Multiwalled Carbon Nanotubes and Nitric Oxide Modulate the Germination and Early Seedling Growth of Barley under Drought and Salinity

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Summary

To evaluate the impacts of various concentrations of multiwalled carbon nanotubes (0, 500, 1000 and 2000 mg L^{-1}) and sodium nitroprusside (0 and 100 µM as nitric oxide (NO) donor) on seed germination and early seedling growth of barley, two separate factorial experiments were conducted based on a randomized complete block design under polyethylene glycol-simulated drought stress and NaCl salinity stress conditions. Based on the results, a concentration-dependent declining trend was observed in barley germination indices upon seed exposure to polyethylene glycol (PEG) and NaCl suspensions. Employing multiwalled carbon nanotubes (MWCNTs) particularly 1000 and 500 mg L^{-1} and sodium nitroprusside (SNP) alleviated the adverse impacts of drought and salinity stresses. However, applied MWCNTs and SNP together were more efficient than suspension alone. The combined application of MWCNTs with SNP increased germination percentage, germination rate, root length, shoot length, vigor index and decreased mean germination time of barley. Similarly, the amount of moisture content and uniformity of seed germination were obviously increased by MWCNTs and SNP under drought and salinity. In contrast, MWCNTs at 2000 mg L^{-1} had an inhibitory impact on barley seed germination, while use of SNP moderated adverse effects of MWCNTs. Generally, it can be concluded that appropriate concentration of MWCNTs is beneficial in improving drought and NaCl salinity tolerance of barley by boosting seed water absorption and increasing the moisture content of seedlings.

Key words

germination, relative water content, stress tolerance

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Introduction

Nanotechnology has undergone remarkable progresses due to the potential applications of nanomaterials (NMs) in numerous industrial and economical fields. The production, disposal, and use of higher amount of nanomaterials will inevitably increase their release into the environment and should have various influences on the biological systems, including plants (Phogat et al., 2016). Therefore, the probability of plant exposure to NMs has increased to a greater extent with the rapid development of research (Parisi et al., 2015). In recent years, the application of engineered nanoparticles (ENPs) has attracted the attention of researchers for agricultural applications given the interactions of nanoparticles with plants through accumulation, physiological and biochemical effects (Arruda et al., 2015). The impact of ENPs in plants varies according to species, composition, and their physical and chemical properties (Maiti et al., 2015).

Carbon-based nanomaterials represent one type of manufactured nanomaterial. These nanoparticles are composed entirely of carbon and are engineered to have specific shapes: spheres, ellipsoids or tubes (Abdalla et al., 2015). The carbon nanotubes consist of two main structures such as single walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) (Deng et al., 2016). The MWCNTs are among the most widely used carbon nanotubes due to their unique nanostructures and extraordinary properties such as large specific surface area, high aspect ratio, high electrical conductivity and remarkable thermal stability (Abdalla et al., 2015). It has attracted a major share of the interest because of the enormous potential for using in agriculture as directed delivery systems for pesticides, water and ionic nutrients (Hamdi et al., 2015; Tiwari et al., 2014; Liu et al., 2009). MWCNTs have an ability to influence the seed germination and plant growth; they can penetrate the cell wall and seed coat, and to provide a suitable delivery system of chemicals to cells (Chen et al., 2015a; Ratnikova et al., 2015). There exist some contradictory reports that the nanoparticle-root cell interaction could cause the change in gene expression, and consequently affect relative root growth and development of plants (Wang et al., 2012; Yan et al., 2013). In contrast, some researchers confirmed the negative role of the MWCNTs on seed germination and plant growth (Andersen et al., 2016; Begum et al., 2014).

Seed germination is an important stage of plant development. Several physiological and biochemical changes occur during seed germination, such as resumption of respiratory activity, activation of repair mechanisms, protein synthesis from stored and newly synthesized mRNA, and reserve mobilization (Shu et al., 2015). The proper course of germination determines the establishment of the mature plant. The percentage of germination, seedling emergence and germination uniformity can be influenced by many abiotic factors. Drought and salinity are among the major abiotic stresses that often cause a series of morphological, physiological and biochemical changes in seed germination and plant growth (Nasri et al., 2015; Osman Basha et al., 2015). In such condition, stress ameliorative compounds can be effective in terms of abiotic stresses. In recent years, much attention has been focused on nitric oxide (NO) effects in plants (Wang et al., 2015). As a signaling molecule, NO serves an important role in numerous physiological processes including developmental, hormonal and environmental responses in plants (Nahar Fancy et al., 2016). NO stimulates plant growth and development, such as promoting seed germination, seedling growth and delaying senescence (Fan et al., 2013; Wang et al., 2015). It has been hypothesized that NO may act as an antioxidant, also evidences have been obtained for the involvement of NO in auxin, ABA and ethylene signaling pathways (Sun et al., 2016). Many researchers reported that NO improves plant tolerance to abiotic stresses such as drought (Santisree et al., 2015) and salinity (Fan et al., 2013). In addition, there are some studies which confirmed that NO can impact the interaction of nanoparticles and plant growth (Chen et al., 2015b). However, little information is gained about plant responses to MWCNTs and NO especially under drought and salinity conditions. The present study was conducted to determine the effects of multiwalled carbon nanotubes and sodium nitroprusside (NO donor) in germination and early seedling growth of barley under drought and salinity stresses.

Materials and methods

Plant material

The seeds of barley (*Hordeum vulgare* L. var. Afzal) were provided by the Seed and Plant Improvement Institute, Karaj, Iran. The average germination rate of barley seeds was greater than 80% as shown by a preliminary test.

Treatments and experimental design

Two separate factorial experiments were arranged based on a randomized complete block design (RCBD) and all germination tests were performed in triplicate. First experiment consisting of three factors: (1) - 0, 500, 1000 and 2000 mg L^{-1} MWCNTs; (2) - 0 and 100 µM SNP, and (3) drought stress (0, -0.3 and -0.6 MPa by PEG 6000). Second experiment consisting of three factors: (1) - 0, 500, 1000 and 2000 mg L^{-1} MWCNTs; (2) - 0 and 100 µM SNP, and (3) salinity stress (0, 50 and 100 mM NaCl).

Multiwalled carbon nanotubes characterization

Commercially available multiwalled carbon nanotubes (MWCNTs, OD 20-30 nm, ID 5-10 nm, purity >95%; length: 10-30 um, ash: <1.5% weight, surface area >110 $m^2.g^{-1}$, true density 2.1 $g.cm^{-3}$) were purchased from Nanosany Co., Ltd., (Iran). The MWCNTs was tested with X-ray diffraction pattern (Figure 1a), thermo gravimetric analysis (Figure 1b), Raman spectrometry (Figure 1c), and SEM (Scanning electron microscope) and TEM (Transmission electron microscopy) images (Figure 1d).

Nanoparticles preparation

In order to obtain properly dispersed and homogeneous mixture of MWCNTs suspensions, an ultra-sonication (100 W/L, 40 KHz) treatment was applied to MWCNTs powders dispersed in distilled water for 30 minutes.

Germination experiments and growth condition

The certified seeds were surface-sterilized using 5% sodium hypochlorite solution for three minutes, then vigorously rinsed three times with sterilized double-distilled water (DDW). Twenty-five seeds were placed in sterilized petri dishes (15 mm × 100 mm) with a single sheet of filter paper (90 mm in diameter, Whatman No.1) moistened with the nine ml of appropriate solutions or distilled water for control. As above mentioned MWCNTs solutions were made by dissolving nanoparticles (500, 1000 and 2000 mg) directly in distilled water. In addition, we used 29.7 mg SNP L^{-1}

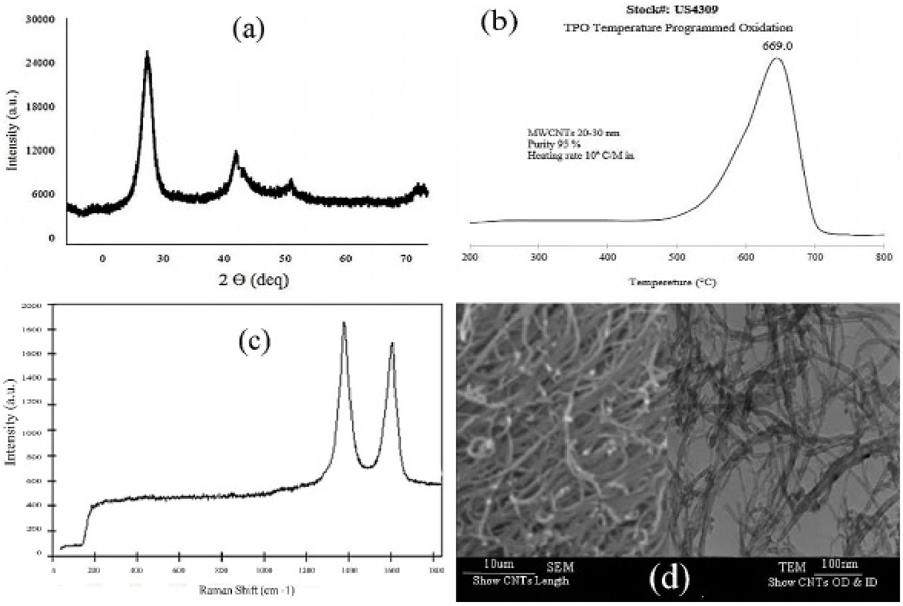

Figure 1. (a) X-ray diffraction pattern of MWCNTs. (b) Temperature programmed oxidation (TPO) pattern of MWCNTs. (c) Raman Spectra of MWCNTs. (d) Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) images of MWCNTs.

for making 100 μM NO suspension. Drought stress was created using polyethylene glycol (PEG 6000), based on equation supplied by Michel and Kaufman (1983) (Eq. 1).

$$\Psi s = -(1.18 \times 10^{-2})\,C - (1.18 \times 10^{-4})\,C^2 + (2.6 \times 10^{-4})\,CT + (8.39 \times 10^{-7})\,C^2T \qquad (1)$$

Where Ψs is the osmotic potential (MPa), C is the PEG concentration (g^{-1} L) and T is room temperature (degrees Celsius).

Salinity stress was made by dissolving 1.46 and 2.96 g of NaCl for 50 and 100 mM salinity levels. Petri dishes were transferred to germinator in dark condition at the 25±1°C temperature.

Determination of germination test and germination indices

Germination tests were performed according to the rules issued by the International Seed Testing Association (ISTA, 2009). Germination was monitored at regular intervals up to 7 days from the beginning of the experiment. Seeds were considered germinated when the radical was emerged to a length of 2 mm. At the end of experiment, the root and shoot lengths were measured. Afterwards, seedling fresh weight (FW) and dry weight (DW; determined by oven drying at 70°C for 24 h) were recorded. The germination indices as well as germination percent (ISTA, 2009) (Eq. 2), germination rate (Maguire, 1982) (Eq. 3), mean germination time (Ellis and Roberts, 1981) (Eq. 4), coefficient of uniformity of germination (El-Katony et al., 2015) (Eq. 5), relative water content (Shalaby et al., 1993) (Eq. 6) and vigor index (Abdul-Baki and Anderson, 1973) (Eq. 7) were calculated using the following equations:

$$\text{Germination percentage (GP)} = (G_f / N) \times 100 \qquad (2)$$

Where, G_f is the total number of germinated seeds at the end of experiment and N is the total number of seeds used in the test.

Germination rate (GR)

$$\text{(GR)} = (a / 1) + (b-a / 2) + (c-b / 3) + \ldots + (n\, n^{-1} / N) \qquad (3)$$

Where, a, b, c and n are numbers of germinated seeds after 1, 2, 3 and N days from the start of imbibition.

$$\text{Mean germination time (MGT)} = \Sigma\, N_i D_i / T \qquad (4)$$

Where, N_i is number of germinated seeds till i^{th} day and D_i is number of days from start of experiment till i^{th} counting and T is total germinated seeds. MGT is a measure of the average length of time required for maximum germination of a seed lot.

Coefficient of uniformity of germination (CUG)

$$\text{(CUG)} = \Sigma\, g_i / \Sigma\, (t - t_i)^2 \times g_i \qquad (5)$$

Where g_i is the number of newly seeds germinated on time t_i from sowing and t is the mean germination time.

$$\text{Relative water content (RWC)} = (F_W - D_W / D_W) \times 100 \qquad (6)$$

Where, F_W is the fresh weight and D_W is the dry weight.

$$\text{Vigor index (VI)} = SL \times GP\% \qquad (7)$$

Where, SL is the seedling length (root length + shoot length) and GP is the germination percentage.

Statistical analysis

Analysis of variance of data was performed based on randomized complete block design (Table 1, 3). The data were analyzed using the SAS 9.3 software. Significant differences among the treatment means were compared by the least significant difference (LSD) test at the 5% level.

Results

Impacts of MWCNTs and SNP under drought stress

According to the results of analysis of variance, drought stress, MWCNTs and SNP treatments were significant for germination percentage, germination rate, root length, shoot length, vigor index, mean germination time, coefficient of uniformity of germination, and relative water contents (Table 1). Most germination indices revealed a gradual decrease in response to increasing PEG concentrations (Table 2). Germination of seeds exposed to -0.3 and -0.6 MPa droughts was delayed and had a reduction in final germination from 16.2 and 26%, respectively, over control (Table 2).

Drought stress significantly reduced GR, RL, SL, VI, RWC and CUG compared with control (Table 2; Figure 2c, e). While, mean germination time (MGT) increased under drought levels (Figure 2a). The MWCNTs had significant effects on germinated seeds grown under stress and non-stress conditions (Table 1). The MWCNTs at 500 mg L^{-1} promoted germination indices in control, also 1000 mg L^{-1} MWCNTs markedly alleviated the adverse effects of drought stress in comparison with the other MWCNTs doses (Table 2 and Figure 2). Drought at -0.3 MPa declined germination percentage and germination rate by 16.2 and 17%, respectively. The MWCNTs (1000 mg L^{-1}) ameliorated reductions for 3 and 4.7%, respectively (Tables 2). Such enhancements were recorded in most other indices. Conversely, MWCNTs at 2000 mg L^{-1} had a deterrent effect on germination, which was evidenced by declining GP, GR, RL, SL, VI, RWC, CUG and delayed MGT at different drought levels (Table 2 and Figure 2). The exogenous SNP (100 μM) alleviated the adverse impacts of drought stress by improving the germination indices. For example, using SNP boosted GP by about 12.8 and 11.5% under -0.3 and -0.6 MPa drought (Table 2). While, the supply of SNP alleviated the deterrent effects of high dose of MWCNTs (2000 mg L^{-1}) and heightened the positive impacts of 500 and 1000 mg L^{-1} MWCNTs. For example, exposure to SNP with MWCNTs (1000 mg L^{-1}) caused stimulated GP, GR, RL, SL and VI by about 10.3, 7.14, 14.6, 11.7 and 20%, respectively, compared with either MWCNTs treatment alone under -0.6 MPa drought (Table 2). As above mentioned, the MGT of barley seeds was increased by rising drought

Table 1. Analysis of variance and coefficient of variations (CV) for germination percentage (GP), germination rate (GR), root length (RL), shoot length (SL), vigor index (VI), mean germination time (MGT), coefficient of uniformity of germination (CUG) and relative water contents (RWC) of barley in response to MWCNTs and SNP under drought stress.

		Probability value (Pr > F)							
Source of variation	df	GP	GR	RL	SL	VI	MGT	RWC	CUG
Replication	2	0.0001	0.0001	0.0001	0.0001	0.0714	0.0001	0.0001	0.0001
Drought (D)	2	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
MWCNTs (M)	3	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SNP (T)	1	0.0001	0.0001	0.0016	0.0007	0.0001	0.0001	0.0041	0.0001
D × M	6	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0022
D × T	2	0.0016	0.0011	0.0385	0.0001	0.0024	0.0001	0.0027	0.0001
M × T	3	0.0001	0.0406	0.0002	0.0001	0.0005	0.0001	0.0001	0.0001
D × M ×T	6	0.0001	0.0058	0.0035	0.0002	0.0005	0.0001	0.0001	0.0001
CV%	-	3.48	6.34	6.72	5.81	10.26	5.63	6.02	5.19

Table 2. Germination percentage, germination rate, root length, shoot length and vigor index of barley seeds in response to MWCNTs and SNP under drought levels.

Treatments			Germination percentage (%)	Germination rate (Seed day^{-1})	Root length (cm)	Shoot length (cm)	Vigor index
Drought (MPa)	MWCNTs (mg L^{-1})	SNP (μM)					
0	0	0	82.00 ± 1.20	2.31 ± 0.12	9.20 ± 0.15	10.22 ± 0.13	1558.10 ± 15.35
		100	85.01 ± 1.50	2.42 ± 0.07	10.00 ± 0.10	10.25 ± 0.22	1605.50 ± 12.18
	500	0	91.00 ± 1.40	2.56 ± 0.20	11.02 ± 0.17	11.20 ± 0.27	1786.08 ± 11.23
		100	98.00 ± 1.08	2.72 ± 0.14	11.20 ± 0.09	12.00 ± 0.17	1976.11 ± 13.20
	1000	0	87.10 ± 2.35	2.50 ± 0.11	11.00 ± 0.11	10.70 ± 0.27	1748.17 ± 15.17
		100	91.00 ± 1.69	2.57 ± 0.15	12.04 ± 0.12	11.53 ± 0.07	1862.00 ± 14.23
	2000	0	70.00 ± 2.50	2.11 ± 0.08	8.00 ± 0.08	8.90 ± 0.24	1244.23 ± 9.98
		100	78.00 ± 3.00	2.30 ± 0.07	9.30 ± 0.67	9.60 ± 0.12	1520.13 ± 13.20
-0.3	0	0	68.70 ± 1.80	1.92 ± 0.19	8.00 ± 0.14	8.00 ± 0.22	1154.21 ± 17.52
		100	78.85 ± 1.23	2.08 ± 0.15	9.90 ± 0.16	9.30 ± 0.24	1482.31 ± 15.68
	500	0	76.90 ± 2.10	1.97 ± 0.17	9.00 ± 0.17	9.60 ± 0.16	1358.50 ± 10.20
		100	81.50 ± 1.45	2.12 ± 0.18	11.10 ± 0.13	10.75 ± 0.22	1520.02 ± 14.00
	1000	0	79.51 ± 1.90	2.20 ± 0.14	10.10 ± 0.12	9.40 ± 0.31	1550.40 ± 15.60
		100	87.50 ± 0.98	2.43 ± 0.10	11.90 ± 0.16	10.40 ± 0.27	1729.10 ± 15.25
	2000	0	59.70 ± 2.00	1.66 ± 0.12	7.80 ± 0.12	7.20 ± 0.12	1000.23 ± 17.63
		100	71.50 ± 1.60	1.88 ± 0.06	9.40 ± 0.09	8.00 ± 0.22	1235.28 ± 13.18
-0.6	0	0	60.61 ± 0.85	1.66 ± 0.11	6.00 ± 0.12	6.39 ± 0.17	802.59 ± 16.21
		100	68.50 ± 0.93	1.80 ± 0.20	7.20 ± 0.12	7.30 ± 0.29	993.71 ± 15.98
	500	0	64.55 ± 1.77	1.67 ± 0.14	7.90 ± 0.16	7.20 ± 0.22	988.25 ± 15.10
		100	70.00 ± 1.60	1.87 ± 0.15	9.10 ± 0.10	8.39 ± 0.30	1221.74 ± 16.20
	1000	0	69.50 ± 1.12	1.95 ± 0.14	9.00 ± 0.13	7.12 ± 0.18	1094.41 ± 17.12
		100	77.50 ± 2.24	2.10 ± 0.18	10.20 ± 0.14	7.97 ± 0.22	1320.11 ± 13.45
	2000	0	52.00 ± 1.75	1.50 ± 0.11	5.00 ± 0.11	5.60 ± 0.17	600.24 ± 14.26
		100	60.70 ± 1.98	1.66 ± 0.20	7.10 ± 0.13	6.40 ± 0.20	840.13 ± 15.01
LSD			4.73	0.115	0.32	0.51	19.81

Means (± SE) within a column are significantly different at $P \leq 0.05$ according to the least significant difference (LSD)

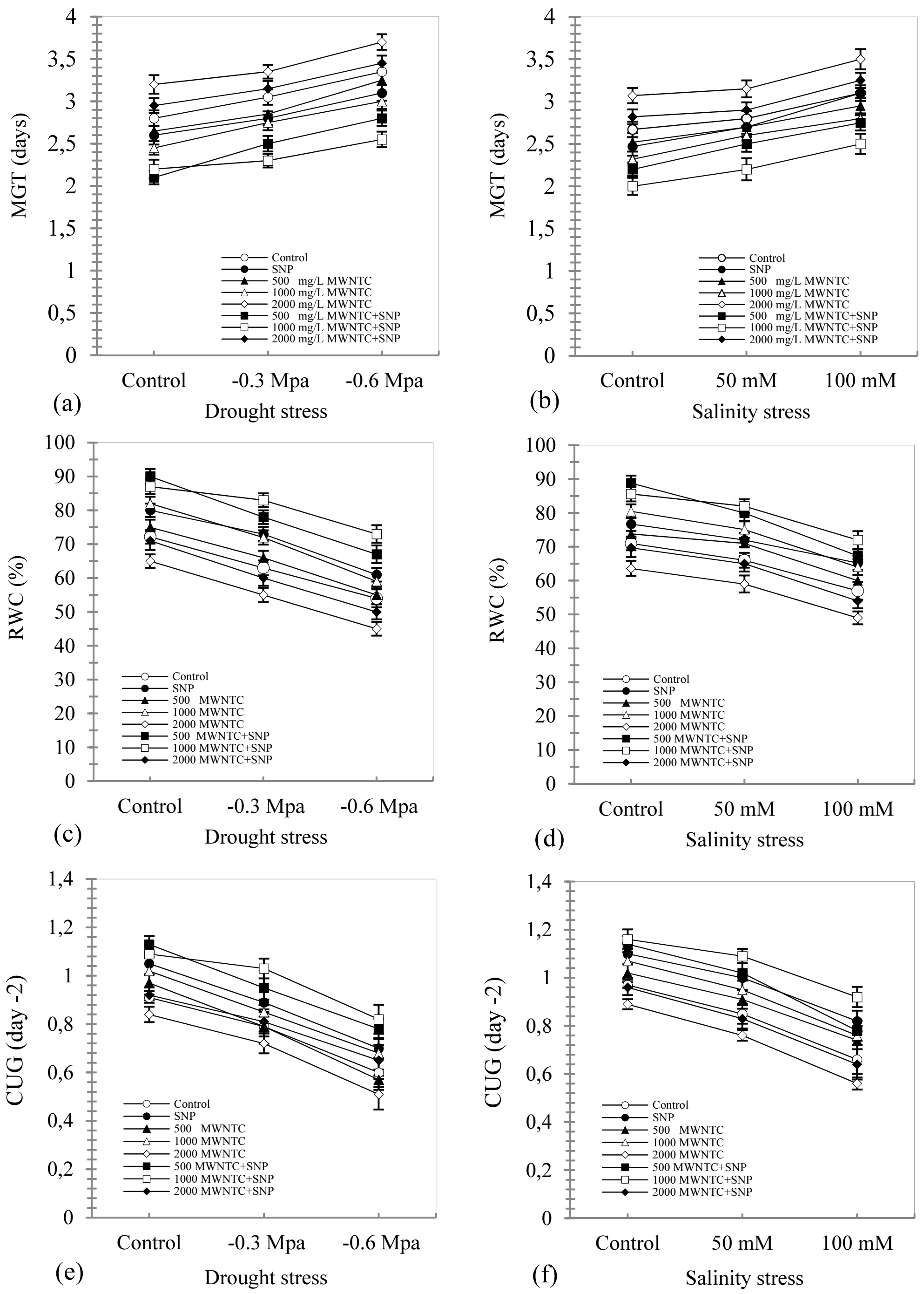

Figure 2. Mean germination time (MGT) under drought (a) and salinity (b) in response to MWCNTs and SNP. Relative water contents (RWC) of barley seedling under drought (b) and salinity (c) in response to MWCNTs and SNP. Coefficient of uniformity of germination (CUG) under drought (e) and salinity (f) in response to MWCNTs and SNP.

severity, while combining application of SNP and MWCNTs reduced MGT under water stress. The lowest MGT with an average of 2.2 and 2.5 days were recorded at MWCNTs (1000 mg L^{-1}) + SNP treatment under -0.3 and -0.6 MPa droughts, respectively (Figure 2a). Such enhancement was recorded in RWC of seedlings that were exposed to SNP + MWCNTs (Figure 2c). The RWC of untreated seeds were 63 and 54% under -0.6 and -0.3 MPa drought levels, while the RWC of treated seeds with the MWCNTs (1000 mg L^{-1}) + SNP was 83 and 74%, respectively (Figure 2c). Also, the supply of SNP with MWCNTs encouraged the CUG as compared with corresponding treatments of MWCNTs. The highest CUG was observed at MWCNTs (1000 and 500 mg L^{-1}) with SNP under -0.3 and -0.6 MPa drought levels (Figure 2e).

Table 3. Analysis of variance and coefficient of variations (CV) for germination percentage (GP), germination rate (GR), root length (RL), shoot length (SL), vigor index (VI), mean germination time (MGT), coefficient of uniformity of germination (CUG) and relative water contents (RWC) of barley in response to MWCNTs and SNP under salinity stress.

		Probability value (Pr > F)							
Source of variation	df	GP	GR	RL	SL	VI	MGT	RWC	CUG
Replication	2	0.0001	0.6665	0.0001	0.0001	0.0001	0.0170	0.0001	0.1541
Salinity (S)	2	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
MWCNTs (M)	3	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SNP (T)	1	0.0001	0.0001	0.0007	0.0001	0.0001	0.0001	0.0205	0.0001
S × M	6	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
S × T	2	0.0179	0.0008	0.0001	0.0077	0.0001	0.0001	0.0010	0.0054
M × T	3	0.0004	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0055
S × M ×T	6	0.0021	0.0472	0.0063	0.0212	0.0001	0.0286	0.0193	0.0090
CV%	-	3.84	4.90	7.03	5.79	6.93	5.68	5.34	7.21

Table 4. Germination percentage, germination rate, root length, shoot length and vigor index of barley seeds in response to MWCNTs and SNP under salinity levels.

Treatments			Germination percentage (%)	Germination rate (Seed day^{-1})	Root length (cm)	Shoot length (cm)	Vigor index
Salinity (mM)	MWCNTs (mg L^{-1})	SNP (μM)					
0	0	0	81.80 ± 2.10	2.61 ± 0.20	9.30 ± 0.18	9.10 ± 0.22	1494.20 ± 13.08
		100	82.00 ± 1.34	2.62 ± 0.11	10.30 ± 0.08	9.75 ± 0.25	1481.21 ± 10.89
	500	0	89.02 ± 2.25	2.77 ± 0.12	11.00 ± 0.11	10.70 ± 0.18	1850.31 ± 11.45
		100	96.00 ± 1.80	2.92 ± 0.18	11.20 ± 0.10	11.50 ± 0.22	1993.20 ± 09.68
	1000	0	88.21 ± 1.20	2.70 ± 0.09	10.80 ± 0.08	10.25 ± 0.13	1798.15 ± 14.00
		100	89.00 ± 1.85	2.78 ± 0.10	11.80 ± 0.15	11.03 ± 0.28	1926.41 ± 11.28
	2000	0	72.00 ± 1.89	2.37 ± 0.08	7.60 ± 0.09	8.25 ± 0.18	1192.00 ± 13.45
		100	76.01 ± 1.40	2.52 ± 0.12	8.79 ± 0.16	8.30 ± 0.31	1227.22 ± 9.69
50	0	0	67.43 ± 2.08	2.32 ± 0.17	8.80 ± 0.13	7.50 ± 0.26	1057.23 ± 14.21
		100	77.21 ± 1.75	2.50 ± 0.18	9.78 ± 0.15	8.70 ± 0.26	1395.08 ± 11.62
	500	0	64.30 ± 2.20	2.27 ± 0.09	9.80 ± 0.14	7.35 ± 0.22	1123.24 ± 14.00
		100	72.34 ± 2.15	2.46 ± 0.10	11.40 ± 0.12	9.35 ± 0.27	1426.32 ± 13.87
	1000	0	73.26 ± 1.95	2.38 ± 0.11	10.90 ± 0.09	8.30 ± 0.16	1325.11 ± 13.74
		100	84.00 ± 2.17	2.60 ± 0.07	11.30 ± 0.11	9.53 ± 0.32	1645.70 ± 11.74
	2000	0	54.20 ± 1.50	2.02 ± 0.19	7.80 ± 0.19	6.60 ± 0.22	802.14 ± 11.45
		100	68.00 ± 1.90	2.25 ± 0.16	9.60 ± 0.20	7.60 ± 0.28	1069.20 ± 13.88
100	0	0	54.00 ± 2.00	1.73 ± 0.11	7.70 ± 0.18	5.80 ± 0.18	719.00 ± 16.10
		100	65.80 ± 1.90	1.99 ± 0.10	9.15 ± 0.16	7.35 ± 0.27	995.12 ± 13.12
	500	0	58.35 ± 1.10	1.82 ± 0.05	9.60 ± 0.09	6.50 ± 0.28	885.24 ± 13.98
		100	61.02 ± 1.34	1.98 ± 0.07	9.70 ± 0.08	7.50 ± 0.24	1045.35 ± 13.05
	1000	0	61.00 ± 2.10	1.87 ± 0.12	8.80 ± 0.14	6.70 ± 0.31	915.27 ± 12.66
		100	68.01 ± 2.25	2.12 ± 0.17	9.80 ± 0.13	8.30 ± 0.22	1135.02 ± 14.48
	2000	0	46.80 ± 1.87	1.57 ± 0.15	6.78 ± 0.11	5.10 ± 0.18	636.14 ± 13.74
		100	55.23 ± 1.85	1.72 ± 0.21	7.81 ± 0.09	5.90 ± 0.17	736.84 ± 15.25
LSD			5.43	0.095	0.41	0.39	21.47

Means (± SE) within a column are significantly different at $P \leq 0.05$ according to the least significant difference (LSD).

Effect of MWCNTs and SNP under salinity stress

According to the analysis of variance of salinity, MWCNTs and SNP as well as their interaction effects were statistically significant for studied parameters (Table 3). There was a progressive rate of inhibition in seed germination with the rise of salinity and different doses of NaCl (50 and 100 mM) which caused deterrent impacts on experiment indices (Table 4). The GP, GR, RL, SL, VI, RWC and CUG were reduced by about 34, 32.9, 17.2, 36.2, 51.8, 19.7 and 31.9%, respectively, under 100 mM salinity. While exposure to different NaCl concentrations increased mean germination time of barley seeds (Table 4 and Figure 2). The germination indices were positively impressed by MWCNTs suspensions under both stress and non-stress conditions. The MWCNTs at both 500 and 1000 mg L^{-1} promoted seed germination, however MWCNTs at 1000 mg L^{-1} was more efficient in comparison to 500 mg L^{-1} (Table 4). The high concentration of MWCNTs (2000 mg L^{-1}) under salinity levels, showed the same behavior as a drought condition. MWCNTs at 2000 mg L^{-1} declined germination indices under both non-stress and stress conditions (Table 4 and Figure 2). Exogenous SNP alleviated the adverse impacts of high dose of MWCNTs and salinity condition on germination indices (Table 4 and Figure 2). In addition, the use of SNP enhanced the positive impacts of MWCNTs at 500 and 1000 mg L^{-1} (Table 4). For instance, treatment by SNP and MWCNTs (1000 mg L^{-1}) solutions in mixture promoted GP, GR, RL and SL by about 10.3, 11.7, 10.2 and 19.27%, respectively, in comparison with the corresponding treatments of MWCNTs under 100 mM salinity (Table 4). The seed vigor index was influenced significantly by MWCNTs and SNP suspensions either solely or in combination compared to untreated seeds. The average vigor index of untreated seeds was 719, while the VI of treated seeds with MWCNTs at 500 and 1000 mg L^{-1} plus SNP were 1045.35 and 1135.02 under 100 mM salinity (Table 4). The MWCNTs treated groups had shorter germination time when SNP was added (Figure 2b). The lowest MGT with an average of 2.1 and 2.6 days were recorded in MWCNTs (1000 mg L^{-1}) + SNP under 50 and 100 mM salinity levels (Figure 2b). Also, the maximum RWC was obtained at 1000 mg L^{-1} of MWCNTs with SNP (Figure 2d). The above mentioned treatments enhanced RWC up to 19.5 and 21%, respectively, under 50 and 100 mM salinity levels (Figure 2d). The CUG was reduced by rising salinity severity, however application of SNP and MWCNTs boosted the CUG of barley seeds. The maximum value of CUG was recorded at combined solutions of MWCNTs (1000 mg L^{-1}) with SNP (Figure 2f).

Discussion

Our study demonstrated that seed germination indices of barley were markedly affected by drought and salinity stresses. The PEG-simulated drought and NaCl induced reduction in germination percentage, germination rate, root and shoot lengths, vigor index, relative water content, coefficient of uniformity of germination and accordingly mean germination time increased. Under drought and salinity, hydration deficit of seeds due to high osmotic potential causes inhibition of the mechanisms leading to the output of the radicle out of the integuments and therefore delay seed germination (Panuccio et al., 2014). This mechanism affects the later stages of growth with a slowdown due to the lack and or unavailability of carbohydrates (Ibrahim, 2016).

Under such conditions, exogenous application of SNP had positive impact on barley seed germination, although it was more efficient under stress condition than the in control. The sodium nitroprusside had similar effects in both drought and salinity conditions and alleviated the adverse effects of mentioned stresses. Generally, NO can protect plants against abiotic stresses by eliminating excessive intracellular reactive oxygen species by boosting antioxidant enzyme activity (Nahar Fancy et al., 2016; Zhang et al., 2016). It was reported previously that, plant treated with 50 μM L^{-1} SNP maintained a high level of RWC and lower content of malondialdehyde (MDA) in *Dendrobium huoshanense* under drought stress (Fan et al., 2012).

In this experiment, most of the indices boosted in seeds treated with different MWCNTs suspensions compared to untreated seeds under both stress and non-stress conditions. The MWCNTs accelerated the process of seed germination and significantly shortened the germination time as compared to the control one. The MWCNTs at 500 mg L^{-1} accelerated the germination and early seedling growth of barley in non-stress condition, while MWCNTs at 1000 mg L^{-1} was more effective under drought and salinity conditions. It is noticeable that the favorable impacts of MWCNTs in drought were more effective than salinity. There are some studies which confirmed that the stress tolerance of plants exposed to nanoparticles was improved by boosting photosynthesis rate, plant water use efficiency and antioxidant activities (Chai et al., 2013; Haghighi and Pessarakli, 2013). Although it remains unclear how CNT can affect seed germination and growth of plants, but the ability of CNT to penetrate the seed coats, activate water channel proteins and increase imbibition rates was pointed out by researches (Lahiani et al., 2013; Ratnikova et al., 2015; Villagarcia et al., 2012). According to the findings reported by Hatami et al. (2017), SWCNTs pretreatment removed the drought-stress block to germination, which could be due to activation of hydrolytic enzyme (α-amylase) in the endosperm; resulting in enhanced germination capacities.

The interaction effects of SNP × MWCNTs × stress demonstrated that most germination indices such as GP, GR and VI were improved by seed exposure to SNP and MWCNTs mixture. In the other word, the supply of SNP heightened the favorable impacts of MWCNTs (500 and 1000 mg L^{-1}) under drought and salinity conditions (Table 1 and 2). It can be concluded temporarily that the use of appropriate dosage of MWCNTs was beneficial in improving the stress tolerance of barley. There are some studies that confirmed that stress tolerance of plants exposed to nanoparticles was improved by boosting photosynthesis rate, plant water use efficiency and antioxidant activities (Chai et al., 2013; Haghighi and Pessarakli, 2013). It was proved that MWCNTs can enhance salinity tolerance of broccoli by affecting the permeability of the root plasma membranes and enhanced aquaporin transduction (Martinez Ballesta et al., 2016). Nanotubes may also affect the water channels in the seed coats (Khodakovskaya et al., 2009). Aquaporins are crucial for root water uptake, seed germination, cell elongation, reproduction and photosynthesis (Maurel, 2007).

Under salinity levels, the combined treatment of MWCNTs and SNP had more effect on shoot length than root length, while such differences were not recorded between root and shoot lengths under drought condition. It can be concluded temporarily that MWCNTs and SNP promote cell elongation of barley seedling. Similarly, the promoting effects of SNP on root and shoot lengths of various plants were reported by several researchers (Sun et al., 2016). It was showed that carbon nanotube (CNTs) can improve the root and shoot growths of various plants such as maize, onion and cucumber (Tiwari et al., 2014). CNTs could improve the root and shoot growths due to increasing cellular metabolism (Flores et al., 2014) and expression of related genes (Wang et al., 2012; Yan et al., 2013).

As above mentioned, the mean germination time of barley seeds was increased by rising drought and salinity severity, while combining application of SNP and MWCNTs reduced MGT under water stress. It seems the MWCNTs treated groups by supply of the SNP had shorter germination time. According to the findings reported by Hatami et al. (2017), the MGT of *Hyoscyamus niger* significantly decreased with applying SWCNTs under drought stress. In other research, Lahiani et al. (2013) showed declined MGT in barley seeds when exposed to MWCNT. It is probable that MWCNTs can accelerate the process of seed germination, germination rate and significantly shorten the germination time. Such an enhancement was recorded in RWC of seedlings which were exposed to SNP + MWCNTs. It is proved previously that the cylindrical shape of carbon nanotubes facilitates water and gas uptake through the testa, and thus facilitate seed germination (Martinez-Ballesta et al., 2016; Yan et al., 2013; Tiwari et al., 2014). Also, the beneficial role of SNP in improving RWC in plants under drought condition was reported by other researchers (Fan et al., 2012). Under current study, the CUG was reduced by rising drought and salinity severity, however application of SNP and MWCNTs boosted the CUG of barley seeds. It seems, uniformity of germination had shown encouraging results using of SNP with MWCNTs. The MWCNTs can enhance CUG of barley due to its role in increasing water uptake through seed coats. Additionally, SNP not only enhanced the CUG of barley seeds, but also intensified the favorable impacts of MWCNTs on CUG. Furthermore, NO is one of these chemical signals and plays a crucial role in stimulating the root system expansion and development (Xu et al., 2017).

According to results, MWCNTs at 2000 mg L^{-1} had a deterrent effect on germination and early seedling growth of barley, which was evidenced by declining GP, GR, RL, SL, VI, RWC, CUG and delayed MGT at different drought levels. The high concentration of MWCNTs (2000 mg L^{-1}) under salinity levels, showed the same behavior as a drought condition. It appears that, high dose of MWCNTs has an inhibitory effect on seed germination and vigor index of barely due to its toxicity. There are some reports that, MWCNTs do not exhibit a positive influence on seed germination in plants, when they received high concentration of CNTs (Andersen et al., 2016; Begum et al., 2014). Begum et al. (2012) reported that in the presence of MWCNTs particularly at 1000 to 2000 mg L^{-1} the growth of red spinach, rice, cucumber and lettuce was inhibited and the root membrane integrity damaged, but chili and soybean displayed no signs of toxicity after exposure to high dosages of MWCNTs. They reported that, the phyto-effect of MWCNTs is species dependent and the induction of ROS was suggested as a main mechanism for MWCNTs phytotoxicity. While, the exogenous use of SNP alleviated toxic and inhibitory impacts of high dose of MWCNTs (2000 mg L^{-1}) on barley germination and early seedling growth. For instance, MWCNTs at 2000 mg L^{-1} declined GP, GR and VI by 11.9, 9.19 and 20.2%, respectively. SNP ameliorated reductions by 7, 3.44 and 18.8%, respectively, under control (Tables 2). In this regard, Chen et al., (2015b) demonstrated that nitric oxide could ameliorate adverse effects of zinc oxide nanoparticles in rice seedlings by scavenging nanoparticle-stimulated ROS. Our results are in harmony with their finding.

Conclusions

The results of our studies showed that drought and salt stresses had deterrent effects on seed germination and early seedling growth of barley. MWCNTS and NO donor had a significant impact on studied traits. The MWCNTs particularly at 500 and 1000 mg L^{-1} promoted seed germination under drought and salinity conditions. It seems that MWCNTs can decrease the inhibition impacts of drought and salinity on seed germination by improving water absorption of seed and increasing moisture content. Application of MWCNTs in drought condition was more effective than salinity stress. On the other hand, high concentrations of MWCNTs (2000 mg L^{-1}) had an inhibitory effect on seed germination and seedling growth of barley. In the current investigation, SNP diminished the adverse impacts of drought and salinity stresses. Application of SNP promoted the favorable impacts of MWCNTs and declined the negative effects of high dose of MWCNTs on seed germination. The relative water content and uniformity of germination as two important germination indices were affected by above mention factors. MWCNTs-treated seeds had higher RWC and germinated more uniformly. Future research is needed to show the physiological modification(s) of the barley seed by MWCNTs in combination with SNP under drought or salinity stresses.

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