^{-2} s^{-1}	T 22 °C N 500 mg L^{-1} PPFD 250 µmol m^{-2} s^{-1}	T 22 °C N 250 mg L^{-1} PPFD 450 µmol m^{-2} s^{-1}

All experimental treatments were performed in 3 replicates in the area, with 20 plants per experimental replication. At the end of the cultivation period, tomato leaves were collected, frozen in liquid nitrogen, freeze-dried (FD-7, SIA Cryogenic and Vacuum Systems, Latvia), and ground.

2.2. Nanoparticle Isolation and Analysis

A kit (2-EPL, Exolitus, Lithuania) was used for the plant-derived nanoparticle (PDEN) isolation from dry plant material. The isolation method is based on the stabilization, precipitation, and purification of exosomes using low-speed centrifugation (Z366, Hermle, Gosheim, Germany). Nanoparticle preparations, isolated from 2.6 g of dry plant material, were resuspended in 0.2 mL of PBS buffer pH 7.2 for size distribution analysis and protein content analysis or in 0.5 mL of 80% methanol for antioxidative activity evaluation.

Nanoparticle tracking analysis (NTA) was performed to evaluate the size distribution and concentration of the PDEN preparations (NanoSight NS300, Malvern Technologies, Malvern, UK). The prepared samples were diluted 1000-fold or 10,000-fold with PBS, and measurement was performed in 5 analytical replications. The mean particle size, the span of particle size distribution, and particle concentration in the PDEN isolate suspensions were obtained. Span = (D90 − D10)/D50, where D10, D50, and D90 mark the points in the particle size distribution up to and including where 10, 50, and 90% of the total number of particles in the sample are contained. PDEN concentrations were re-calculated to represent the number of nanoparticles per g of plant dry weight (DW).

The Bradford method was used to evaluate the protein content in PDEN isolates resuspended in PBS. Bovine serum albumin (0.05–1.0 mg mL^{-1}) was used for quantification. Aliquots of 10 µL of sample or standard were mixed with 190 µL of diluted Bradford

reagent, and absorption was measured at 595 nm (Spectro-star Nano microplate reader, BMG Labtech, Ortenberg, Germany). PDEN protein content was expressed as mg of protein per g of plant dry weight (DW).

Total RNA content was evaluated using the TRIzolTM reagent (InvitrogenTM, Life Technologies Limited, Cambridge, UK). The procedure was performed according to the manufacturer's protocol. A sample of 100 µL of PDEN solution was mixed with 250 µL TRIzolTM reagent and incubated for 5 min to allow for the complete dissociation of the nucleoprotein complex. Then, 50 µL of chloroform was added for lysis, and centrifugation was performed (15 min at 12,000× *g* at 4 °C). The mixture was separated into a lower red phenol–chloroform layer and a colorless upper aqueous phase layer containing RNA. The upper layer was collected and mixed with 125 µL isopropanol, incubated for 10 min at 4 °C, and centrifuged for 10 min at 12,000× *g* at 4 °C to precipitate the RNA. The pellet was resuspended in 250 µL of 75% ethanol. Then, the sample was vortexed and centrifuged for 5 min at 7500× *g* at 4 °C. The supernatant was discarded, and the RNA was air-dried for 10 min. The pellet was resuspended in 20 µL of RNAse-free water and incubated in a heat block at 55 °C for 10 min. The RNA content was quantified by absorbance at 260 nm and 280 nm wavelengths using a µDopTM Plate (InvitrogenTM, Life Technologies Limited, Cambridge, UK) and a VarioskanTM Lux multimode microplate reader, Thermo Fisher, Waltham, MA, USA. RNA content in the PDEN preparations was expressed as µg of RNA per g of plant dry weight (DW).

Antioxidative activity was evaluated in plant material extracts and PDEN preparations. Plant extracts were prepared by grinding 0.01 g of dry plant material with 5 mL of 80% methanol, and after incubation for 24 h, centrifugation was performed (15 min at 4500 rpm; Z366, Hermle, Gosheim, Germany). PDENs resuspended in 80% methanol were used directly for the analysis of their antioxidative properties, DPPH and ABTS free-radical scavenging activity, and ferric-reducing antioxidant power (FRAP). Each measurement was performed with 3 replications.

For the DPPH (2-diphenyl-1-picrylhydrazyl) analysis [26], a 126.8 µM DPPH (Sigma-Aldrich, Burlington, MA, USA) solution in methanol was prepared. A volume of 290 µL of the DPPH solution was mixed with 20 µL of the PDEN solution. The absorbance was read at 515 nm (Spectro-star Nano microplate reader, BMG Labtech, Ortenberg, Germany) at the 16th min. The DPPH free-radical scavenging activity was expressed as mmol of DPPH per g of dry plant weight (µmol g^{-1} DW) or per PDEN preparation isolated from 1 g of dry plant weight.

For the ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) analysis [27], radical cations were obtained by incubating the 7 mM ABTS solution with 2.45 mM $K_2S_2O_8$ for 12–16 h in the dark. A 20 µL aliquot of the prepared sample was mixed with 290 µL of the diluted (1:7) incubated ABTS solution, and the absorbance was measured after 11 min at 734 nm (Spectrostar Nano microplate reader, BMG Labtech, Ortenberg, Germany). The ABTS free-radical scavenging activity of tomato-leaf material extracts and PDEN preparations was expressed as mmol ABTS scavenged per g of dry plant weight (µmol g^{-1} DW) or per PDEN preparation isolated from 1 g of dry plant weight.

For the FRAP assay [28], the working solution was prepared by mixing 300 mM acetate buffer pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \times 6H_2O$ at a ratio of 10:1:1 ($v/v/v$) [28]. A 20 µL aliquot of the sample was mixed with 290 µL of freshly prepared working solution and incubated in the dark for 30 min. Then, the absorbance was read at 593 nm (Spectrostar Nano BMG Labtech microplate reader, Ortenberg, Germany). A calibration curve was determined using 0.005–0.5 mM $Fe_2(SO_4)_3$ (Iron (III) sulfate; Sigma-Aldrich, Burlington, MA, USA). The FRAP is expressed as µmol of Fe^{2+} reduced per g of dry plant weight (DW) or per PDEN preparation isolated from 1 g of plant DW.

2.3. Metabolic Activity Evaluation

The impact of the PDEN preparations on HaCaT keratinocyte (CLS Cell Lines Service GmbH, Eppelheim, Germany) metabolic activity was evaluated. Cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin solution at 37 °C and 5% CO_2. The cells were grown in a T25 flask until 80–90% confluency and then detached with 0.05% Trypsin–EDTA solution. The metabolic activity was evaluated using the PrestoBlueTM Cell Viability reagent (InvitrogenTM, Life Technologies Limited, Cambridge, UK) according to the manufacturer's protocol. The cells were seeded into a 96-well plate at 2500 cells/well density. In the first step, cells were treated with PDENs from 'Betalux' tomato leaves and cultivated under high (450 μmol m^{-2} s^{-1}) PPFD, and those possessing the highest antioxidative activities were selected for concentration screening at 10^6, 10^7, 10^8, 10^9, and 10^{10} particles mL^{-1} and incubated for 48 h. Then, 10 μL of PrestoBlueTM reagent was mixed with 90 μL of warm cell culture medium. The plate was incubated for 2 h at 37 °C in the dark. After the incubation, the absorption assessment was performed with a VarioskanTM Lux multimode microplate reader (InvitrogenTM, Life Technologies Limited, Cambridge, UK) at a wavelength of 570 nm, using 600 nm as the reference. The results were expressed as an average percentage of the control cells and the standard deviation. A concentration of 10^9 particle mL^{-1} was selected for further experiments. In the second step, the effect of other samples on the keratinocyte metabolic activity was evaluated. The procedure was the same as described earlier.

2.4. Statistical Analysis

The results are presented as the average ($\overline{x}$) of 3 replicates ± standard deviation (SD). ANOVA Tukey's HSD test (p = 0.05) and multivariate principal component analysis (PCA) were performed for result modeling. Data were processed using MS Excel with compatible XLStat 2022.3.1 (Addinsoft, France) statistical software.

3. Results

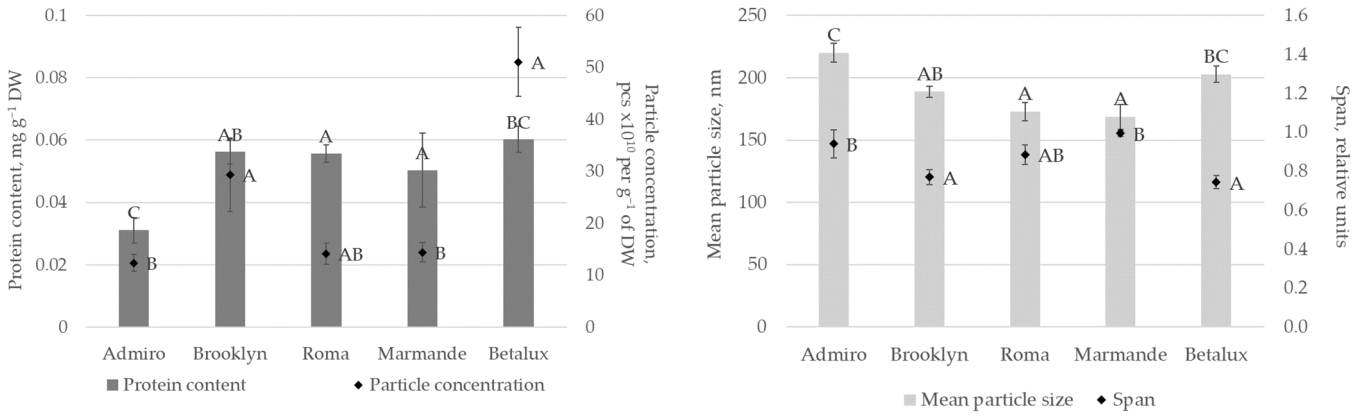
3.1. Impact of Tomato Genotype

Cultivated tomato genotypes from different growth strategies are not specific to vertical farming and were investigated for experimental purposes. The 'Brooklyn' and 'Admiro' tomato varieties are indeterminate hybrids for commercial cultivation; 'Marmande' is an indeterminate variety more popular for amateur greenhouses; 'Roma' is a variety of determinate growth, while 'Betalux' is a dwarf tomato variety. At a young seedling age (5–6 fully developed leaves), the determinate or indeterminate growth strategy has no significant impact on the accumulated fresh plant weight or height, except for the dwarf 'Betalux' variety (Table 2). 'Admiro' and 'Roma' tomato seedlings accumulated significantly higher fresh and dry plant weights, indicating the highest biomass productivity. However, dwarf tomato 'Betalux' seedlings, despite having a lower fresh weight and leaf area, were distinguished by a relatively high biomass accumulation rate and height ratio, indicating their suitability for cultivation in height-limiting vertical-farming conditions.

Table 2. Biometric parameters of young tomato plants of different genotypes, cultivated under CEA conditions ($\overline{x} \pm SD$, $n = 3$). Different letters indicate statistically significant differences between means within the same column according to Tukey's HSD test at the confidence level of p = 0.05.

Tomato Genotype	Fresh Weight, g	Dry Weight, g	Height, cm	Leaf Area, cm^2
Admiro	25.0 ± 1.1 C	2.92 ± 0.15 D	31.7 ± 0.5 D	348 ± 33 B
Brooklyn	22.4 ± 0.7 B	2.08 ± 0.12 BC	32.0 ± 1.6 D	328 ± 30 B
Roma	27.0 ± 0.9 C	2.47 ± 0.18 C	23.7 ± 0.5 B	380 ± 11 B
Marmande	21.7 ± 0.5 B	1.81 ± 0.10 BC	27.0 ± 0.8 C	321 ± 20 B
Betalux	18.8 ± 0.1 A	1.49 ± 0.06 A	18.3 ± 0.5 A	221 ± 16 A

PDENs from the leaves of different tomato genotypes exhibited distinct protein and nanoparticle yields and size distributions (Figure 1), but the results are not directly associated with growth traits of the different tomato genotypes. Preparations derived from leaves of the 'Admiro' tomato contained 45% lower protein content compared with other tomato genotypes (Figure 1a). In terms of nanoparticle concentration in preparations derived from the same weight of dry tomato leaves, 'Brooklyn' and 'Betalux' are defined by 1.9 and 3.6 times higher particle numbers compared with the others. The particle size distribution parameters are presented in Figures 1b and A1. Although the mean particle size varies within a relatively narrow interval of 169–220 nm, 'Brooklyn' and 'Betalux' tomato-leaf-derived nanoparticle preparations are characterized by lower span values, indicating a narrower, more uniform particle size distribution.



(**a**) (**b**)

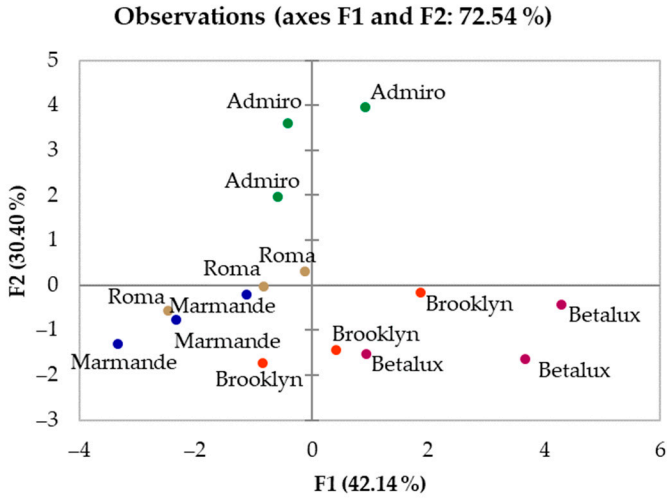
Figure 1. Yield and size properties of nanoparticles derived from the leaves of different tomato cultivars ($\overline{x} \pm SD$, $n = 3$). (**a**) Protein amount, equivalent to the nanoparticle yield from 1 g of dry plant weight (primary *Y* axis) and nanoparticle yield per gram of dry weight (secondary *Y* axis); (**b**) Mean particle size (primary *Y* axis) and span (secondary *Y* axis). Different letters indicate statistically significant differences between means according to Tukey's HSD test at the confidence level of $p = 0.05$. DW—dry plant weight.

For their direct application, the biochemical properties of PDENs should evaluated. Table 3 summarizes the antioxidative properties of different varieties of tomato leaves and their nanoparticle preparations. Although leaves of the tomato varieties 'Admiro' and 'Marmande' were defined by 1.2 and 1.5 times higher DPPH and 1.1 and 1.2 times higher ABTS free-radical scavenging activity—'Marmande' by 1.2 times higher FRAP compared with leaves of the other tomato genotypes—there were no signifficant differences in the free-radical scavenging activities of the tomato-leaf-derived nanoparticle preparations, and no FRAP activity was determined in them.

The principle component analysis shown in Figure 2 confirms that nanoparticle preparations derived from the leaves of young tomato seedlings of different cultivars contain genotype-specific properties. 'Betalux' tomato-leaf nanoparticle preparations, according to their factor loadings and in agreement with previous data, are distinguished by a higher particle concentration, particle size, and size distribution span. Although differences in the antioxidative properties of the PDEN are insignificant, according to the PCA analysis, 'Admiro' leaf preparations tend to have higher antioxidative activity. Based on these trends, and as its dwarf morphology is suitable for CEA cultivation, the 'Betalux' tomato variety was selected for further experiments.

Table 3. Antioxidative properties of tomato-leaf material and leaf-derived nanoparticle preparations ($\bar{x} \pm SD$, $n = 3$). Different letters indicate statistically significant differences between means within the same column according to Tukey's HSD test at the confidence level of p = 0.05. n.d.—not determined.

Tomato Cultivar	DPPH Scavenging Activity, mmol g^{-1} DW		ABTS Scavenging Activity, µmol g^{-1} DW		FRAP, µmol Fe(II) g^{-1} DW	
	Plant Material	Nanoparticle Preparation	Plant Material	Nanoparticle Preparation	Plant Material	Nanoparticle Preparation
Admiro	321 ± 6 B	1.07 ± 0.13 A	1741 ± 33 C	3.54 ± 0.31 A	131 ± 2 C	n.d.
Brooklyn	273 ± 5 A	1.08 ± 0.07 A	1526 ± 17 A	2.64 ± 0.55 A	119 ± 1 A	n.d.
Roma	261 ± 4 A	0.99 ± 0.14 A	1555 ± 17 AB	3.13 ± 0.20 A	123 ± 2 AB	n.d.
Marmande	386 ± 9 C	1.12 ± 00.5 A	1874 ± 30 D	2.41 ± 0.43 A	159 ± 3 D	n.d.
Betalux	263 ± 12 A	0.85 ± 0.18 A	1642 ± 36 B	3.10 ± 0.37 A	128 ± 1 BC	n.d.



	F1	F2
Protein content	0.286	**0.679**
Conc., pcs per g of DW	**0.871**	0.434
Mean	**0.661**	0.660
Mode	**0.903**	0.344
Span	**0.697**	0.476
DPPH	0.775	0.019
ABTS	0.398	**0.671**

Figure 2. Principal component analysis and factor loadings, indicating distinct properties of the nanoparticle preparations derived from the leaves of different tomato genotypes ($n = 3$).

3.2. Impact of Cultivation Parameters

The main parameters of cultivation environment, temperature, lighting intensity, and nitrogen nutrition had a pronounced impact on the growth of the young 'Betalux' tomato (5–6 fully developed leaves) (Table 4). Environmental temperature and lighting PPFD had a more pronounced impact on tomato growth parameters than nitrogen nutrition at the investigated levels. Higher temperature (26 °C) resulted in 16% higher fresh plant weight, while a lower temperature (18 °C) resulted in 23% higher dry weight. Both high- and low-lighting PPFD reduced the tomato height and leaf area, but high light (450 µmol m^{-2} s^{-1}) did not inhibit biomass accumulation. Only low light (150 µmol m^{-2} s^{-1}) resulted in 66% lower fresh weight and 52% dry weight compared with the optimal 250 µmol m^{-2} s^{-1} lighting PPFD, demonstrating remarkable productivity losses per cultivation area.

Properties of the PDENs obtained from leaves of the 'Betalux' tomato cultivated under different conditions are presented in Table 5 and Figure A2 (NTA size distribution charts). The protein content of PDEN preparations was reduced by ~1.9 times when the tomatoes were cultivated under non-optimal conditions for any cultivation parameter. The same trend was observed for the particle concentration per dry tomato plant weight, where temperatures both higher and lower than 22 °C resulted in ~2 times lower particle concentration, higher and lower nitrogen nutrition resulted in 2.3 and 2 times lower particle concentration, and higher and lower lighting PPFD led to 1.8 and 2 times lower nanoparticle concentration, respectively. A more pronounced impact of cultivation conditions was on the RNR content in PDEN preparations. A higher temperature of 26 °C resulted in 10 times lower RNR content, while an 18 °C temperature resulted in 60% lower RNR

content, compared with the optimal temperature. Nitrogen nutrition moderately affected the RNR content in PDENs while low light (150 µmol m^{-2} s^{-1}) reduced the RNR content by 5.3 and high light (450 µmol m^{-2} s^{-1}) by 2.4 times. Mean particle size in the PDEN preparations varied only slightly (from 197 to 229 nm) upon exposure to different cultivation parameters. Higher temperature resulted in 10% higher mean particle size and lower lighting intensity led to 12% higher mean particle size compared with optimal cultivation conditions. However, deviation from optimal conditions led to higher span values and a more scattered particle size distribution (Table 5, Figure A2), especially under low lighting conditions (150 µmol m^{-2} s^{-1}).

Table 4. Biometric parameters of young 'Betalux' tomato plants cultivated under different temperatures, nitrogen nutrition, and lighting intensity parameters ($\overline{x} \pm SD$, $n = 3$). Different letters indicate statistically significant differences between means within the same column according to Tukey's HSD test at the confidence level of p = 0.05.

Parameter		Fresh Weight, g	Dry Weight, g	Height, cm	Leaf Area, cm^2
Temperature	18 °C	17.5 ± 0.3 A	1.84 ± 0.11 B	13.7 ± 0.5 A	189 ± 12 A
	22 °C	18.8 ± 0.1 A	1.49 ± 0.06 A	18.3 ± 0.5 B	221 ± 16 B
	26 °C	21.0 ± 0.0 B	1.71 ± 0.10 AB	21.0 ± 0.8 C	232 ± 37 B
Nitrogen nutrition	0 mg L^{-1}	21.2 ± 1.0 B	1.68 ± 0.08 A	23.7 ± 0.5 C	229 ± 21 A
	250 mg L^{-1}	18.8 ± 0.1 A	1.49 ± 0.06 A	18.3 ± 0.5 A	221 ± 16 A
	500 mg L^{-1}	20.8 ± 0.9 AB	1.52 ± 0.10 A	20.7 ± 0.5 B	194 ± 22 A
PPFD	150 µmol m^{-2} s^{-1}	12.4 ± 1.8 A	0.78 ± 0.13 A	14.6 ± 0.4 A	165 ± 25 A
	250 µmol m^{-2} s^{-1}	18.8 ± 0.1 B	1.49 ± 0.06 B	18.3 ± 0.5 B	221 ± 16 B
	450 µmol m^{-2} s^{-1}	17.5 ± 0.1 B	1.71 ± 0.05 B	14.4 ± 0.1 A	151 ± 9 A

Table 5. Yield and size properties of nanoparticles derived from the leaves of the 'Betalux' tomato, cultivated under different temperature, nitrogen nutrition, and lighting intensity parameters ($\overline{x} \pm SD$, $n = 3$). Different letters indicate statistically significant differences between means according to Tukey's HSD test at the confidence level of p = 0.05. DW—dry plant weight.

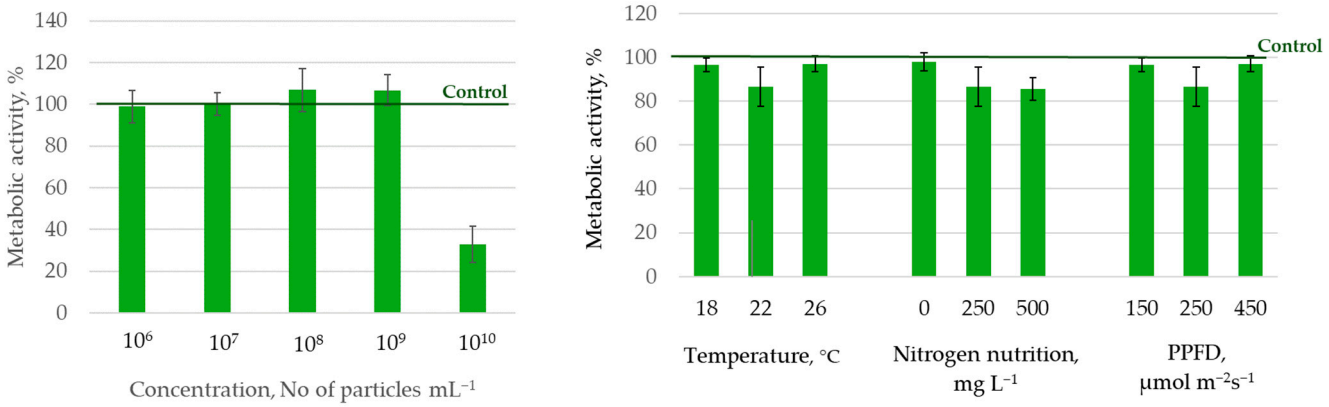
Parameter		Protein Content, mg g^{-1} DW	Particle Concentration, pcs $\times 10^{10}$ per g^{-1} of DW	RNR Content, mg g^{-1} DW	Mean Particle Size, nm	Span
Temperature	18 °C	0.031 ± 0.004 A	26.6 ± 2.63 A	1.91 ± 0.01 B	201 ± 2 A	0.94 ± 0.02 B
	22 °C	0.057 ± 0.004 B	51.0 ± 6.66 B	3.23 ± 0.01 C	203 ± 6 A	0.74 ± 0.03 A
	26 °C	0.029 ± 0.001 A	23.9 ± 1.28 A	0.33 ± 0.09 A	227 ± 3 B	0.96 ± 0.05 B
Nitrogen nutrition	0 mg L^{-1}	0.032 ± 0.004 A	25.3 ± 4.28 A	3.61 ± 0.01 C	214 ± 7 B	0.91 ± 0.05 B
	250 mg L^{-1}	0.057 ± 0.004 B	51.0 ± 6.66 B	3.23 ± 0.01 B	203 ± 6 AB	0.74 ± 0.03 A
	500 mg L^{-1}	0.027 ± 0.005 A	38.4 ± 2.44 AB	2.88 ± 0.02 A	197 ± 2 A	0.82 ± 0.01 AB
PPFD	150 µmol m^{-2} s^{-1}	0.031 ± 0.001 A	24.2 ± 1.64 A	0.60 ± 0.02 A	229 ± 10 B	1.04 ± 0.02 B
	250 µmol m^{-2} s^{-1}	0.057 ± 0.004 B	51.0 ± 6.66 B	3.23 ± 0.01 C	203 ± 6 A	0.74 ± 0.03 A
	450 µmol m^{-2} s^{-1}	0.032 ± 0.002 A	28.2 ± 1.65 A	1.30 ± 0.04 B	212 ± 8 AB	0.83 ± 0.06 A

Tomato-leaf material possesses relatively high antioxidative properties (Table 6). Moreover, a lower environmental temperature resulted in 20% higher DPPH and ~10% higher ABTS free-radical scavenging activities. Non-optimal nitrogen nutrition conditions only slightly lowered the ABTS free-radical scavenging activity and FRAP, while all measured antioxidative parameters rose with increasing lighting PPFD. The DPPH free-radical scavenging activity and FRAP of tomato leaves increased by ~40% and the ABTS free-radical scavenging activity—64% when the lighting PPFD rose from 150 to 450 µmol m^{-2} s^{-1}. However, though PDEN preparations can be characterized by measurable antioxidative activity (0.03–0.3% compared with leaf DPPH and ABTS free-radical scavenging activities), different cultivation environment parameters did not significantly affect their properties.

Table 6. Antioxidative properties of tomato-leaf material and leaf-derived nanoparticle preparations ($\bar{x} \pm SD$, $n = 3$). Different letters indicate statistically significant differences between means within the same column according to Tukey's HSD test at the confidence level of $p = 0.05$. n.d.—not determined.

Parameter		DPPH Scavenging Activity, mmol g^{-1} DW		ABTS Scavenging Activity, µmol g^{-1} DW		FRAP, µmol Fe(II) g^{-1} DW	
		Plant Material	Nanoparticle Preparation	Plant Material	Nanoparticle Preparation	Plant Material	Nanoparticle Preparation
Temperature	18 °C	318 ± 5 B	0.93 ± 0.03 A	1785 ± 19 C	4.38 ± 1.48 A	138 ± 2 B	n.d.
	22 °C	263 ± 12 A	0.85 ± 0.18 A	1642 ± 36 B	3.10 ± 1.48 A	128 ± 1 A	n.d.
	26 °C	259 ± 6 A	0.83 ± 0.04 A	1530 ± 5 A	2.27 ± 0.30 A	121 ± 4 A	n.d.
Nitrogen nutrition	0 mg L^{-1}	269 ± 6 A	1.03 ± 0.01 A	1467 ± 24 A	2.75 ± 0.48 A	115 ± 2 A	n.d.
	250 mg L^{-1}	263 ± 12 A	0.85 ± 0.18 A	1642 ± 36 B	3.10 ± 0.37 A	128 ± 1 B	n.d.
	500 mg L^{-1}	264 ± 7 A	0.97 ± 0.04 A	1456 ± 44 A	2.39 ± 0.51 A	113 ± 3 A	n.d.
PPFD	150 µmol m^{-2} s^{-1}	229 ± 5 A	0.67 ± 0.37 A	1320 ± 58 A	4.38 ± 1.48 A	120 ± 1 A	n.d.
	250 µmol m^{-2} s^{-1}	263 ± 12 B	0.85 ± 0.18 A	1642 ± 36 B	3.10 ± 0.37 A	128 ± 1 B	n.d.
	450 µmol m^{-2} s^{-1}	323 ± 11 C	0.83 ± 0.04 A	2174 ± 23 C	2.27 ± 0.30 A	164 ± 4 C	n.d.

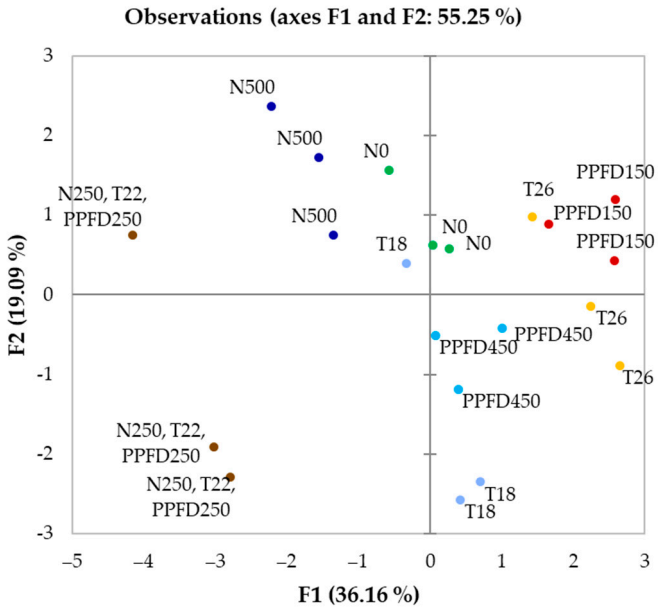
Although there was no direct correlation with the antioxidative properties of PDEN preparations, the 10^9 particles mL^{-1} particle concentration (Figure 3a) had an inhibiting effect on HaCaT keratinocyte metabolic activity compared with the control, and the highest impact (lowest metabolic activity; 15% lower compared with control) was determined for PDEN preparations obtained from tomato leaves cultivated under optimal conditions (T22 °C, N 250 mg L^{-1}, 250 µmol m^{-2} s^{-1}) and a higher (N 500 mg L^{-1}) nitrogen nutrition level (Figure 3b).



(**a**) (**b**)

Figure 3. The impact of nanoparticle preparations on keratinocyte metabolic activity. (**a**) Selection of the PDEN concentration using the 'Betalux' leaf preparation derived from tomato plants cultivated under 450 µmol m^{-2} s^{-1} lighting PPFD; (**b**) The impact of different cultivation temperature, nitrogen nutrition, and lighting photosynthetic photon flux density (PPFD) conditions on the metabolic activity ($\bar{x} \pm SD$, $n = 3$) of the 'Betalux' tomato nanoparticle preparation (10^9 particle mL^{-1}).

The principal component analysis and factor loadings (Figure 4) confirm previous data showing that optimal cultivation conditions (T22 °C, N 250 mg L^{-1}, 250 µmol m^{-2} s^{-1} and) are marked by higher protein and RNA content and higher metabolic activity according to the F1 component, while lower and higher temperatures and the highest investigated PPFD are distinguished according to the F2 component; namely, the antioxidative properties.



	F1	F2
Particle concentration	−0.788	−0.315
Protein content	**−0.693**	−0.237
RNR content	**−0.798**	0.076
Mean	**0.782**	−0.007
Mode	0.259	−0.556
Span	**0.843**	0.178
DPPH	−0.243	**0.663**
ABTS	−0.101	**−0.601**
Fe	−0.044	**−0.645**
Metabolic activity	**0.644**	−0.436

Figure 4. Principal component analysis and factor loadings, indicating distinct properties of nanoparticle preparations derived from the leaves of the tomato variety 'Betalux', cultivated under different temperature, nitrogen nutrition, and lighting photosynthetic photon flux density (PPFD) conditions ($n = 3$).

4. Discussion

Tomato breeding trends have long been steered toward higher productivity, environmental and disease resistance, and fruit uniformity, while in recent years, the aroma properties of tomato fruits have also been included in the breeding targets [29,30]. Moreover, the need for specific modifications to the plant architecture to obtain petite sizes suitable for controlled environment cultivation conditions is rising [31]. However, currently, there is no published information on how genotypes or cultivation conditions affect the properties of PDEN preparations isolated from tomatoes or other horticultural plants. In this study, it was expected that different plant genotypes from determinate or indeterminate growth strategies and with higher or lower resistance would also differ in terms of their material properties for PDEN isolation. According to the results, the tomato genotype had a pronounced impact on biomass accumulation at the seedling (5–6 fully developed leaves) stage, with the highest biomass accumulating in the determinate 'Roma' and indeterminate 'Admiro' tomato varieties. However, a high leaf biomass productivity rate does not ensure a high PDEN yield. The 'Admiro' tomato accumulated the highest biomass, but the PDEN preparations obtained from the leaves of this tomato genotype contained ~45% lower protein content. Also, lower-concentration PDENs were obtained from 'Admiro', 'Roma', and 'Marmande' leaves. The results confirm presumptions that the PDEN yield and properties will depend on the cultivated tomato genotype. 'Brooklyn' and 'Betalux' tomato cultivars were selected as the most potent materials for PDEN isolation due to their high isolate protein content and 1.9 and 3.6 times higher nanoparticle yield. The mean particle size did not differ remarkably between cultivars (varying between 169 and 220 nm), but the percentile values of size variation (span) indicate that a narrower, more uniform particle size distribution [32] is also characteristic for the 'Betalux' and 'Brooklyn' tomatoes. Due to its consistent PDEN profile, dwarf morphology, relatively high biomass accumulation rate, and height ratio, indicating its suitability for cultivation in height-limiting CEA cultivation, the 'Betalux' tomato was selected for further experiments on the cultivation parameter impact on PDEN quality. It did not supersede other tomato genotypes in its leaf antioxidative properties, but it was determined that PDEN preparations' DPPH and ABTS free-radical scavenging activities do not directly correlate with the properties of their source plant material.

To ensure high nanoparticle yield and bioactive properties from plant sources, isolation methods have been developed purposefully, and the necessity for standardized procedures has been highlighted when seeking result uniformity and reproducibility [4,5,33]. It was also noticed in the studies with Arabidopsis plants that revising the plant growth environment to stimulate PDEN release from plant cells is also a crucial approach for enhancing their yield [34,35]. However, there is no extended research on PDEN yield and properties in horticultural and medicinal plants in response to their cultivation conditions. In this study, we contributed to filling this gap by cultivating young 'Betalux' tomato plants under different temperature, nitrogen nutrition, and lighting intensity parameters. It is well known that tomato growth, productivity, and physiological indices depend remarkably on the cultivation environment conditions [30,36]. Our results showed that tomato biomass growth was more affected by environmental temperature and lighting PPFD than nitrogen nutrition at the investigated levels. The optimal conditions of 22 °C temperature, 250 mg L^{-1} nitrogen nutrition, and 250 µmol m^{-2} s^{-1} lighting PPFD were used as a reference. Deviations in environmental temperature resulted in higher plant dry-weight accumulation, as the non-optimal temperature often resulted in thicker leaves [37]. However, deviations from the optimal environmental temperature, both lower and higher, resulted in ~2 times lower PDEN protein content and particle concentration. Although insufficient (N0; no supplemental nitrogen nutrition) and excess (N500 mg L^{-1}) nitrogen did not limit tomato growth, it resulted in 2 and 1.3 times lower nanoparticle yield from tomato leaves. Light intensity is also known as a growth-limiting factor, which was also valid for dwarf tomatoes under controlled environment conditions [38,39]. In agreement with Zheng et al. [39], a light intensity of ~250 µmol m^{-2} s^{-1} resulted in the highest biomass of tomato seedlings cultivated in CEA. Low light, 150 µmol m^{-2} s^{-1}, resulted in 66% lower fresh weight and 52% dry weight compared with optimal conditions, demonstrating remarkable productivity losses per unit of cultivation area. High light, 450 µmol m^{-2} s^{-1}, while inhibiting tomato height, did not affect biomass accumulation. However, both high and low temperatures remarkably affected the PDEN protein content, particle concentration, and RNR content, and reduced PDEN sample uniformity by affecting the size distribution of the nanoparticles. Investigated non-optimal cultivation parameters, especially lighting intensity, are in agreement with findings by other authors [39,40] in showing enhanced tomato-leaf antioxidative properties; however, this higher DPPH and ABTS free-radical scavenging activity was not transferred to the PDEN preparations. Despite this, an inhibitory effect of PDEN preparations on HaCaT keratinocyte metabolic activity, compared with the control, was observed, and the highest impact (lowest metabolic activity; 15% lower compared to control) was determined for PDEN preparations obtained from tomato leaves cultivated under optimal conditions. This confirms the necessity for further genotype-specific investigations on the source material and standardization of the cultivation procedures for the uniform yield, quality, and reproducible biological activity of PDEN preparations.

5. Conclusions

The yield, size distribution, and biological activity of tomato-leaf-derived exosome-like preparations differ remarkably depending on the source material, plant genotype, and cultivation conditions. The leaves of tomatoes of different genotypes differ by up to 3.6 times in PDEN protein content and nanoparticle concentration. For the dwarf tomato 'Betalux', optimal temperature, nitrogen nutrition, and lighting intensity conditions resulted in the highest nanoparticle yield and the most uniform particle distribution. Deviation from optimal cultivation conditions in CEA resulted in reduced tomato biomass and reduced PDEN protein and nanoparticle yield. Therefore, CEA cultivation technologies for the PDEN isolation from tomato material should be developed depending on the needs of the plants. Further, the utilization of residue tomato plants cultivated in other horticultural systems and materials for PDEN isolation should be revisited to determine the possible impacts of different genotypes and variable cultivation conditions on the quality and uniformity of PDEN preparations.

Author Contributions: Conceptualization, A.V.; methodology, G.S. and A.J.; formal analysis, K.L.; investigation, E.M. and Z.B.; writing—original draft preparation, A.V.; writing—review and editing, A.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Lithuanian Research Council, grant number S-MIP-21-25.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: The authors are grateful to researchers Rasa Bernotienė and Deimantė Narauskaitė from the Lithuanian University of Health Sciences for their support in elaborating the method.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of the data; in the writing of the manuscript; or in the decision to publish the results.

Appendix A

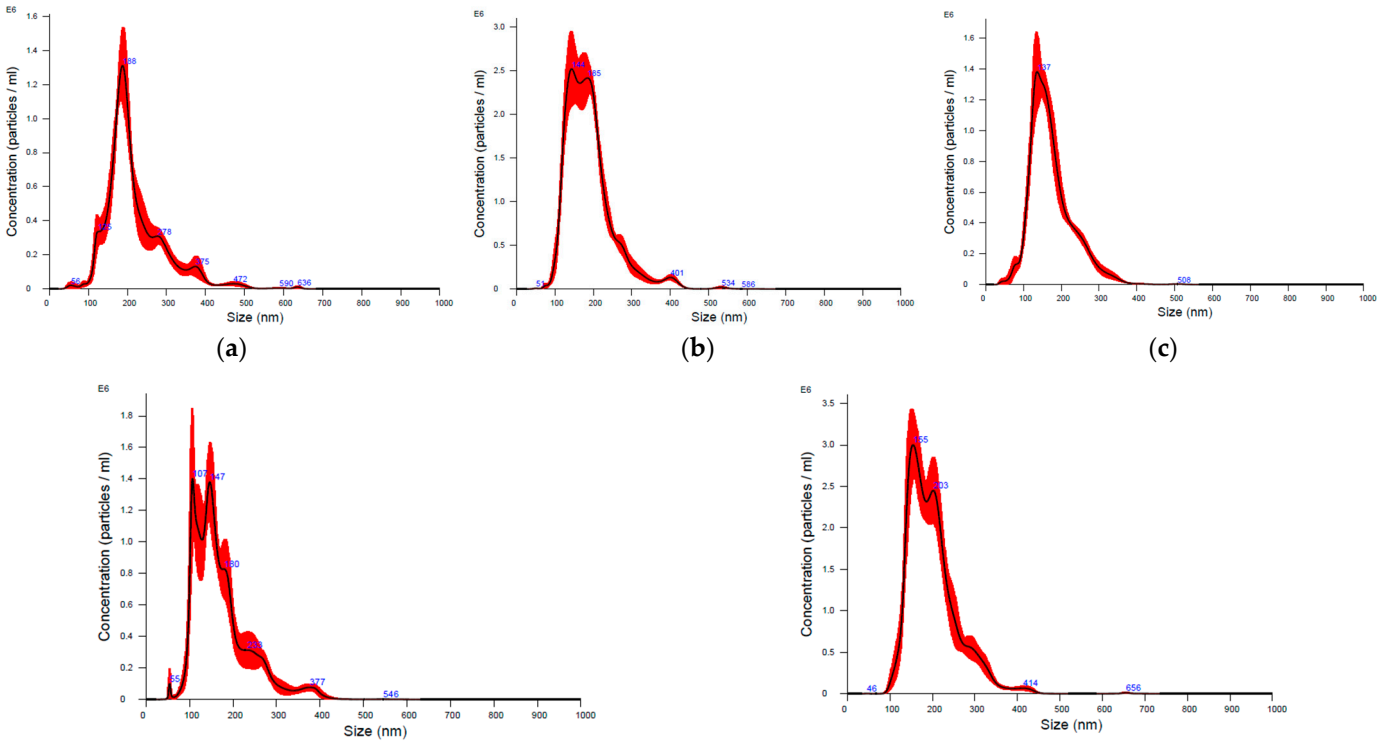


Figure A1. Particle size distribution, according to nanoparticle tracking analysis (NTA), in the nanoparticle preparations (10,000× dilution) from the leaves of different tomato genotypes that had been cultivated in CEA. (**a**) Admiro; (**b**) Brooklyn; (**c**) Roma; (**d**) Marmande; (**e**) Betalux. Black lines in the graph represent the average value and the width of the red band represents the standard error of the mean of the particle size distribution (n = 5).

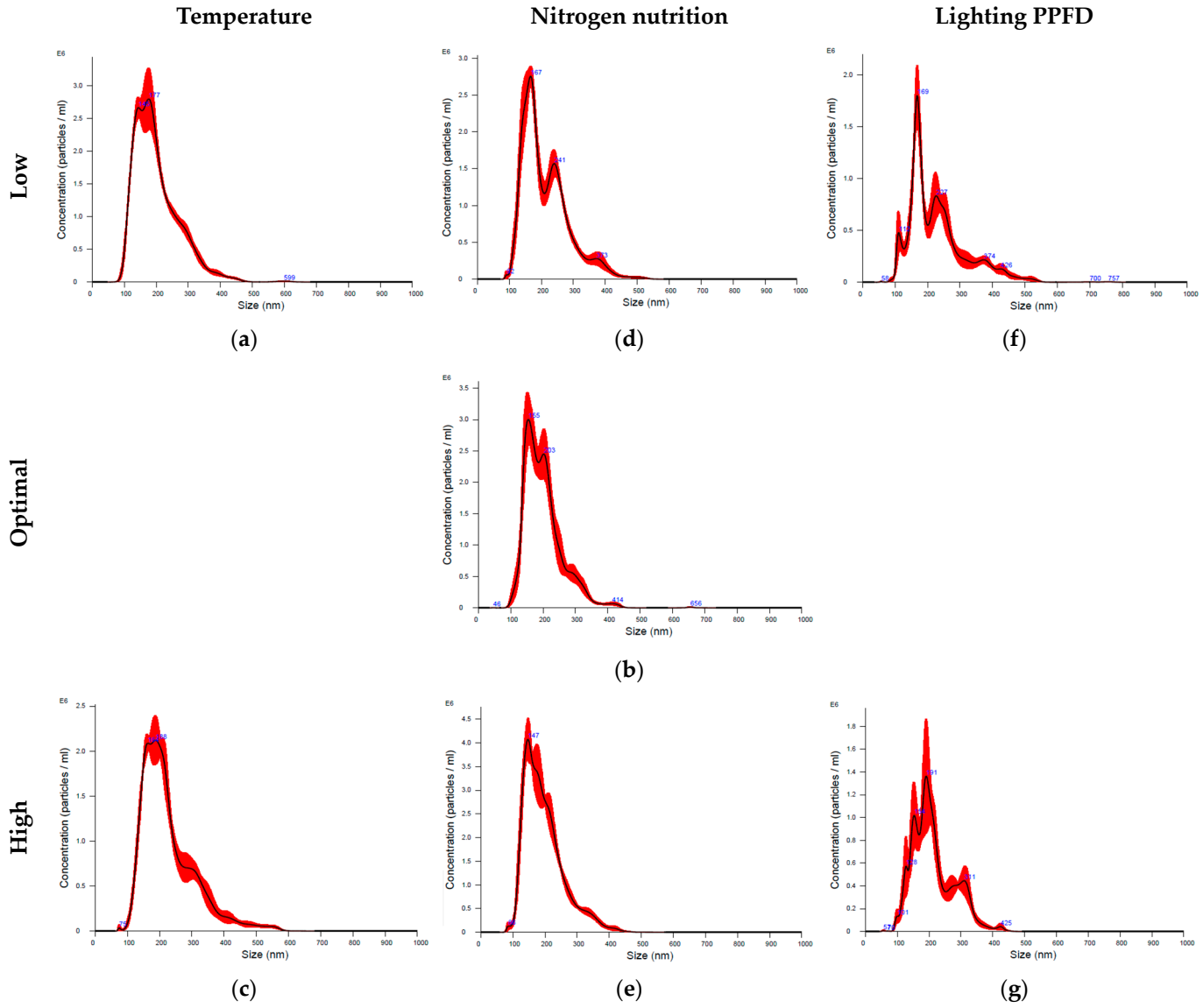


Figure A2. Particle size distribution, according to nanoparticle tracking analysis (NTA), in leaf-derived nanoparticle preparations (10,000× dilution) from tomatoes cultivated under different temperature, nitrogen nutrition, and lighting photosynthetic photon flux density (PPFD) conditions: (**a**) T 18 °C; (**b**) T 22 °C, N 250 mg L^{-1}, PPFD 250 µmol m^{-2} s^{-1} (reference); (**c**) T 22 °C;(**d**) N 0 mg L^{-1}; (**e**) N 500 mg L^{-1}; (**f**) PPFD 150 µmol m^{-2} s^{-1}; (**g**) PPFD 450 µmol m^{-2} s^{-1}. Black lines in the graph represent the average value and the width of the red band represents the standard error of the mean of the particle size distribution (n = 5).

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