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Research Article

Mitigation of Drought Stress in Potato Cultivars in vitro Culture by Application of Silicon Nanoparticles

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Abstract:

Nano Silicon exerts advantageous impacts on several crops, particularly under biotic and abiotic stress conditions. Nano Silicon may influence biochemical, physiological, and photosynthetic processes, hence mitigating drought stress. The impact of nano Si on potato plants during drought stress remains unclear. This study aimed to assess the impact of nano Si application on various growth and microtuber characteristics. The potential of growth and microtubrization of four potato cultivars "Spunta (SP.), Lady Rosetta (LD.), Diamant (D.), and Agria (A.)" treated with two concentrations of nano-silicon Si-NPs (50 and 100 mg L^{-1}) exposed to drought stress, induced by two concentrations of polyethylene glycol (PEG 40 and 80 g L^{-1}) were studied. Treatment that consisted of PEG application with the absence of nano Si application showed a decline in vegetative measurements, and a clear weakness in growth, when comparing the results to the control treatment. While the presence of both concentrations of Si-NPs in the culture media reduced the negative effect of PEG due to drought stress that the plants were exposed to, Under PEG -induced water stress, cv. Diamant was the highest value for most vegetative and microtubers parameters except survival% and biomass%, in all treatments. It can be recommended that the addition of Si-NPs alleviates the effects of drought stress simulated with PEG in vitro for all growth and microtubers parameters and it must be evaluated under field conditions.

1. Introduction

Potato (*Solanum tuberosum* L.) is an annual horticultural crop. With maize, wheat, and rice as the top three stable food crops, it is currently the fourth biggest. In Egypt, the farmed area encompassed 175,161 hectares, yielding around 5 million tons, with an average of 28 tons per hectare. Egypt yearly exports around 684,735 tons of potatoes, valued at over 266 million dollars (FAO-STAT, 2019). Potatoes are propagated asexually through tubers, which are imported annually from several European nations for summer planting. Egypt imported around 152,198 tons yearly, valued at over 103 million dollars. Tuberization is a complicated developmental process that may be promoted in vitro to reduce the importation of tubers and produce disease-free potatoes (Morais et al., 2018). Egypt is in a dry zone where precipitation is rare, and the desert comprises most of its area with deep nonrenewable groundwater reservoirs (El-Agha et al., 2024). Drought stress is a significant environmental challenge in several global locations, particularly in arid and semi-arid areas. It induces alterations in plants' physiological, morphological, biochemical, and molecular characteristics, adversely impacting their quantity and quality of plant growth and yield (Rouphael et al., 2012). The potato exhibits significant sensitivity to water deficiency (Epstein and Grant, 1973; Van Loon, 1981). This sensitivity is due to its diminutive and superficial root system, rendering the plant inefficient in water absorption (Gregory and Simmonds, 1992). Short periods of water deficiency may lead to decreased tuber development, yield, and quality (Dalla Costa et al., 1997). Drought stress lowers potato production by reducing plant growth and is jeopardized by abiotic stress, which is further intensified by global warming. Potatoes are recognized as crops that utilize water efficiently. This crop species is highly susceptible to water deficiencies because of its shallow and low-density root architecture (Ruttanaprasert et al., 2016). Because of their tiny size, structural characteristics, and increased surface-to-volume ratio, Nanoparticles promote the growth of plants and protect them from several abiotic stresses (Agrawal and. Rathore, 2014). "NPs have been widely used in many aspects of agriculture and can be beneficial in mitigating abiotic stresses such as salinity and drought.

The application of Si-NPs in soil amendment, irrigation, and foliar spray significantly enhanced plant productivity and the quality of fruits and grains under stress conditions, while also supporting root growth and photosynthetic CO_2 absorption (Shang et al., 2019). Water stress generally leads to a reduction in the absorption of minerals including nitrogen, sodium, calcium, iron, zinc, copper, manganese, and silicon. However, Si-NPs enhance the levels of nitrogen, potassium, and other nutrients, leading to the aggregation of Si-NPs in plant leaves, which triggers stomatal closure to mitigate water loss. Water stress increases the creation of reactive oxygen species (ROS), resulting in excessive ROS generation and subsequent oxidative damage to plants. Si-NPs enhanced. antioxidative enzyme activity and decreased ROS concentration under stress conditions (Shrishti et al., 2023).

The purpose of this study was to examine the impact of drought stress caused by Polyethylene Glycol (PEG 40 & 80 gL^{-1}), on the growth characteristics of four potato cultivars: "Spunta (SP.), Lady Rosetta (LD.), Diamant (D.), and Agria (A.)" at two different concentrations of Si-NPs (50 and 100 mgL^{-1}). The effectiveness of Si-NPs in decreasing adverse consequences of drought on potato plants in vitro was evaluated.

2. Materials and Methods

To achieve the aim of the research, the following steps were taken: This study was performed in the *Plant Tissue Culture Laboratory under the Department of Horticulture, Faculty of Agriculture, Suez Canal University* in 2023-2024. Four potato cultivars from various maturity cohorts, including early genotype (*Spunta*), mid-early genotype (*Lady Rosetta and Diamant*), one late maturing (*Agria*), were brought from the *Egyptian Ministry of Agriculture* Certified seed tubers from 4 potato genotypes "*Spunta (SP.), Lady Rosetta (LD.), Diamant (D.), and Agria (A.)*" were allowed to grow in plastic pots filled with peatmoss and vermiculite, till germination occurred. Sprouts (2-4 cm) were gathered and cleaned with tap water for 15 minutes. Sprout tips were surface sterilized in a laminar air-flow hood with 70% ethanol solution for 30 s. then sodium hypochlorite (2%) for 10 min and washed thrice using sterile distilled water. Sprouts were cultured in a 15 cm test tube (one meristem/test tube) containing 10 ml of (MS) Murashige and Skoog (1962) in the absence of a Plant Growth Regulator (PGR). The medium pH was set to 5.8 before autoclaving at "110 kPa for 20 min at 120°C". Cultured explants were maintained in a controlled environment at (25±2°C). Whole plantlets containing about Ten nodes have been used for further in vitro propagation using single-node cuttings. Nodal segments with a single bud (explant) were cultured in 250 mL glass jars, having 40 mL of full-strength MS fortified with concentrations of Kinetin (1mgL^{-1}) for multiplication. The unit consisted of five explants/jars. Repeat each multiplication subculture 2-3 times for each 35 days in the same media content. After obtaining sufficient potato plantlets, the potato explant of four genotypes is transferred to special media components to start work in the experiments.

Application of Si-Nanoparticles and Induction of Drought Stress: For drought stress induction polyethylene glycol (PEG-Sigma 6000 was used to exert a water deficiency in the nutrient medium necessary for the growth and development of the plantlet to cause changes of growth, similar to those produced by the drying of the soil.) were added to the MS with two concentrations (40 or 80 g L^{-1}) together with *Si-NPs (Nanoparticles (Sigma)* with 25 nm size) at two levels (50 and 100 mg L^{-1}), were used to assess the impact of Si-NPs treatment on the drought stress tolerance of potato plants in vitro. Combinations of *Si-NPs and PEG* concentrations are given in Table 1.

Table 1. Combinations of Si-NPs and PEG concentrations were used in this study.

PEG (g L^{-1})	Si-NPs (mg L^{-1})	Treatments	
0	0	T1	0gL^{-1} PEG + 0 mg L^{-1} Si-NPs
40	0	T2	40gL^{-1} PEG + 0 mg L^{-1} Si-NPs
	50	T3	40gL^{-1} PEG+ 50 mg L^{-1} Si-NPs
	100	T4	40gL^{-1} PEG+ 100 mg L^{-1} Si-NPs
80	0	T5	80gL^{-1} PEG+ 0 mg L^{-1} Si-NPs
	50	T6	80gL^{-1} PEG+ 50 mg L^{-1} Si-NPs
	100	T7	80gL^{-1} PEG+ 100 mg L^{-1} Si-NPs

T1 control treatment: single nodes are grown in a traditional multiplication media. Treatments T2 till T7: single nodes culture in media with different concentrations of PEG and Si-NPs. Five explants were placed in each culture and kept in the growth room, all the previous Cultures were maintained in a growth chamber at a constant temperature of 25±2ºC, under illumination with a photoperiod of 16 hours. After 4 weeks from culture plantlets were collected for morphological traits, assessment including survival%, shoot height (cm), number of nodes/plantlet, fresh weight (gm), dry weight (gm), and biomass% (were measured using equation = dry weight/fresh weight x100). To evaluate in vitro microtuberization under the seven treatments as mentioned above, 30 ml of sterilized liquid MS medium supplemented with a high sucrose concentration (80 g L^{-1}) was introduced into each jar holding the developing plantlets following the removal of the jar cap in a laminar airflow hood. Cultures were maintained in darkness at 18-20ºC for two months. Microtubers generated from each treatment were collected, and data were recorded on the quantity and weight (yield) of microtubers per jar. The percentage of tuber development under seven treatments was determined relative to the control.

Statistical analysis:

This investigation was conducted as a split plot in a completely randomized design with 10 replicates used in a factorial arrangement (two-way ANOVAs). Each plot consists of five jars. Cultivars in main plots and combinations of Si-NPs and PEG concentrations treatment (PEG x Si-NPs) in sub-plots. The means of treatment for all the traits were subjected to computational analysis of variance based on the model proposed by (Steel and Torrie, 1960). The means of genotypes for all the traits were compared using the least significant difference (L.S.D) at a P-value < 5%. Data were subjected to statistical analyses using a computer program Costat software (version 6.311).

3. Results and Discussion

3.1. Vegetative growth & microtubers parameters:

Mean values attributable to various amounts of Si-NPs, PEG, and their interaction were significant for most growth parameters of in vitro potatoes (Table 2). The greatest values for all vegetative and microtuber parameters were obtained in the control treatment (T1) for all four experimental potato cultivars. On the other hand, plants that had water stress induced by PEG application, in the treatment medium (T2 & T5) showed a decline in all vegetative measurements, a clear weakness in growth, and microtuber parameters when comparing the results to the first control exp. for all four experiment potato cultivars, Table (2) and Figure (2). The notable decrease ($p < 0.05$) in growth parameters of in vitro plants treated with PEG applications may be attributed to reduced water uptake, leading to decreased cell division and elongation. (Farooq, et al., 2009). , however, potato plants grown in media with 40 &80 gL^{-1} PEG and in the presence of two different concentrations of Si-NPs, were also shown to have a very positive effect on all vegetative growths and microtubers measurements in general, when compared with plants growing under drought stress in treatments T2&T5 experiment media. the results showed that despite the existence of a strong influence responsible for drought in the Media of T3, T4, T6 &T7 exp., the presence of a nano-silicon compound in the culture media reduced the negative effect of PEG due to drought stress that the plants were exposed to and that the results of the vegetative and microtuber measurements of the T3, T4, T6 &T7 exp., were as close as possible to the first control experiment. as presented in (Table 2) and Figure. (1&2). these results are congruent with (Mathur and Roy, 2020; Mukarram et al., 2021) Reports indicate that nano-silicone provides a protective influence on plants via modulating antioxidant systems, and the control of phytohormones due to Si supplementation enhances drought stress tolerance. A recent study has shown that SiNPs enhance the drought stress tolerance of roses (Rosa damascena Mill.) caused by PEG through lowering H_2O_2 levels and raising antioxidant enzyme activity, (Hajizadeh et al., 2022).

Also, the findings align with those of Gowayed et al. (2017), who observed that SiO2-NPs treatment at 50 ppm enhanced shoot and root length, the roots number, and callus fresh weight in both potato varieties, Proventa and Santecvs., under saline conditions. The beneficial impact of silicon on plant growth can be attributed to the elevated concentration of GA_3, which promotes cell division and elongation, as observed in Salvia splendens under high-temperature conditions, as reported by Soundararajan et al. (2014). While there were significant differences between potato cultivars, both cv. Spunta & cv. Lady Roseta had the highest survival values in all treatments, on the other hand, cv. Diamant was the highest value for most vegetative and microtuber parameters except survival% and biomass%, in all treatments as presented in Table (2).

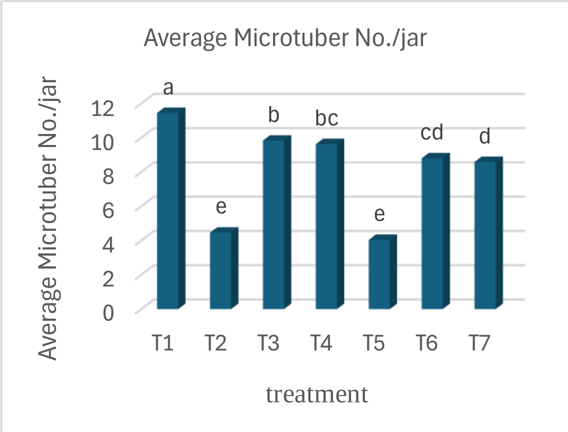
We documented in (Figure 1) the different vegetative growth & microtuber parameters of plantlets Responses to drought conditions differed according to the amounts of PEG application alone (T2 &T5) and with Si-NPs treatment (T3, T4, T6 &T7) compared with control treatment (T1). It was determined that $40gL^{-1}$ and 80 g L^{-1} PEG application decreased all vegetative and microtuber parameters, while Si-NPs by both concentrations with PEG treatment were found to reduce the negative effect of drought stress on all vegetative and microtuber parameters (Figure 1) and there are no significant different between the two Si-NPs concentration and there effect. The findings agree with those of (Crusciol et al., 2009), who demonstrated that the application of silicon at 284.4 mg/dm^3 of soil mitigated stem and branch lodging while enhancing potato tuber weight during drought conditions, with soil water potential ranging from −0.020 to −0.050 MPa. (Saadatian et al., 2021) discovered that foliar treatment of Si-NPs at concentrations between 0.8 and 3.2 mmol had a greater effect than ionized Si in enhancing the physiological features and production of potato mini-tubers.

The findings from this work indicate that the presence of Si-NPs mitigates the impacts of drought stress induced by PEG in vitro, corroborating existing literature. Outcomes from in vitro systems do not consistently predict in vivo effects applications may require testing under field conditions. Moreover, despite the enormous expansion and advancement of nanotechnology, the spectrum of products remains prohibitively expensive for many nations, particularly poor ones, rendering them economically inaccessible in certain regions. But it is well-recognized that drought causes significant product and production losses globally every year (Santini et al., 2022). Efforts to mitigate drought are considered essential to potatoes, which are sensitive to water stress. Consequently, silicon supplementation is believed to enhance tolerance in plants exposed to drought stress.

Table 2. Influence of interaction between (PEG and Si-NPs) treatments and potato cultivars on vegetative growth & microtubers parameters.

Cultivars	Treatment	Vegetative growth characters						Microtubers parameters	
		Survival%	Shoot height	No. of nodes/ plantlet	Fresh weight(g) Plantlets/ jar	Dry weight Plantlets/ jar	Biomass%	Average Microtuber No./jar	fw of Mi-crotu-ber/jar(g)
Spunta	T1	100 a	14.47 ab	16.50a	2.05 a-d	0.73cde	35.59c-f	11.40a	0.90c-g
	T2	63.67 k	7.87 ij	8.13ef	1.07 hi	0.25ijk	22.91ghi	4.47f	0.30h
	T3	96.33 bcd	13.7 a-d	14.37bc	1.80 b-f	0.70c-g	38.80a-f	9.53b-e	0.79efg
	T4	93.33 def	13.73a-d	14.43bc	1.67 efg	0.66e-h	39.72a-e	9.50cde	0.77fg
	T5	46.33 n	4.87 l	5.60g	0.93 i	0.22jk	24.69gh	4.23f	0.30h
	T6	87.00 g	11.83e-h	12.93cd	1.76 c-f	0.77bcd	44.40ab	8.57de	0.76fg
	T7	82.00 h	11.63fgh	12.57d	1.76 c-f	0.71c-g	40.50a-d	8.13e	0.71g
Lady Rosetta	T1	100a	14.30abc	15.83ab	2.09 ab	0.78abc	37.34c-f	11.70a	1.13ab
	T2	60.33 l	7.47 ij	8.53ef	1.27 h	0.24jk	18.72hij	4.53f	0.38h
	T3	97.33 ab	13.33b-e	14.90b	1.79 b-f	0.64e-h	35.94c-f	10.33a-d	0.93c-f
	T4	92.67ef	13.9 a-d	15.17ab	1.80 b-f	0.67d-g	37.35c-f	10.03a-d	0.89c-g
	T5	44.00 no	4.4 l	5.43 g	1.09 hi	0.19k	17.35ij	3.67f	0.35h
	T6	87.00 g	11.03 h	12.30d	1.64 fg	0.71c-g	44.60a	8.90de	0.86c-g
	T7	81.67 h	11.03 h	12.43 d	1.63 fg	0.62gh	38.43b-f	8.63de	0.87c-g
Diaman	T1	100 a	14.97 a	16.60a	2.28 a	0.87a	38.06c-f	11.37ab	1.21a
	T2	56.00 m	8.27 i	9.63e	1.35 gh	0.34i	24.99g	4.60f	0.43h
	T3	94.67 b-e	14.03abc	15.43ab	2.07 abc	0.71c-g	34.56def	9.23de	1.03abc
	T4	90.67 f	13.9 a-d	15.07ab	2.00 a-e	0.73c-f	36.26c-f	8.97de	0.98b-e
	T5	42.67 o	4.23 l	5.30g	1.16 hi	0.19k	16.87j	4.23f	0.39h
	T6	81.33 h	11.63fgh	13.07cd	1.91 b-f	0.69c-g	36.24c-f	8.63de	0.90c-g
	T7	72.67 j	12.37d-h	13.33cd	1.94 b-f	0.66e-h	34.05ef	8.50de	0.92c-f
Agria	T1	100 a	13.97abc	15.63ab	2.02 a-d	0.83ab	40.96abc	11.33abc	1.03a-d
	T2	54.33 m	6.53 jk	7.93f	1.19 hi	0.30ij	24.98g	4.23f	0.37h
	T3	96.67 bc	13.17b-f	14.33bc	1.73 def	0.62gh	35.93c-f	10.23a-d	0.87c-g
	T4	93.67 c-f	12.80c-g	14.47bc	1.79 b-f	0.62gh	34.45ef	10.03a-d	0.89c-g
	T5	44.00 no	5.13 kl	6.30g	1.15 hi	0.18k	16.82j	4.03f	0.37h
	T6	76.00 i	11.27 gh	12.50d	1.78 b-f	0.63fgh	35.50c-f	9.10de	0.85c-g
	T7	80.33 h	11.27 gh	12.40d	1.75 c-f	0.57h	32.81f	9.10de	0.83d-g
The main factor of Genotype									
Spunta		81.24 a	11.16 ab	12.08 ab	1.58 b	0.58 ab	35.23 a	7.98 a	0.65 c
Lady Rosetta		80.43 a	10.68 b	12.09 ab	1.62 b	0.55 bc	32.82 b	8.26 a	0.77 ab
Diamant		76.86 b	11.32 a	12.63 a	1.82 a	0.60 a	31.57 b	7.93 a	0.84 a
Agria		77.86 b	10.59 b	11.94 b	1.63 b	0.54 c	31.64 b	8.30 a	0.74 b

• Means with the same letters are not significantly different at $p \leq 5$.



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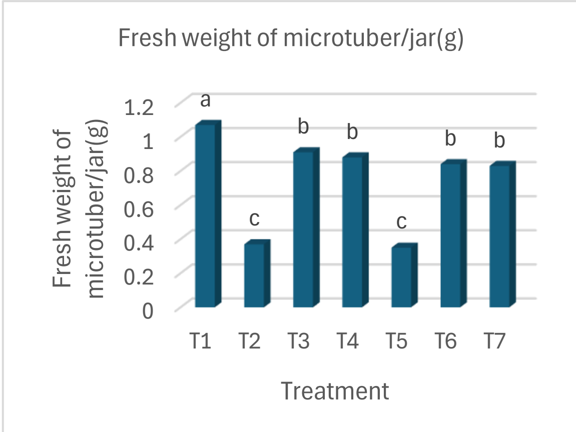

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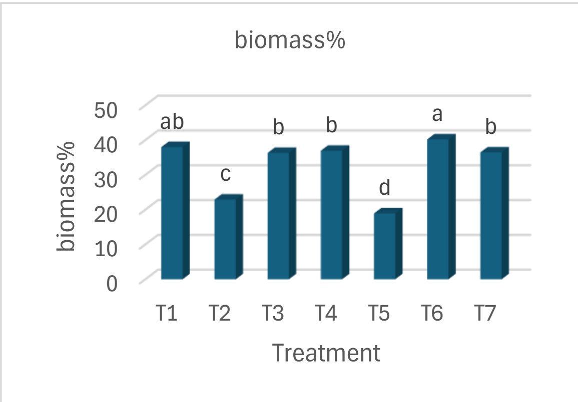

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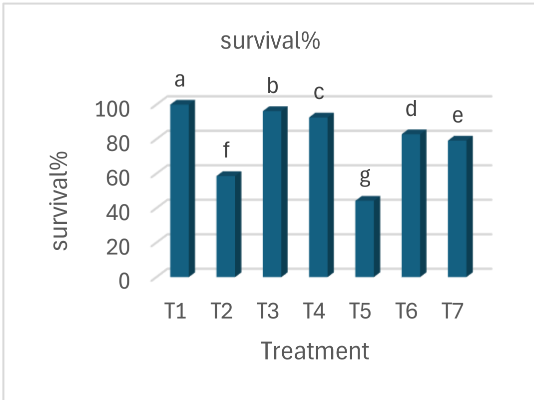

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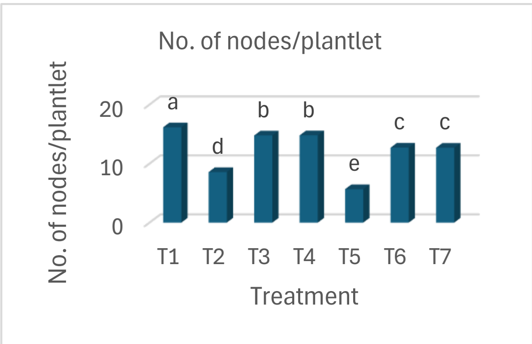

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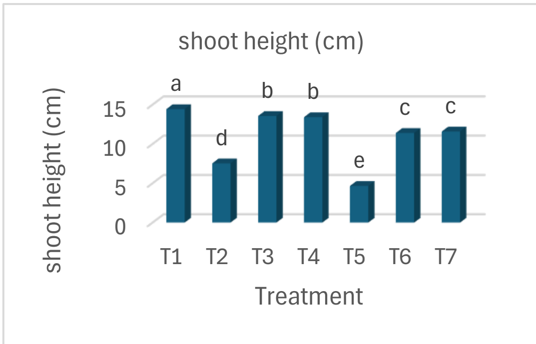

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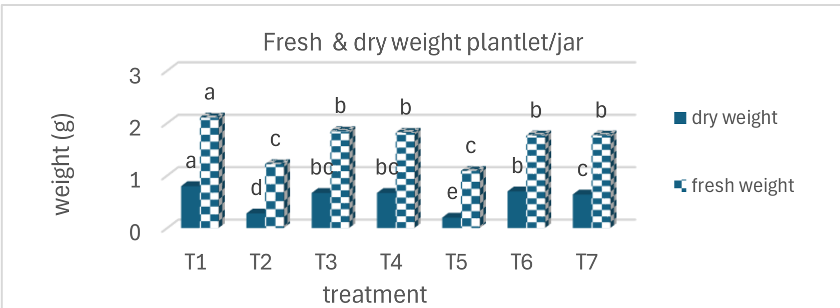

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Figure 1. Mean performance for treatment on the studied variable in different cultivars. Common letters are not significant ($p < 0.05$).

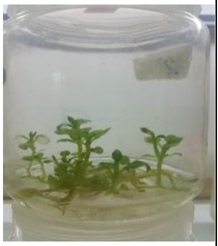
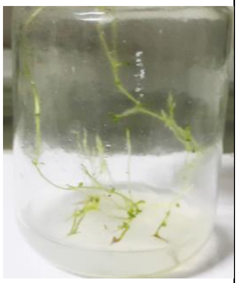
Treatment	Cultivars			
	Spunta	**Lady Rosetta**	**Diamant**	**Agria**
T1				
T2				
T3				
T4				

Figure 2. Presented overall growth of potato of four cultivars in control and under drought stress T2 compared with T3 & T4 after application of Si-NPs.

4. Conclusion

In general, the addition of Si-NPs at both concentrations improved vegetative traits compared to drought-exposed plants. Where nanocomposites are considered promising compounds in many uses, especially in plant production, especially Si-NPs, improve the ability of plants to grow under difficult environmental conditions such as salinity and Drought, which has become a seriou phenomenon that threatens plant production in general due to climate changes, especially in arid and semi-arid countries.

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