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ORIGINAL PAPER

The effects of ZnO NPs applied to donor plant on *in vitro* gynogenesis efficiency in long-day onion (*Allium cepa* L.)*

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Abstract

The incorporation of nanomaterials into agricultural biotechnologies has introduced a new approach to modern agriculture. Among the most widely used nanoparticles in agricultural nanotechnology are zinc oxide (ZnO) nanoparticles (NPs). This study aims to evaluate the effects of ZnO NPs on donor plant growth and *in vitro* gynogenesis efficiency. The plant materials used in this study were long-day onion cultivars, namely cv. Delfos, cv. Nun 7194, and the Suluova genotype. Overall, ZnO NPs treatments significantly influenced morphological characteristics in all genotypes. The 60 mg L^{-1} ZnO NPs treatment generally resulted in the highest values for plant growth parameters, while 80 mg L^{-1} was more effective for leaf length and for some flowering traits in cv. Delfos and cv. Nun 7194. In contrast, maximum leaf width in cv. Nun 7194 was obtained at 100 mg L^{-1}. These findings indicate that the optimal ZnO NPs concentration varied among genotypes and traits, with 60 mg L^{-1} ZnO NPs treatment emerging as more effective. Furthermore, this concentration also resulted in more efficiency of *in vitro* gynogenesis, with eight putative gynogenic plantlets exhibiting both root and shoot development, three from cv. Delfos, three from cv. Nun 7194, and two from the Suluova genotype.

Keywords: *Allium cepa* L., gynogenesis, nanoparticles (NP), onion, ZnO NPs

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INTRODUCTION

Nanotechnology is an emerging field of research with diverse applications across science and technology, particularly involving nanoparticles (NPs) (Baranowska-Wójcik et al. 2020). This technology enables the design of conventional chemicals and materials at the nanoscale, imparting novel and unique properties (Halıcı et al. 2021). In recent years, with advancements in technology, nanoparticles have gained significant importance in various sectors, including industry, biomedicine, healthcare, and agriculture (Singh et al. 2018). The integration of nanotechnology and nanomaterials into agricultural biotechnologies has introduced a new dimension to modern agriculture. Nanomaterial-based technologies are continuously expanding to address the growing global challenges of limited food and water resources (Sabir et al. 2014). Zinc oxide nanoparticles (ZnO NPs) are among the most widely utilized nanomaterials in agricultural nanotechnology (Hussain et al. 2019). To meet the nutritional demands of the growing global population, it has become essential to adopt innovative technologies in horticultural crop production to enhance agricultural efficiency and ensure food security (Nechitailo et al. 2018, Kızılırmak 2022). Nanotechnology has various applications in horticulture, including increasing crop yield, extending post-harvest shelf life, improving storage conditions, and enhancing packaging and transportation of processed products (Ditta et al. 2015). In addition to these applications, nanoparticles exhibit antifungal and antiviral properties, making them valuable as fertilizers, pesticides, and plant growth regulators in agriculture (Ghidan et al. 2017). The effectiveness of nanofertilizers is enhanced due to their reduced dimensions at the nanometer scale, which facilitates their uptake by plants (Singh et al. 2018). The application of nanofertilizers, particularly via foliar spraying, is more efficient as it enables the gradual and controlled release of essential nutrients required by plants (Kah et al. 2018). Additionally, this method reduces fertilizer consumption compared to both soil applications and conventional fertilization techniques (Rossi et al. 2019). Nanoparticles applied to the leaf surface penetrate various tissues through stomata or trichomes and are subsequently translocated within the plant (Uzu et al. 2010). Several studies have reported that foliar-applied nanoparticles enhance fruit yield and quality in various fruit and vegetable species (Mottaleb et al. 2021, Kızılırmak 2022).The most widely produced nanoparticles worldwide include zinc (Zn), gold (Au), and silver (Ag) – Khot et al. (2012). Zinc, classified as a metallic element, is the only metal that is essential for the function of six enzyme groups (Vallee and Falchuk 1993). As a vital micronutrient, metallic zinc plays a crucial role in plant growth and development by participating in various enzymatic and physiological processes (Misra et al. 2005). Zinc is involved in carbohydrate metabolism, protein and nucleic acid synthesis (Hänsch et al. 2009), cell division (Li et al. 2017), maintenance of cell membrane integrity, and chloro-

plast activity (Brown et al. 1993). Additionally, it contributes to auxin and gibberellin biosynthesis and plays an active role in mitigating abiotic and biotic stress factors (Eisvand et al. 2018). Zinc oxide nanoparticles (ZnO NPs) are inorganic compounds that appear as a white powder and can be synthesized using both chemical methods, such as precipitation, vapor transport, and hydrothermal treatment and biological methods involving various plant extracts (Sabir et al. 2014, Ali et al. 2018).

Nowadays, biotechnological methods offer significant advantages in accelerating breeding programs, enabling the rapid development of pure lines for variety candidates and, particularly, parental lines used in hybrid breeding. In onion breeding studies, the advancement of a single generation typically requires 2-3 years. Consequently, when conventional breeding methods are employed, achieving homozygous pure lines is a time-consuming process (Yarali, Yanmaz 2013, Khar et al. 2018, Singh et al. 2018). The doubled haploidization technique significantly reduces the time required for line purification, a process that traditionally takes several years, to just a few months, thereby providing substantial time savings in hybrid cultivar breeding programs (Murovec, Bohanec 2012, Yarali, Yanmaz 2013). In studies conducted on *Allium* species, flower bud culture has emerged as a prominent gynogenetic method (Khar et al. 2018, Mathapati et al. 2019). The genotype and growing conditions of the donor plant are key factors influencing the efficiency of gynogenic haploid embryo production in onion (Michalik et al. 2000, Murovec, Bohanec 2012, Fayos et al. 2015).

This study aims to investigate, for the first time, the effect of ZnO NPs treatments, as nano fertilizers, on plant growth (plant height, number of leaves, leaf length, leaf width, flower stalk length and number, umbel diameter and length and number of flower buds) and *in vitro* gynogenesis efficiency. By introducing a novel approach to onion breeding, an economically significant vegetable species, this research seeks to contribute to breeding programs and take a crucial step toward developing new onion cultivars through the improved breeding material.

MATERIALS AND METHODS

Growing donor plants

The plant materials used in this study were long-day onion cultivars, namely cv. 'Delfos', cv. 'Nun 7194', and the Suluova genotype. To prepare the soil in the plot designated for donor plant growing, deep ploughing was carried out in the autumn. Before to bulb planting, fertilization was applied based on soil analysis results (Table 1) to promote plant growth. The plots were made ready for planting by applying 8-12 kg of nitrogen, 8-10 kg of phosphorus, and 12-15 kg of potassium fertilizer per decare (Vural et al. 2000).

Table 1

Soil analysis results of plots

N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	pH	Organic matter (%)	Lime (%)
0.24	3.51	349.11	2961.75	481.09	1.05	0.94	18.41	7.19	2.59	9.21

Since the onion genotypes/cultivars used in the study possess a long-day characteristic, their vernalization requirements must be met to ensure flowering (Yalamalle 2016, Bağcı et al. 2021). Therefore, the onion bulbs were planted in the prepared plots on December 5, 2023, with a spacing of 50×50 cm between and within rows (Yarali 2014). In the study, 210 bulbs were used for each cultivar/genotype (3 replications × 10 plants × 7 ZnO NPs treatments).

ZnO NPs treatments to donor plants

ZnO NPs treatments were applied to onion plants as foliar sprays at doses of 0, 20, 40, 60, 80, 100, and 120 mg L^{-1}, following the methodology of Fouda et al. (2020). The applications were performed at 55, 70, and 85 days after planting using a 1.2 L hand-held Hudson RL Flomaster sprayer (García-López et al. 2019). Chemically synthesized ZnO NPs (dispersion, <100 nm particle size by TEM; ≤40 nm average particle size) from Sigma--Aldrich were used in the study.

In vitro gynogenesis studies

Unopened flower buds intended for *in vitro* culture were collected from donor plants that had been treated with ZnO NPs, 3-5 days prior to anthesis (Yarali Karakan 2020, Lestari et al. 2021). For surface sterilization, the flower buds were immersed in 70% ethanol for 3 minutes, followed by treatment with a 15% clorox solution (containing 0.9% sodium hypochlorite and 0.1% Tween-20) for 30 min and subsequently rinsed three times with distilled water (Yarali Karakan 2019-2020). The BDS medium (Dunstan, Short 1977), was used as the nutrient medium (Yarali, Yanmaz 2013). The BDS medium was supplemented with 2 mg L^{-1} 2,4-D, 2 mg L^{-1} 6-BAP, and 0.7% agar, and its pH was adjusted to 5.8 (Khar et al. 2018, Adhiyamaan et al. 2019, Şahinalp, Yarali Karakan 2019, Yarali Karakan 2020). Surface-sterilized flower buds were cultured at a density of 15 buds per petri dish, each containing 15 ml of BDS culture medium, which had been sterilized in an autoclave at 121°C for 20 minutes. To prevent possible contamination, the petri dishes were sealed with parafilm and incubated in a growth chamber at 25°C under a 16/8 h light/dark photoperiod (Adhiyamaan et al. 2019, Yarali Karakan 2019-2020, Lestari et al. 2020). In this study, 35 replicates (petri dishes) were used for each genotype and treatment, with 15 buds per petri dish, resulting in a total of 11.025 flower buds cultured. After 6-7 weeks, the buds that had developed in the induction medium were transferred to the subculture (B1) medium (Yarali, Yanmaz 2017).

Statistical analysis

The experiment was conducted using a completely randomized design to evaluate the effects of "genotype/cultivar x ZnO NPs treatments" on plant growth and *in vitro* gynogenesis efficiency. Statistical analyses were performed using the JMP Pro 14 software package (SAS Institute, NC, USA), with a significance level set at 5%. Analysis of variance (ANOVA) was conducted, and Tukey's test was applied to identify statistically significant differences among groups. Additionally, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed to reveal the relationships between the parameters examined and ZnO NPs treatments. The distribution of parameters and genotypes in the PCA was determined using the first two principal components (PC1 and PC2).

RESULTS AND DISCUSSION

The effect of ZnO NPs treatments on the donor plant growth

To evaluate the effects of ZnO NPs treatments on plant development, plant height, leaf length, leaf width, number of leaves, number of flower stalks, and flower stalk length were measured (Laware, Raskar 2014, Fouda et al. 2020). Additionally, to assess the impact of ZnO NPs treatments on the development of flower buds for *in vitro* culture, umbel length, umbel diameter, and the number of flower buds were recorded. The results revealed that highest plant height and leaf number values were obtained from 60 mg L^{-1} ZnO NPs treatment in all genotypes, and decreases in both of them at 80 mg L^{-1}, 100 mg L^{-1} and 120 mg L^{-1} treatments. The greatest leaf length was observed at 80 mg L^{-1} ZnO NP treatment in both cv. 'Delfos' and the Suluova genotype, whereas cv. 'Nun 7194' exhibited comparable values at 20 mg L^{-1}, 40 mg L^{-1}, and 80 mg L^{-1} ZnO NPs treatments. The highest leaf width values varied among genotypes, being recorded at 60 mg L^{-1} ZnO NP treatment in the Suluova genotype, at 80 mg L^{-1} in cv. 'Delfos', and at 100 mg L^{-1} in cv. 'Nun 7194' (Table 2).

The effects of ZnO NPs treatments on the number of flower stalks, flower stalk length, umbel length, umbel diameter and number of flower bud obtained from donor plants are presented in Table 3. The maximum number of flower stalks was observed at 40 and 60 mg L^{-1} ZnO NPs treatments in the Suluova genotype and at 80 mg L^{-1} treatment in cv. 'Delfos', whereas no significant differences were detected in cv. 'Nun 7194'. Among all genotypes, the greatest flower stalk length and umbel diameter were recorded at 60 mg L^{-1} ZnO NPs. The longest umbel length was achieved at 60 mg L^{-1} for the Suluova genotype and cv. 'Nun 7194', while cv. 'Delfos' attained its maximum at 80 mg L^{-1}. Additionally, the highest number of flower buds was observed at 60 mg L^{-1} in the Suluova genotype and cv. 'Delfos', whereas cv. 'Nun 7194' reached its peak at 80 mg L^{-1}. As the concentration of ZnO NPs increased,

Table 2

The effect of different ZnO NPs treatments on the development of donor plants

Cultivar/ Genotype	ZnO NPs (mg L^{-1})	Plant height (cm)	Number of leaves (per plant)	Leaf length (cm)	Leaf width (mm)
Delfos	Control	58.75±8.77^{a-f}	19.50±2.77^{a-e}	27.87±4.58^{b-e}	10.63±0.70^{f}
	20	53.00±6.23^{e-g}	20.87±5.33^{a-c}	23.25±7.66^{c-f}	11.23±2.81^{f}
	40	54.50±7.50^{c-g}	19.00±5.78^{a-e}	28.12±5.08^{b-e}	12.11±2.95^{ef}
	60	66.50±3.66^{ab}	22.75±4.71^{a}	29.37±3.58^{b-e}	12.82±2.51^{d-f}
	80	59.25±8.49^{a-e}	22.50±3.70^{ab}	45.50±3.11^{a}	12.92±2.82^{c-f}
	100	54.75±8.68^{c-g}	12.25±2.43^{fg}	26.37±3.06^{b-f}	12.45±1.78^{d-f}
	120	53.87±10.13^{c-g}	13.75±2.05^{eg}	27.25±4.39^{b-f}	12.31±3.36^{d-f}
Nun 7194 F1	Control	65.25±5.82^{abc}	15.62±3.46^{c-g}	28.50±5.68^{b-e}	14.08±4.03^{a-f}
	20	63.62±7.79^{a-e}	20.00±2.61^{a-d}	33.12±8.57^{b}	12.05±1.87^{ef}
	40	67.37±5.34^{a}	17.37±2.13^{a-g}	34.37±3.20^{b}	13.50±3.01^{b-f}
	60	67.62±5.39^{a}	21.62±3.02^{ab}	31.87±8.40^{bc}	15.47±2.84^{a-e}
	80	64.75±4.80^{a-d}	18.25±2.37^{a-e}	33.37±9.07^{b}	17.02±1.37^{a-c}
	100	63.00±4.65^{a-e}	11.62±2.77^{g}	31.25±4.65^{bc}	17.33±1.44^{ab}
	120	64.25±6.98^{a-e}	14.87±2.79^{d-g}	31.12±8.65^{b-d}	15.43±2.58^{a-e}
Suluova	Control	46.00±5.34^{g}	14.50±2.32^{d-g}	18.12±3.90^{f}	10.40±0.73^{f}
	20	53.37±6.86^{d-g}	17.87±2.35^{a-f}	21.37±2.50^{ef}	17.41±1.27^{ab}
	40	57.50±3.89^{a-g}	16.75±1.83^{b-g}	25.37±1.84^{b-f}	17.15±0.75^{ab}
	60	61.12±4.01^{a-e}	20.00±4.40^{a-d}	22.75±3.05^{c-f}	17.85±2.14^{a}
	80	55.00±3.66^{b-g}	17.37±1.59^{a-g}	28.62±3.37^{b-e}	16.35±1.02^{a-d}
	100	58.50±3.42^{a-f}	17.50±2.20^{a-g}	26.62±2.66^{b-f}	10.03±0.71^{f}
	120	47.25±5.54^{fg}	15.62±3.06^{c-g}	21.62±3.58^{d-f}	12.78±2.53^{d-f}

Results are expressed as the mean value ± standard deviation for plant height, number of leaves, leaf length and leaf width. Column values (different small letters) are significantly different (ANOVA, and Tukey's HSD test, $p<0.05$).

leaf yellowing and tip necrosis were observed at the cv. 'Delfos' and cv. 'Nun 7194', as well as in the Suluova genotype, particularly at the 100 and 120 mg L^{-1} treatments. Specifically, it was recorded in 6 and 7 plants for both cv. 'Delfos' and cv. 'Nun 7194', and in 3 and 2 plants for Suluova, respectively.

The biplot analysis indicated that the first principal component (PC1) accounted for 48.7% of the total variation, while the second principal component (PC2) explained 16.9%. Together, these two components captured 65.6% of the total variation (Figure 1). PCA results showed a strong positive correlation among leaf length, umbel length, flower stalk length, plant height, umbel diameter, and number of flower buds, while the number of flower stalks was positively associated with the number of leaves. In contrast, leaf width was independent from the other traits. The 60 mg L^{-1}

Table 3

The effect of different ZnO NP s treatments to the donor plants on flowering

Cultivar/ Genotype	ZnO NPs (mg L^{-1})	Number of flower stalks (per plant)	Flower stalk length (cm)	Umbel length (mm)	Umbel diameter (mm)	Number of flower buds (per umbel)
Delfos	Control	4.00±1.85^{a-c}	89.37±5.28^{d-g}	58.47±11.48^{c-e}	72.20±7.53^{c-f}	1406.12±226.54^{c-e}
	20	3.25±1.38^{a-c}	85.62±8.21^{e-h}	55.52±11.47^{d-f}	66.95±6.31^{e-h}	1656.00±206.69^{a-d}
	40	3.00±1.51^{a-c}	71.12±5.59^{h_1}	58.83±5.75^{c-e}	72.25±8.87^{c-f}	1596.50±131.14^{a-d}
	60	4.50±3.02^{ab}	103.75±7.49^{b-d}	60.46±5.95^{c-e}	77.21±8.93^{b-e}	1676.25±125.08^{a-c}
	80	5.12±2.35^{a}	102.87±13.22^{b-e}	76.78±14.36^{ab}	68.30±3.63^{d-g}	1395.62±258.90^{de}
	100	3.75±1.48^{a-c}	81.12±11.89^{g-i}	59.60±3.22^{c-e}	60.23±4.11^{g-i}	1175.87±108.22^{ef}
	120	2.62±1.06^{a-c}	98.00±5.50^{b-g}	56.77±5.74^{d-f}	70.25±5.33^{c-g}	1579.50±131.16^{a-d}
Nun 7194 F1	Control	2.87±0.99^{a-c}	105.62±7.76^{b-d}	64.00±7.53^{c-e}	85.62±4.09^{b}	1522.00±205.30^{a-d}
	20	2.62±1.76^{a-c}	106.62±8.05^{b-d}	54.10±6.90^{ef}	74.65±9.63^{b-f}	1562.62±60.58^{a-d}
	40	3.75±1.75^{a-c}	105.62±7.19^{b-d}	62.41±2.78^{c-e}	80.11±2.77^{b-d}	1080.87±112.83^{fg}
	60	3.25±0.88^{a-c}	126.62±17.31^{a}	77.08±5.14^{a}	102.87±11.31^{a}	1640.25±113.61^{a-d}
	80	2.75±1.28^{a-c}	107.75±4.59^{bc}	64.27±2.95^{b-e}	81.32±3.97^{bc}	1730.12±121.22^{a}
	100	1.62±0.51^{c}	93.87±7.05^{c-g}	55.96 ±2.68^{d-f}	75.57±4.48^{b-f}	1694.50±177.83^{ab}
	120	2.37±1.30^{bc}	112.12±8.83^{ab}	70.46±8.62^{a-c}	84.48±7.56^{b}	1699.25±99.15^{ab}
Suluova	Control	2.62±0.74^{a-c}	85.50±23.26^{f-h}	52.26±7.75^{d-f}	55.34±6.62^{hi}	992.37±97.96^{f-h}
	20	3.62±0.91^{a-c}	92.75±3.88^{c-g}	59.36±7.13^{c-e}	63.70±6.17^{f-i}	867.00±153.94^{gh}
	40	4.50±1.41^{ab}	95.37±5.44^{b-g}	59.37±4.21^{c-e}	79.65±3.38^{b-d}	1454.25±102.21^{b-d}
	60	4.75±1.28^{ab}	102.00±5.80^{b-f}	67.50±3.28^{a-d}	84.56±5.20^{b}	1636.25±76.02 $^{a-d}$
	80	2.62±1.06^{a-c}	65.50±5.68^{i}	45.38±4.80^{f}	54.16±4.88^{i}	839.00±149.80^{gh}
	100	2.50±1.19^{a-c}	70.62±4.86^{hi}	45.15±5.84^{f}	66.90±7.85^{e-h}	811.50±198.83 ^{gh}
	120	3.50±1.77^{a-c}	85.00±8.03^{f-h}	52.57±3.90^{ef}	59.50±6.14^{g-i}	776.75±120.02^{h}

Results are expressed as the mean value ± standard deviation for number of flower stalks, flower stalks length, umbel length, umbel diameter and number of flower buds. Column values (different small letters) are significantly different (ANOVA, and Tukey's HSD test, $p<0.05$).

ZnO NP treatment was closely related to most favorable traits, whereas higher concentrations and the control groups showed limited or negative effects. The results of two-way hierarchical clustering analysis (HCA) also revealed that ZnO NPs treatments were divided into different groups in terms of the examined parameters (Figure 2), and 60 mg L^{-1} ZnO NPs treatment, which was found to be more efficient on plant development, forms a separate group in all genotypes. Nanoparticles are used in different areas such as improving seed germination, increasing plant growth and yield, providing genetic modification of plants, increasing the production of bioactive compounds and protecting plants against disease agents (Wang et al. 2016). Insufficient zinc in plants causes abnormal growth, late ripening and product loss (Broadley et al. 2007), while high concentrations damage cell functions (Andreji´c et al. 2018). The use of ZnO NPs to eliminate these limitations is one of the current topics being researched today (Du et al. 2019). The effect of nanoparticles, which cause both positive and negative morphological and physiological changes, on plants

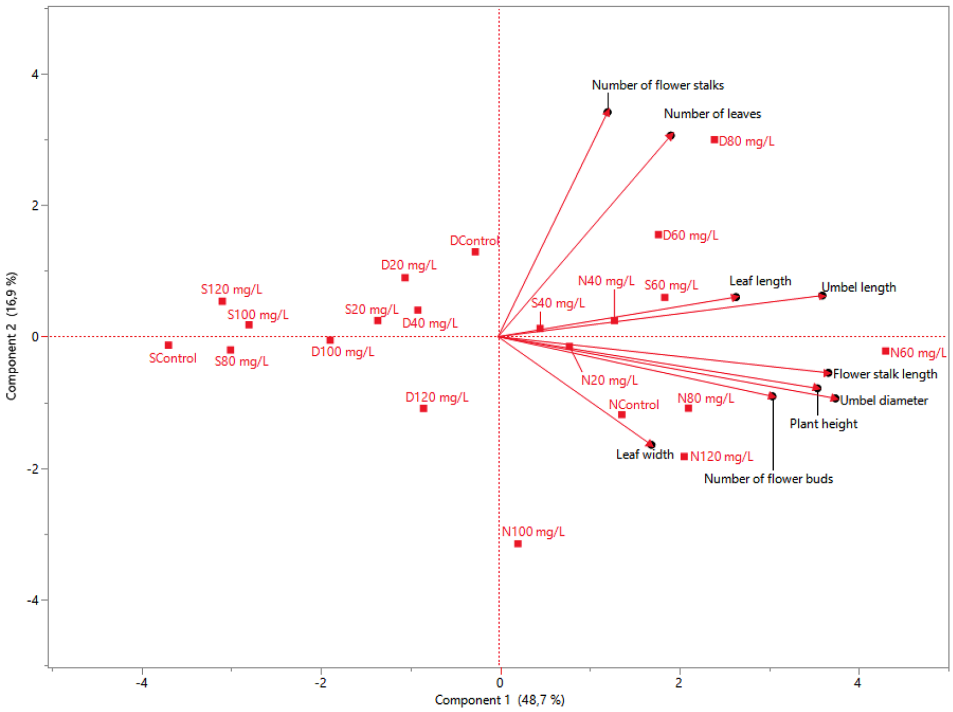

Fig. 1. Biplot graph relationships between ZnO NPs treatments and plant growth parameters obtained through PCA: D – Delfos, N – Nun 7194 F1, S – Suluova

varies depending on the type of nanoparticle, size, concentration, application method and duration, plant genotype and developmental stage (Baskar et al. 2018). Studies have reported that ZnO NPs are more effective in plant growth and other physiological activities due to greater leaf penetration than zinc sulfate (Lopez-Vargas et al. 2018). The results obtained from the research revealed that low concentrations of ZnO NPs used as foliar fertilizer had a positive effect on morphological characteristics in onions. Similarly, Pérez-Velasco et al. (2020), in their study investigating the effects of ZnO NPs treatments on plant growth in tomatoes (*Solanum lycopersicum* L.), reported that plant height, stem diameter and dry weight of plant organs (leaves, stems and roots) in tomatoes applied with ZnO NPs increased significantly. Kızılırmak (2022) found that foliar application of ZnO NPs to cucumber (*Cucumis sativus* L.) increased yield and the highest yield increase was obtained from the treatment of 75 mg L^{-1} concentration. In a study examining the effects of different concentrations of silver nanoparticles on fresh onion (*Allium cepa* L.), it was reported that the best values in terms of plant height and leaf width were obtained from 50 mg L^{-1} AgNPs treatments (Akhoundnejad et al. 2019). Contrary to these findings, Laware and Raskar (2014) reported that the best results on plant development and flowering in onion were obtained from 20 and 30 µg ml^{-1} ZnO NPs applications.

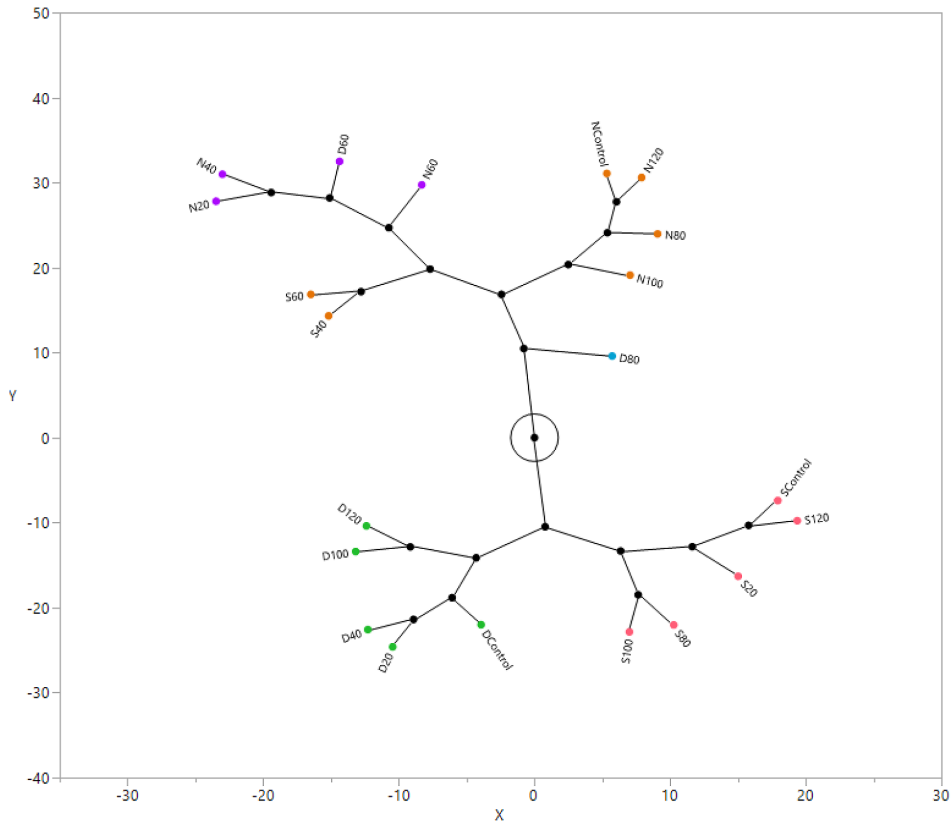

Fig. 2. The constellation plot obtained from the HCA based on ZnO NPs treatments and plant growth parameters: D – Delfos, N – Nun 7194 F1, S – Suluova

The Effect of different ZnO NPs treatments applied to donor plants on gynogenesis efficiency

In the study, it was determined that ZnO NPs treatments to donor plants promoted the growth of flower buds in *in vitro* culture, and the plumpy rate was affected by the treatments (Table 4). In fact, when compared with the control, it was observed that plumpy explants occurred in 92.0% and 91.6% of the explants at 20 mg L^{-1} ZnO NPs treatment in cv. 'Delfos' and cv. 'Nun 7194', respectively, while in the Suluova genotype, the highest plumpy explant rate was obtained from the 100 mg L^{-1} ZnO NPs treatment a rate of 88.9%. Callus formation in plumpy explants in culture generally decreased with increasing ZnO NPs concentration. The highest callus formation rate was determined as 8.8% in cv. 'Delfos' with 40 mg L^{-1} ZnO NPs treatment, 5.9% in cv. 'Nun 7194' (120 mg L^{-1} ZnO NPs treatment) and 4.9% in Suluova genotype (60 mg L^{-1} ZnO NPs treatment). The rate of hyperhydric explants in culture media and the rate of non-responsive explants increased depending on the increase in ZnO NPs treatments. It was

Table 4

In vitro flower buds development and the number of gynogenic plantlets

Cultivar/ Genotype	ZnO NPs (mg L^{-1})	No of cultured explants	Plumpy		Callus formation		Hyperhydricity		Non responsive		Gynogenic plantlets	
			No	%	No	%	No	%	No	%	No	%
Delfos	Control	525	313	59.6	18	5.7	5	0.9	189	36.0	0	0.00
	20	525	483	92.0	22	4.5	7	1.3	13	2.5	1	0.21
	40	525	385	73.3	34	8.8	3	0.6	103	19.6	1	0.26
	60	525	359	68.4	16	4.4	4	0.8	146	27.8	1	0.27
	80	525	322	61.3	8	2.4	7	1.3	188	35.8	0	0.00
	100	525	434	82.7	11	2.5	12	2.3	68	12.9	0	0.00
	120	525	384	73.1	9	2.3	8	1.5	124	23.6	0	0.00
Nun 7194 F1	Control	525	306	58.3	12	3.9	3	0.6	204	38.8	0	0.00
	20	525	481	91.6	17	3.5	6	1.1	21	4.0	0	0.00
	40	525	397	75.6	21	5.2	11	2.1	96	18.3	0	0.00
	60	525	434	82.7	19	4.3	9	1.7	63	12.0	2	0.46
	80	525	424	80.8	22	5.2	2	0.4	77	14.7	1	0.24
	100	525	362	68.9	13	3.6	10	1.9	140	26.7	0	0.00
	120	525	409	77.9	24	5.9	7	1.3	85	16.2	0	0.00
Suluova	Control	525	432	82.3	7	1.6	2	0.4	84	16.0	1	0.23
	20	525	354	67.4	13	3.7	5	0.9	153	29.1	0	0.00
	40	525	407	77.5	17	4.2	7	1.3	94	17.9	0	0.00
	60	525	423	80.6	21	4.9	11	2.1	70	13.3	1	0.24
	80	525	440	83.8	11	2.5	9	1.7	65	12.4	0	0.00
	100	525	467	88.9	8	1.7	5	0.9	45	8.6	0	0.00
	120	525	360	68.6	13	3.6	7	1.3	145	27.6	0	0.00

determined that ZnO NPs treatments applied to donor plants affected gynogenesis efficiency in *in vitro* culture. The 60 mg L^{-1} ZnO NPs treatment, which was found to have a more positive contribution to plant growth parameters than others, was also more efficient for obtaining gynogenic plantlets *in vitro* culture. In the study, a total of eight putative gynogenic plantlets were obtained from which shoot and root development was observed, three from the cv. 'Delfos', three from the cv. 'Nun 7194' and 2 from the Suluova genotype (Table 4 and Figure 3). These plantlets emerged directly from the ovules rupturing the ovary wall (Fayos et al. 2015, Yarali, Yanmaz 2017). Embryo development did not occur from the explants with callus formation.

The growing conditions of the donor plants are one of the important factors affecting *in vitro* gynogenesis (Yarali, Yanmaz 2013). In the detailed literature review on onion, no study was found that revealed the effect of nanoparticle applications on *in vitro* gynogenesis efficiency during the cultivation of donor plants. In this study, it was determined that different

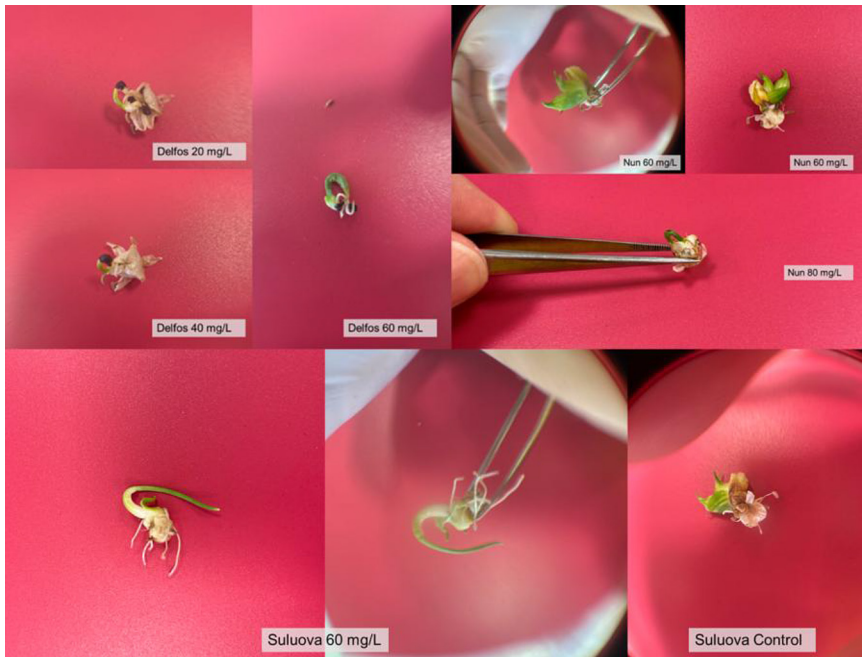

Fig. 3. Development of gynogenic plantles from ovary wall

concentrations of ZnO NPs applied to the donor plants as foliar fertilizer affected the gynogenesis efficiency in *in vitro* culture. In our research, it was determined that different concentrations of ZnO NPs applied to donor plants in *in vitro* culture directly affected the development of flower buds taken from these plants and indirectly affected the induction of gynogenic plantlets. The rate of hyperhydricity in explants in culture media and the rate of non-responsive explants increased depending on the increase in concentration of ZnO NPs. Hyperhydricity, which generally occurs due to the effect of high humidity and nutrient balance in the culture media, is a negative situation for the healthy development of plants due to excessive water intake of explants.

CONCLUSIONS

The results of the study revealed that low concentration of ZnO NPs had a positive effect on donor plant growth, whereas higher concentrations, particularly 100 mg L^{-1} and 120 mg L^{-1}, caused leaf yellowing and tip necrosis. The 60 mg L^{-1} ZnO NPs treatment generally resulted in the highest plant height, leaf number, flower stalk length, and umbel diameter, while 80 mg L^{-1} enhanced leaf length and several flowering traits, particularly

in cv. 'Delfos' and cv. 'Nun 7194'. Leaf width responses varied among genotypes, with maxima at 60 mg L^{-1} in Suluova, 80 mg L^{-1} in cv. 'Delfos', and 100 mg L^{-1} in cv. 'Nun 7194'. The longest umbel length was achieved with 60 mg L^{-1} ZnO NPs for Suluova genotype and cv. 'Nun 7194', while the highest value for cv. 'Delfos' was recorded at 80 mg L^{-1} ZnO NPs treatment. The highest number of flower buds was recorded at 60 mg L^{-1} ZnO NPs treatment for Suluova genotype and cv. 'Delfos', whereas for cv. 'Nun 7194', the highest value was recorded at 80 mg L^{-1} ZnO NPs treatment. ZnO NPs treatments also improved *in vitro* responses by enhancing callus formation and gynogenic induction. The 60 mg L^{-1} ZnO NPs treatment was the most efficient, yielding a total of eight putative gynogenic plantlets (three from cv. 'Delfos', three from cv. 'Nun 7194', and two from Suluova genotype), all of which developed shoots and roots.

Author contributions

Conceptualization – F.Y.K., and B.B.A., methodology – F.Y.K, B.B.A., Y.Ö., and B.E.Ç. – software, F.Y.K., validation – F.Y.K., and B.B.A., investigation – F.Y.K., Y.Ö., and B.E.Ç, data curation – F.Y.K., and Y.Ö., writing-original draft preparation – F.Y.K., Y.Ö., and B.E.C., writing-review and editing – F.Y.K.., Y.Ö., and B.B.A., visualization – F.Y.K. All authors have read and agreed to the published version of the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest related to this article.

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