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NANOTECHNOLOGY APPLICATION FOR SALINITY TOLERANCE ENHANCEMENT OF MICROPROPAGATED *GARDENIA JASMINOIDES* ELLIS PLANT

Lobna S. Taha[1], Nora M. Youssef[1], Iman M. El-Sayed[1] and Walaa H. Salama[2]

[1]Ornamental Plants and Woody Trees Dept., National Research Centre, Dokki, Giza, Egypt.
[2]Department of Molecular Biology, Genetic Engineering and Biotechnology Division, National Research Centre, Dokki, Giza, Egypt.

Abstract

Gardenia jasminoides Ellis is widely used as screens, hedges, borders or ground overs in the field of ornamental plants. Besides, it has been included in traditional medicine due to its homeostatic, antiphlogistic, analgesic, anti-inflammatory and antipyretic effects. This work aimed to alleviate the abiotic effects of salinity stress using some elements nanoparticles. The experiment was carried out to assess the effect of BA concentration (0.0, 0.5 and 1.0 mg/l) on *in vitro* propagation ability of *Gardenia jasminoides* Ellis plant and mitigate the effect of different levels of salinity stress (0.0, 2000, 4000 and 6000 ppm) on the plant using two concentrations (2.5 and 5.0 mg/l) of some elements Nanoparticles (Zn, Ti and Fe NPs). MS supplemented with BA at 1.0 mg/l combined with NAA (0.2 mg/l) was the suitable *in vitro* propagation medium and the proliferated shoots couldn't form any roots under all used salinity levels. At 2000 ppm of salinity, using high concentration (5 mg/l) of Ti NPS had the best results for shooting and rooting abilities. While, adding 5 mg/l of Zn or Fe NPs with 4000 ppm of salinity induced shoot proliferation, elongation and rooting capability. Increasing NaCl salt to the highest level (6000 ppm) increased the phenolic, flavonoids and proline contents of shootlets. The highest values of these compounds were noticed when 2.5mg/l of Ti NPs was added the culture medium that contained 4000 ppm salinity.

Key words: Gardenia, *in vitro*, salinity and Nano-elements.

Introduction

Gardenia jasminoides Ellis, a native of Indochina and China. It is an ornamental and medicinal woody plant. This plant is a member of the family Rubiceae, is an evergreen shrub, the flowers are white and very fragrant. The double-flowered form is usually used for ornamental purposes, whereas, the single-flowered form is used as a medicinal plant (Mizukam, 1989). The leaves are glossy, leathery and dark green (Duhoky and Rasheed, 2010). *Gardenia jasminoides* can be used as cut flower, screens, hedges and garden shrub. Also, it is a popular pot plant in many European countries and the US. (Wilkins, 1986). The leaves and fruits of this plant are used as antibacterial, demulcent, diuretic properties, fever, jaundice, sore throat, bloody stools and dysuria (Hayashi *et al.,* 1992). The crude extract of *Gardenia jasminoides* is capable of inhibiting various types of cancer cells (Neugut, 1996).

In traditional propagation, terminal cutting of *G. jasminoides* produced a low proliferation rate, anyway, Successful employment of *in vitro* techniques for propagation, maintenance and manipulation of plant germplasm has been possible for a great number of plant species. Micropropagation of *Gardenia jasminoides via in vitro* organogenesis using modified Murashige and Skoog (1962) medium (MS) gave higher generation rate per each starting plant (Suprasanna and Bapat, 2005 and Wu *et al*., 2012).

Under normal conditions, plants are exposed to diverse biotic and abiotic stress factors. Salinity as abiotic stress factor in plants leading to series physiological, morphological, biochemical and molecular changes, inhibits germination and growth causes reduced yields and huge damage in the overall economy (Ashraf, 2002). Salinization may be the result of natural processes, but happens more frequently due to human effect on the environment through a series of agronomic activities, such as irrigation (Szabolcs, 1994). In nature salinity is often

***Author for correspondence :** E-mail : norayousif20@yahoo.com

present in soils which are concurrently affected by drought and/or heat stress. Salinity in plants leads to dehydration, osmotic and ionic stress, inhibition of protein synthesis and photosynthesis, chlorosis and necrosis, growth deficiency and production of highly reactive oxygen species (ROS) (Munns and Tester, 2008).

Nanotechnology is the application and study of small-sized materials (1–100 nm), a specified quality that makes these tiny entities unique. Thus, one of the new strategies is application of nanoparticles to improve plant performance and growth under salinity stress (Duhan *et al*., 2017). Nanoparticles are able to modify the production quality and the tolerance to abiotic stresses when used in small quantities (Khan, 2016). Nanoparticles interact with plants causative many physiological and morphological changes, which likely is positive or negative effects on plant growth and development, the effect of engineered nanoparticles (ENPs) on plants depends on the chemical composition, surface covering, size, reactivity and most importantly the dose and plant species Where varies from plant to plant (Khodakovskaya *et al*., 2012).

The present study was to evaluate the negative effects of abiotic stresses (saline condition) and application of nanoparticles (zinc, iron and Titanium oxides) for amelioration of salinity stress on growth *Gardenia jasminoides* Ellis *in vitro*.

Materials and Methods

This work was achieved during seasons of 2018–2019 on Gardenia jasminoides Ellis at Tissue Culture Technique Lab., Central laboratories, Department of Ornamental Plants and Woody Trees, National Research Center (NRC), Egypt.

Plant materials

Gardenia Young shoots were taken from the commercial farm "Egypt Green". The shoots were divided into nodal explants which were washed in soapy water and rinsed with running tap water for one hour. The explants were soaked for 1 min in 70% ethanol solution under aseptic condition in laminar air flow cabinet, followed by further sterilization for 10 min in Clorox 20% (v/v) with a few drops of Tween-20 as emulsifier and rinsed three times with sterile distilled water. After that, they were immersed in 0.1% mercuric chloride (MC) for 10 min followed by rinsing three times.

Culture Conditions

Cultures were incubated under controlled conditions in the growth chamber. The incubation temperature was 24±2°C. The photoperiod was 16 hours light /8 hours darkness, controlled automatically. Illumination intensity was 3000 lux from cool fluorescent lamps.

Experiment procedure

The explants were cultured on a basal MS medium (Murashige and Skoog, 1962) which was enriched with sucrose at 25 g/l and 8 g/l agar. The pH of the culture medium was adjusted to 5.7± 0.1. Culturing was done in 200 ml glass jars containing 25 ml of the medium.

In vitro propagation ability

To establish an *in vitro* culture of *Gardenia jasminoides*, three concentrations of (BA) Benzylaminopurine (0.0, 0.5 and 1.0 mg/l) were tested individually or combined with 0.2 mg/l of (NAA) Naphthalene Acetic acid.

In vitro propagation ability under saline condition

The obtained micropropagated shoots from the best optimized culture medium were used for examine the *in vitro* propagation ability under different salinity levels (0.0, 2000, 4000 and 6000 ppm).

For shooting and rooting ability improvement under saline condition, explants were cultured on MS media containing two concentrations (2.5 and 5.0 mg/l) of some elements Nanoparticles of Zinc Oxide (Zn NPs), Titanium Oxide (Ti NPs) and Ferric Oxide (Fe NPs).

Each treatment consisted of six replicates (jars) with three explant per jar. After ten weeks of treatments for each experiment, number and length of shootlets, leaves number, rooting percent as well as roots number and lengths were recorded.

The specification of used nanoparticles the present experiment was indicated in table 1 and Fig. 1.

Chemical constituents

Total phenolic content

The total phenolic content was measured according to Velioglu *et al*., (1998) Fifty µL of the extract was mixed

Table 1: Specification of used Nanoparticles in the experiment.

Specification		Test method
Nano- Zinc Oxide		
Phase	ZnO	XRD
Particle size	<30 nm	TEM
Surface area	~ 20m^2/gm	BET (P/Po: up to 0.35
Nano- Titanium Oxide		
Phase	anatase	XRD
Particle size	<50 nm	TEM
Surface area	>100m^2/gm	BET (P/Po: up to 0.35
Nano- Iron Oxide		
Phase	hematite	XRD
Particle size	<50 nm	TEM
Surface area	>50m^2/gm	BET (P/Po: up to 0.35

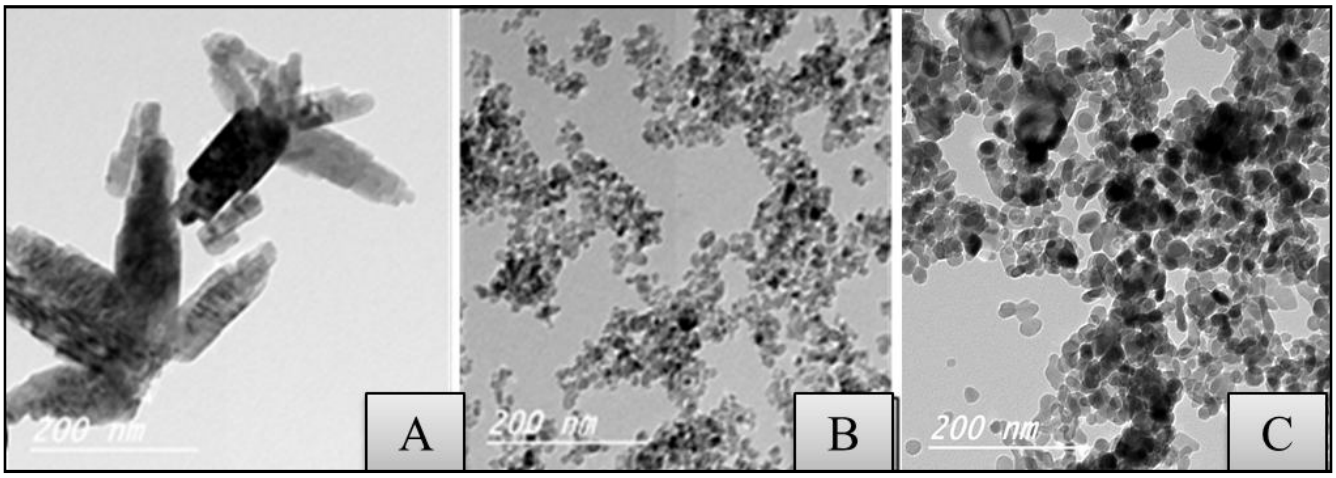

Fig. 1: Scanning electron microscopy image of A: Nano- Zinc, B: Titanium and C: Iron Oxides.

with 100 µL Folin–Ciocalteu reagent, 850µL of methanol and allowed to stand for 5 min at ambient temperature. A 500 µL of 20% sodium carbonate was added and the mixture was incubated for 30 min at room temperature. Absorbance was measured at 750 nm. Gallic acid was used as standard for the calibration curve and total phenolic content was expressed as mg gallic acid equivalent (GAE) / g tissue.

Total flavonoid content

The total flavonoid content was determined using modified colorimetric method described previously by Zhishen *et al*., (1999) and used catechin as a standard. The extract (250 µL) was mixed with 1.25 µL distilled water and 75 µL of 5 % $NaNO_2$ solution. After 6 min, 150 µL of 10% $AlCl_3$ solution was added to the mixture. 0.5 ml of 1 M NaOH and 275 µL of distilled water were added to the mixture 5 min later. The absorbance of the solutions at 510 nm was measured. The results were expressed as mg catechin equivalent (CE) /g tissue.

Proline determination

The proline was determined according to Carrilo *et al*., (2011) 500 µl ethanolic extract (80% ethanol) or 100 µl of 5-2-1-0.5-0.2 mM proline standard completed with up to 400µl of ethanol: water (40: 60 v/v) is added to 1ml of reaction mixture (ninhydrin 1% (w/v) in acetic acid 60% (v/v), ethanol 20% (v/v)) and incubated at 95°C for 20 min. After that, the mixture was cooled at room temperature and centrifuged 1min at 10000 rpm. The supernatant was read at 520 nm with uv-spectrophotometer.

Proline in $nmol.mg^{-1}$ FW or in $\mu mol.g^{-1}$ FW = (Abs (extract – Abs (blank) /slope *Volextract/Volaliquot)*(1/ FW.

Experimental design and data analysis

The data were analyzed through analysis of variance ANOVA and the treatments' means were compared for significance by Duncan's New Multiple Range test at 0.05% level of probability (Duncan 1955) using COSTATV-63.

Results and Discussion

***In vitro* propagation ability**

To establish an *in vitro* culture of *Gardenia jasminoides*, three concentrations of BA (0.0, 0.5 and 1.0 mg/l) were tested individually or combined with 0.2 mg/l of NAA table 2. As illustrated in the tabulated data, more proliferated and elongated shootlets with the highest number of leaves (4.60, 34.67 mm and 48.33, respectively)

Table 2: Effect of BA concentration and NAA on *in vitro* propagation ability of *Gardenia Jasmnoides* plant.

Character Treatments	Shootlet number/ explant	Shootlet length (mm)	Leaves number/ explant	Rooting %	Roots number/ shootlet	Root length (mm)
Control 0.0	1.10 d	22.5 c	34.00 d	-	-	-
BA 0.5 mg/l	1.15 d	23.5 c	35.00 cd	-	-	-
BA 1mg/l	1.53 c	30.67 b	38.67 bc	-	-	-
BA 0.5 mg/l +NAA 0.2 mg/l	3.53 b	35.33 a	40.67 b	11.33 b	1.33 a	8.67 b
BA 1mg/l + NAA 0.2 mg/l	4.60 a	34.67 ab	48.33 a	33.33 a	2.00 a	15.33 a

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

beside the best rooting results that were presented in rooting %, roots number and length (33.33%, 2.0 and 15.33 mm) were noticed when MS culture medium was supplemented with the high concentration of BA (1.0 mg/l) combined with NAA (0.2 mg/l) as compared to control and other treatments. It can be noticed that using BA individually at any concentration decreased the proliferated shoots and none rooting was formed with absence of auxin in the culture medium. This indicates the important role of both cytokinin and auxin in the culture medium to regulate the growth and development of plant. Confirmed results were found by Husen and Pal (2007) who stated that supplementation of auxin to the microshoots resulted in strength roots through induction of the internal enzymes that regulate various biochemical pathways and subsequently induce cell division and elongation. Sayd *et al*., (2010) on *Gardenia jasminoides*, noticed that the proliferation was increased in case of using 1 mg/l of BA.

In vitro propagation ability under saline condition

The obtained micropropagated shoots from the best optimized culture medium (MS+ BA1.0 + NAA 0.2 mg/l) were used for examining the *in vitro* propagation ability under different salinity levels (0.0, 2000, 4000 and 6000 ppm) as shown in table 3. The data revealed that the proliferated and elongated shoots as well as number of leaves could be obtained at highest values (5.50, 31 and 36.5, respectively) under saline condition at 2000 ppm with no significant differences between these values and those of control (0.0 of salinity). Increasing the salt concentration in the culture media to 4000 and 6000 ppm decreased these values to lowest ones. Under this investigation, the proliferated shoots couldn't form any roots under all used salinity levels. Similar results were found by Fathy *et al*., (2019) on *Populus alba,* mentioned the adverse effect of salt concentration increasing in the culture medium on the *in vitro* shoot proliferation and led to non-rooting of shoots. The positive effect of NaCl low concentration on *in vitro* shoot proliferation might be attributed to increasing of osmolarity (Flowers and Lauchil, 1983). Earlier reports mentioned that the roots are the first plant organs that effected and most sensitive ones by salt stress (Siler *et al*., 2007). The internal water balance was readjusted as a result of excess salinity by accumulation of Na and reducing of K, Ca and sugar concentrations (Brinker *et al*., 2010). Hejazi-Mehriz *et al*., (2013) indicated the positive correlation of Na concentration in shoot to membrane permeability that was damaged as a result of Na uptake.

Effect of some elements nanoparticles (NPs) on *in vitro* propagation ability under saline condition

The role of used element NPs to alleviate salinity stress on *in vitro* propagation was noticed as declared in table 4. At 2000 ppm of salinity, using high concentration (5 mg/l) of Ti NPs had the best results for shooting ability. Also, this stimulating effect stands out clearly on rooting ability (rooting%, root length and number) that were in highest values (83.3%, 19.5 mm, 6.6, respectively) followed by those obtained (66.6%, 12.6 mm and 5.0, respectively) when low concentration (2.5 mg/l) of Fe NPs was used at the same level of salinity (2000 ppm), as compared to control which caused the lowest ones (33.3%, 14.6 mm and 2.4, respectively). The effects of element NPs varied with increasing salinity level to 4000 ppm. Adding 5 mg/l of Zn or Fe NPs at this level to the culture medium assist the explants to grow with high shoot proliferation (6.3 and 6.0, respectively) and elongation (40.0 and 33.3 mm, respectively) followed by 2.5 mg/l of Ti NPs that also had induced particularly rooting capability comparing with other treatments. However, none rooting was noticed with increasing salinity level to 6000 ppm even when any concentration of all used elements NPs was used. Similar foundation by Shankramma *et al*., (2015) reported that using of nanomaterial causes an increase in plant growth. The inhibition effect of most NPs treatments on rooting ability suggested that the effect of nanoparticles on plant can be beneficial for growth and development or non-beneficial to prevent root growth (Zhu *et al*., 2008). This might be due to the ability of nanoparticles to damage the

Table 3: Effect of different salinity levels on *in vitro* propagation ability of *Gardenia jasminoides* plant.

Character Treatments	Shootlet number/ explant	Shootlet length (mm)	Leaves number/ explant	Rooting %	Roots number/ shootlet	Root length (mm)
Control (0.0 of salinity)	4.75 a	34 a	43.75 a	32..67	2.13	13.33
NaCl at 2000 ppm	5.50 a	31 a	36.5 ab	-	-	-
NaCl at 4000 ppm	3.53 b	18.33 b	28.00 b	-	-	-
NaCl at 6000 ppm	3.03 b	16.00 b	26.67 b	-	-	-

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

plasma membrane and deplete the internal calcium (Chichiricco and Poma, 2015).

Total phenols, flavonoids and proline

Data in table 5 indicates the chemical constituents of shootlets under effect of various salinity levels (0.0, 2000, 4000 and 6000 ppm) individually and in combination of the levels (2000 and 4000 ppm) that the plantlets could form rooting with some selected elements NPs. Concerning the effect of salinity, increasing of phenolic,

Table 4: Effect of some elements nanoparticles (NPs) on *in vitro* propagation ability of *Gardenia jasminoides* plant under different salinity levels.

Character Treatments	Shootlet number/ explant	Shootlet length (mm)	Leaves number/ explant	Rooting %	Roots number/ shootlet	Root length (mm)
Control	5.0 ab	33.2 a-d	49.5 ab	33.3 c	2.4 bc	14.6 ab
2000 ppm NaCl +2.5 mg/l Zn NPs	6.0 a	32.7 a-d	51.3 ab	33.3 c	1.6 bc	7.8 b-d
2000 ppm NaCl +5 mg/l Zn NPs	4.3 ab	35.5 a-d	45.3 bc	16.6 d	1.6 bc	5.8 b-d
2000 ppm NaCl +2.5 mg/l Ti NPs	6.3 a	42.51 ab	40.5 bc	33.3 c	3.3 a-c	9.3 b-d
2000 ppm NaCl +5 mg/l Ti NPs	6.6 a	43.33 a	58.3 a	83.3 a	6.6 a	19.5 a
2000 ppm NaCl +2.5 mg/l Fe NPs	6.3 a	45.0 a	28.0 de	66.6 b	5.0 ab	12.6 a-c
2000 ppm NaCl +5 mg/l Fe NPs	5.0 ab	37.33 ab	24.0 de	16.6 d	1.0 bc	3.5 cd
4000 ppm NaCl +2.5 mg/l Zn NPs	4.3 ab	30.2 b-e	25.3 de	0.0 d	0.0	0.0
4000 ppm NaCl +5 mg/l Zn NPs	6.3 a	40.0 ab	44.5 bc	16.7 d	0.3 c	11.7 a-c
4000 ppm NaCl +2.5 mg/l Ti NPs	4.6 ab	38.3 ab	15.5 ef	16.6 d	0.3 c	1.66 d
4000 ppm NaCl +5 mg/l Ti NPs	3.3 ab	32.3 a-d	19.0 ef	0.0	0.0	0.0
4000 ppm NaCl +2.5 mg/l Fe NPs	5.0 ab	18.33 e	61.0 a	0.0	0.0	0.0
4000 ppm NaCl +5 mg/l Fe NPs	6.0 a	33.3 a-d	25.5de	16.6 d	0.3 c	1.66 d
6000 ppm NaCl +2.5 mg/l Zn NPs	3.0 ab	22.5 c-e	19.5 ef	0.0	0.0	0.0
6000 ppm NaCl +5 mg/l Zn NPs	2.0 b	19.16 e	27.0 de	0.0	0.0	0.0
6000 ppm NaCl +2.5 mg/l Ti NPs	2.0 b	21.7 d	7.0 f	0.0	0.0	0.0
6000 ppm NaCl +5 mg/l Ti NPs	3.3 ab	21.66 de	23 de	0.0	0.0	0.0
6000 ppm NaCl +2.5 mg/l Fe NPs	4.0 ab	35.8 ab	41.5 be	0.0	0.0	0.0
6000 ppm NaCl +5 mg/l Fe NPs	4.6 ab	35.0 a-c	35.5 cd	0.0	0.0	0.0

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

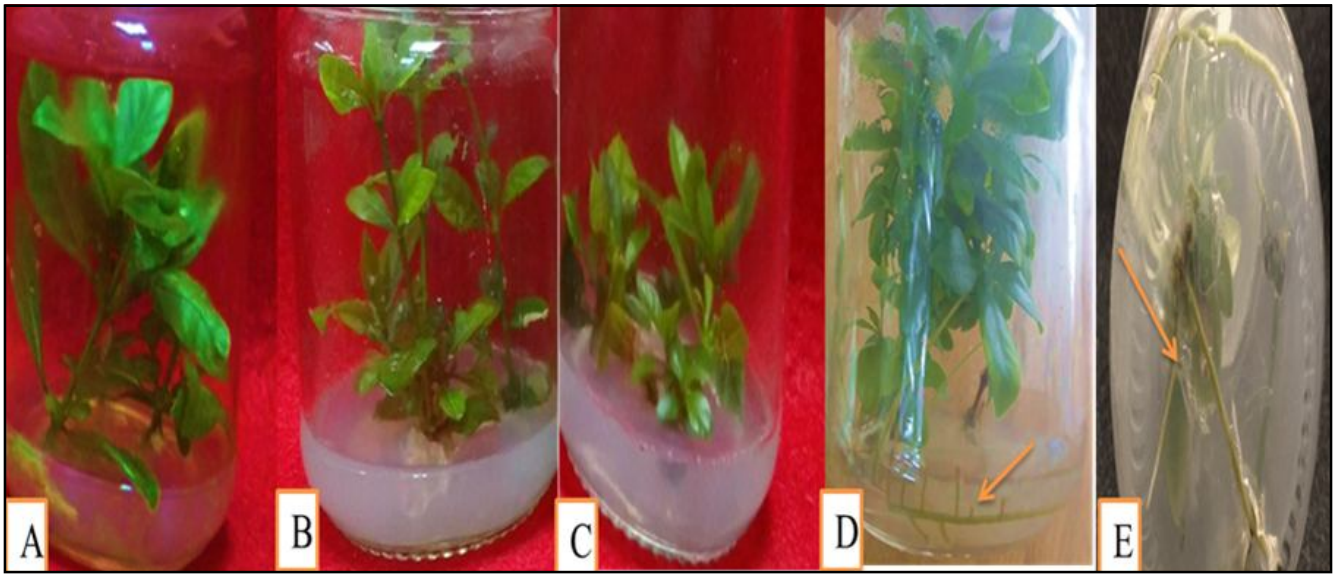

Fig. 2: *In vitro* Growth behavior of *Gardenia jasminoides*, (A): MS culture medium supplemented with BA (0.5 mg/l) +NAA (0.2 mg/l), (B): Shoot proliferation with application of NaCl at 2000 ppm, (C): Shoot multiplying with application of NaCl at 6000 ppm, (D): Shoot proliferation and rooting induction *in vitro* with application of NaCl at 2000 ppm +5 mg/l Ti NPs and (E): rooting on plantlets treated with NaCl at 2000 ppm with 2.5 mg/l Fe NPs.

Table 5: Effect of different salinity levels and some elements nanoparticles (NPs) on total phenolic, total flavonoid and proline contents of *Gardenia jasminoides* plantlets (n=3, value= mean± SD).

Treatments	mg gallic /g tissue	mg catechin/g tissue	Proline Conc. (nmol/mg)
Control	4000± 400	2800±200	0.36±0.04
S1(NaCl at 2000ppm)	4050± 90	2124± 124	0.33± 0.028
S2(NaCl at 4000ppm)	4849± 105	3816± 180	0.36± 0.03
S3(NaCl at 6000ppm)	5243± 191	4218± 100	0.78± 0.065
S1+ 2.5mg/l Zn NPs	4900±300	3000±280	0.40±0.04
S1+ 5 mg/l Ti NPs	5000±500	3200±300	0.40±0.035
S1+ 2.5 mg/l Fe NPs	6430±420	4000±320	0.55±0.04
S2+ 5mg/l Zn NPs	5240±350	3500±250	0.50±0.045
S2+ 2.5mg/l Ti NPs	7500±200	4300±350	0.60±0.035
S2+ 5mg/l Fe NPs	6000±300	3800±200	0.50±0.025

Data are mean of three replicates ± SD at P≥0.01.

flavonoids and proline contents of shootlets to the highest values (5243 mg gallic/g tissue, 4218 mg catechin/g tissue and 0.78 nmol/mg, respectively) were recorded with increasing NaCl salt to the highest level (6000 ppm) in the culture medium comparing with control. Rice-Evans *et al*., (1997) observed that increasing the flavonoids production may protect the plant against environmental abiotic stress such as salinity. Also, the positive effect of salinity on proline concentration may due to the role of that osmoprotectants in decrease of stress-induces cellular acidification, adjustments of osmotic and sub-cellular structure for recovery (Tan *et al*., 2008).

The influence of combined some elements NPs with salinity levels (2000 and 4000 ppm) on these constituents comparing with control and other treatments were recorded in table 5. The highest values (7500 mg gallic/g tissue, 4300 mg catechin/g tissue and 0.60 nmol/mg, respectively) were noticed when 2.5mg/l of Ti NPs was added to the culture medium contained 4000 ppm salinity followed by salinity at 2000 ppm supplemented with 2.5 mg/l Fe NPs. Similar results by Mohammed and Al-oubaidi (2015) indicated the increase of gallic content in *Cicer arietinum* plant with TiO_2 NPs at 4.5-6mg/l. It can be also observed that using all selected elements NPs at various salinity levels had positive effect on the estimated constituents as compared to control. This suggests that micronutrients can reduce the environmental stress impressions such as salinity (Wang *et al*., 2011). This could explain the *in vitro* propagation ability that was correlated with the estimated biochemical assays in this work.

Conclusion

In vitro propagation ability of *Gardenia jasminoides* under saline condition to 4000 ppm stress could improved when the explants were cultured on MS medium supplemented with some elements NPs. This study will help the producers to mitigate the abiotic effects on the growth of the plant in saline soils such as some new lands.

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