doi: 10.17221/15/2016-JFS

Increasing drought resistance of *Alnus subcordata* C.A. Mey. seeds using a nano priming technique with multi-walled carbon nanotubes

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ABSTRACT: To evaluate the effects of nano priming on seed germination of *Alnus subcordata* (Caucasian alder) C.A. von Meyer under drought stress, the present research was conducted using multi-walled carbon nanotubes (MWCNTs; 0, 10, 30, 50, and 100 $mg \cdot l^{-1}$) at 6 levels of drought stress (0, –2, –4, –6, –8, and –10 bar) through a factorial experiment (5 priming levels with 4 replications). After priming, the seeds were placed into a germinator at 21°C. Results revealed that nano priming at the concentration of 100 $mg \cdot l^{-1}$ led to the highest germination rate and percentage at all levels of drought stress. Also, the highest values of seed vigour index and root and stem lengths and dry weights were observed at nano carbon treatment with 30 $mg \cdot l^{-1}$. Considering the obtained results, it was concluded that nano priming could result in boosted resistance of Caucasian alder seeds against drought stress, so that the seed tolerance increased from –4 bar (without nano priming treatment, i.e. reference sample) to –8 bar upon applying 100 $mg \cdot l^{-1}$ nanotubes. Based on the results of the present research, it is suggested that the seed nano priming technique with MWCNTs can be applied in order to increase the seed and seedling tolerance of other members of the genus *Alnus* Miller.

Keywords: polyethylene glycol 6000; seed vigour index; seed coat permeability; drought tolerance; alder tree; nano carbon

Seed germination is the first and most sensitive stage of plant growth (Song et al. 2008) and the onset of a physiological process that needs water absorption (Adhikari et al. 2013). Thus, the percentage of seed germination depends on the permeability of the seed coat and the amount of water available in the germination circumference (Khodakovskaya et al. 2012). In the germination phase, drought stress occurs along with water reduction and seed bed osmotic potential, which decreases germination rate and percentage as well as seedling growth (Kaya et al. 2006). The ability of seed germination under drought stress ensures better plant establishment leading to enhanced plant performance (Baalbaki et al. 1999). One of the efficient techniques for higher seed germination rate and percentage, steadier and faster seedling emergence, and resistance against biological (pests) and environmental (drought, salinity, and low temperature) stresses is seed priming through different materials and solutions (Ashraf, Foolad 2005; Afzal et al. 2008). The commonly used materials in a priming technique include KNO_3, KH_2PO_4, NaCl, mannitol, and polyethylene glycol (Haghighi, Pessarakli 2013). Seed priming with nanomaterials is an efficient and developing technique for increasing seedling vigour and growth performance applied in some plant species (Dehkourdi, Mosavi 2013).

Materials of the particle size < 100 nm in at least one dimension are generally called nanomaterials. The minuscule size and great surface area of nanoparticles allow them to exhibit unique physical, chemical, and biological characteristics used in water purification, wastewater treatment, environmental remediation, food processing and packaging, industrial and household purposes, medicine, and smart sensor development, as compared to bulk materials (Monica, Cremonini 2009; Khot et al. 2012).

From the literature reviewed, application of nanomaterials is a novel and developing technique in agriculture due to its high potential for improving seed germination and growth, plant protection, pathogen detection, and pesticide/herbicide residue detection (Khot et al. 2012; Siddiqui, Al-Whaibi 2014). Response of plants to nanoparticles depends on the plant species, their growth stages, and the nature of nanomaterials (Nair et al. 2010).

Amongst all the nanomaterials presently used, carbon nanotubes (CNTs; single- and multi-walled), due to their specific and unique characteristics, have been extensively considered by the researchers of miscellaneous sciences such as biomedicine, biosensors, and tissue engineering. The given materials have recently proved to be of advantage in plant sciences and various fields of agriculture as well (Tripathi et al. 2011; Wang et al. 2012; Chai et al. 2013). However, the positive effect of low dosages of multi-walled carbon nanotubes (MWCNTs) on germination and root growth has also been confirmed for *Raphanus sativus* Linnaeus, *Brassica napus* Linnaeus, *Lolium perenne* Linnaeus, *Cucumis sativus* Linnaeus, and *Zea mays* Linnaeus (Lin, Xing 2007; Larue et al. 2012). Carbon nanotubes can boost water uptake through increasing the number of new pores existing in the seed coat. There is likewise a possibility that CNTs can facilitate water ingress into the cells through both influencing water pathways in the seed coat and regulating their performance (Khodakovskaya et al. 2009).

According to the findings reported by Siddiqui, Al-Whaibi (2014), MWCNTs (at 5 to 500 $\mu g \cdot ml^{-1}$) could increase the growth rate of tobacco cells by 55 to 64%. Nevertheless, there exists scanty information on the effects of nanomaterials, in particular CNTs, on plant physiology and growth, which calls for immediate attention (Khodakovskaya et al. 2012; Haghighi, da Silva 2014).

The genus *Alnus* (Alder) Miller is the only one amongst the six genera of Betulaceae having the potential of nitrogen fixation through symbiosis with soil filamentous bacteria, namely *Frankia* Brunchorst (Benson, Silvester 1993; Chen et al. 1999; Chen, Li 2004).

The genus *Alnus*, along with its members, is always considered as one of the main elements leading to the spread of temperate forests in the northern hemisphere. Alders in general grow well at riverbanks, swamplands, coastal strips, and the areas where the underground water table is high enough to be accessible for the roots (Claessens et al. 2010).

Alnus subcordata (Caucasian alder) C.A. von Meyer is among the few tree species of this genus reaching to heights of even above 40 m in climax Hyrcanian forests. The mentioned species is observed in an area ranging from the southern coasts of the Caspian sea (as a pioneer species) to elevations of over 2000 m a.s.l. Caucasian alder is deemed as a highly and commercially valuable species exclusively existing in Hyrcanian forests due to its widespread application in timber and furniture industries. Seed germination of the given species only occurs in areas containing a high water supply. To the best of the authors' knowledge, this is the first documented paper dealing with the role of drought stress in seed germination and seedling growth as well as the threshold of Caucasian alder seed germination under drought stress. Considering that only few researches have been conducted on the effects of MWCNTs on seed germination and plant growth (in particular for valuable forest trees) under drought stress, this research was carried out aimed at evaluating the effect of different dosages of MWCNTs on multi-purpose species of Caucasian alder under laboratory drought stress.

MATERIAL AND METHODS

Plant material. The investigation was performed as factorial experiment under a completely randomized design with 4 replications in 2014. The simultaneous effects of two nano priming and drought stress factors were analysed. To evaluate the effect of MWCNTs on Caucasian alder seed germination under drought stress, the seeds of *A. subcordata* were supplied from Koloudeh Seed Research Centre of Forest Trees, Amol, Mazandaran province, Iran. The initial characteristics of the supplied seeds based on ISTA (2009) are presented in Table 1.

Table 1. Initial characteristics of seeds supplied from the Koloudeh Seed Research Centre of Forest Trees, Mazandaran province, Iran

Seed provenance	Geographic coordinates	Altitude (m a.s.l.)	Purity (%)	Moisture content (%)	Weight of 1,000 seeds (g)	Number of pure seeds per kg
Kelardasht	36°28'27''N 51°06'23''E	1,599	97	10.9	2.03	492,492.5

Table 2. Properties of used multi-walled carbon nanotubes (MWCNTs)

Nanostructure	MWCNTs
Manufacturing method	chemical vapour deposition
Purity	> 98 wt% (carbon nanotubes, from TGA and TEM), > 99 wt% (carbon content)
Outside diameter	5–15 nm (from HRTEM, Raman spectroscopy)
Inside diameter	3–5 nm
Length	~ 50 μm
Ash	< 1.5 wt% (TGA)
Electrical conductivity	> 100 $s \cdot cm^{-1}$
Specific surface area	> 233 $m^2 \cdot g^{-1}$ (BET method)
Tap density	0.27 $g \cdot cm^{-3}$
True density	~ 2.1 $g \cdot cm^{-3}$

TGA – thermogravimetric analysis, TEM – transmission electron microscopy, HRTEM – high-resolution transmission electron microscopy, BET method – Brunauer-Emmett-Teller method

Preparation of carbon nanotubes. The multi-walled carbon nanotubes used in this research were supplied from the US Research Nanomaterials Corporation (Houston, Texas, USA). The characteristics of MWCNTs are given in Table 2. A uniform mixture of MWCNTs was prepared after suspending in distilled water, and then sonicating at 40 KHz (100 W) for 30 min. Homogeneous suspensions of MWCNTs at different concentrations of 10, 30, 50, and 100 $mg \cdot l^{-1}$ were prepared.

Fig. 1 also illustrates scanning and transmission electron microscopy images of MWCNTs used in this research.

Seed treatment with carbon nanotubes. Caucasian alder seeds were first sterilized with Benomyl fungicide (2 $g \cdot l^{-1}$; INCOAGRO, Guayaquil, Ecuador) and then rinsed thrice with distilled water. The seeds were immersed (at the above-mentioned concentrations) in CNTs suspensions to be primed and then placed on an orbital shaker for 24 h to prevent from the suspension precipitation in the course of priming. To neutralize the effect of water uptake by seeds, distilled water was also used in the control treatment (0 $mg \cdot l^{-1}$ of CNTs), and the mentioned treatment was stirred along with the other ones. Then, all the primed seeds were rinsed with distilled water for 2 min. Ultimately, the primed seeds were dried at room temperature and in darkness, and when the moisture of the primed seeds reached the level prior to priming, nano priming process was finished (Brancalion et al. 2008).

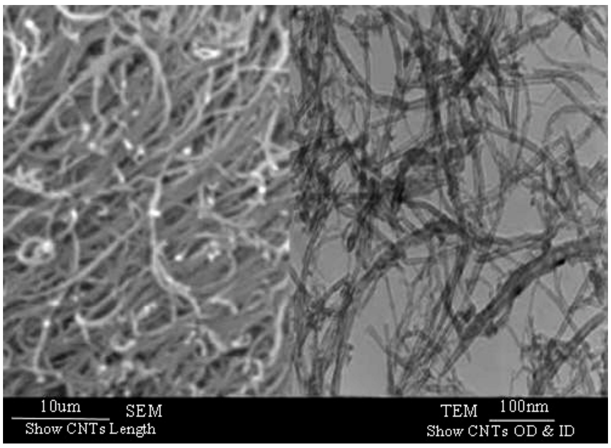

Fig. 1. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) image of the used multi-walled carbon nanotubes (MWCNTs)

OD – outside diameter, ID – inside diameter

Laboratory experiment. Drought stress levels (0, –2, –4, –6, –8, and –10 bar) were set using a polyethylene glycol 6000 solution (Merck KGaA, Darmstadt, Germany). Michel, Kaufmann (1973) equation was used to prepare the given solutions (Eq. 1):

$$\Psi_s = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T \quad (1)$$

where:

Ψ_s – polyethylene glycol 6000 osmotic potential (bar),

C – dosage (g) of polyethylene glycol 6000 in 1 kg distilled water,

T – solution temperature (°C).

All the treated and untreated seeds were placed into 6 cm^2 Petri dishes between two layers of filter paper (25 seeds per treatment with 4 replications). Deionized water was added to each Petri dish as needed. In order to avoid fungal infection in the Petri dishes, Wattman filter papers were replaced every 3 days. 5 ml of the polyethylene glycol solution (at the specified concentration) was added to each Petri dish to set the intended drought stress levels. Then, the Petri dishes were randomly placed into a germinator under constant temperature of 21°C and 65% relative humidity with a photoperiodic regime of 16 h light/8 h dark at fluorescent light of 1,000 lux.

Table 3. Calculation methods for the germination characteristics

Equation	Reference
GP = $(n/N) \times 100$	Kumar et al. (2016)
GR = $\Sigma(ni/ti)$	Kulkarni et al. (2007)
MGT = $\Sigma(ni \times ti)/\Sigma n$	Sharma et al. (2013)
SVI= GP × mean (Sl + Rl)/100	ISTA (2009)

GP – germination percentage, n – number of germinated seeds per day, N – total number of seeds, GR – germination rate, ni – number of germinated seeds between scoring intervals, ti – number of days since the test was started, MGT – mean germination time, SVI – seed vigour index, Sl – shoot length, Rl – root length

Measuring germination characteristics. Counting the number of the germinated seeds began upon observing the first germination and continued until no seed germination was observed in 3 consecutive days. Germination was specified when the emergent radicle reached 2 mm length and monitored daily (Batool et al. 2015). Germination traits were calculated using the equations presented in Table 3.

At the end of the experiment, the root and shoot were separated, and their respective lengths were measured, and then placed into an oven (70°C) for 48 h.

Statistical analysis. All experiments were carried out using factorial experiments in a completely randomized design (general linear model analysis). Data were tested for normality and homogeneity of variances (Kolmogorov-Smirnov and Levene's tests, respectively) before doing the analysis of variance. Then, comparison of mean differences was conducted by Tukey's HSD test at different levels of probability ($P \leq 0.05$ and 0.01). Spearman's correlation test was also used to establish relationships between germination characteristics. All statistical analyses were conducted using the SPSS software (IBM Corporation, New York, USA).

RESULTS

Germination of the treated seeds started 3 days after the test commencement, but as for the seeds devoid of nano-treatment, the experiment was terminated 21 days after the first germination was observed.

The results of two-way analysis of variance revealed that the effects of drought stress and nano priming were statistically significant on all the studied germination traits (Table 4).

The results of means comparison (Fig. 2) indicated that at all levels of nano priming, and likewise control treatment (0 mg·l^{-1} nano carbon), drought stress had a decreasing effect on germination percentage, germination rate, seed vigour index, and root lengths and dry weights. On the other hand, nano priming showed a favourable and significant effect on increasing the seed tolerance to drought stress, so that it enhanced as MWCNTs concentration increased. For instance, the seed resistance against drought stress rose from –4 (without nano priming) to –8 bar (100 mg·l^{-1} nano priming) as nano concentration increased from 0 (control) to 100 mg·l^{-1}.

Growth performance

The means comparison of growth parameters including shoot length, root length, and shoot and root dry weights indicated that 30 mg·l^{-1} (MWCNTs) and control treatments, respectively, possessed the longest and the shortest roots and shoots at all levels (Fig. 2), and likewise, the maximal and minimal root and shoot dry weights were observed in 30 mg·l^{-1} (MWCNTs) and control treatments, respectively, so that 30 mg·l^{-1} nano priming (at –2 and –4 bar drought stress levels) increased shoot length by 200%, compared to that of the control.

Table 4. Two-way analysis of variance for the physiological and seed germination traits of *Alnus subcordata* C.A. von Meyer under nano priming and drought stress treatments (presented values are mean square)

Source of variance	*df*	GP	GR (seed·day^{-1})	MGT (day)	SVI	Root length (mm)	Shoot length (mm)	Root dry weight (mg)	Stem dry weight (mg)
Nano priming	4	1,357.04**	5.71**	35.44**	228.41**	99.89**	194.04**	2.93**	22.39**
Drought	4	19,983.04**	110.03**	84.40**	1,688.24**	361.21**	640.00**	9.86**	62.83**
Nano priming × drought	16	28,240^{ns}	0.92**	50.92**	26.52**	9.83**	20.64**	0.29**	2.49**
Error	70	52,578	0.27	4.00	5.29	1.80	0.81	0.06	0.32
Total	95								

GP – germination percentage, GR – germination rate, MGT – mean germination time, SVI – seed vigour index, **statistically significant at 99% confidence interval, ns – not significant

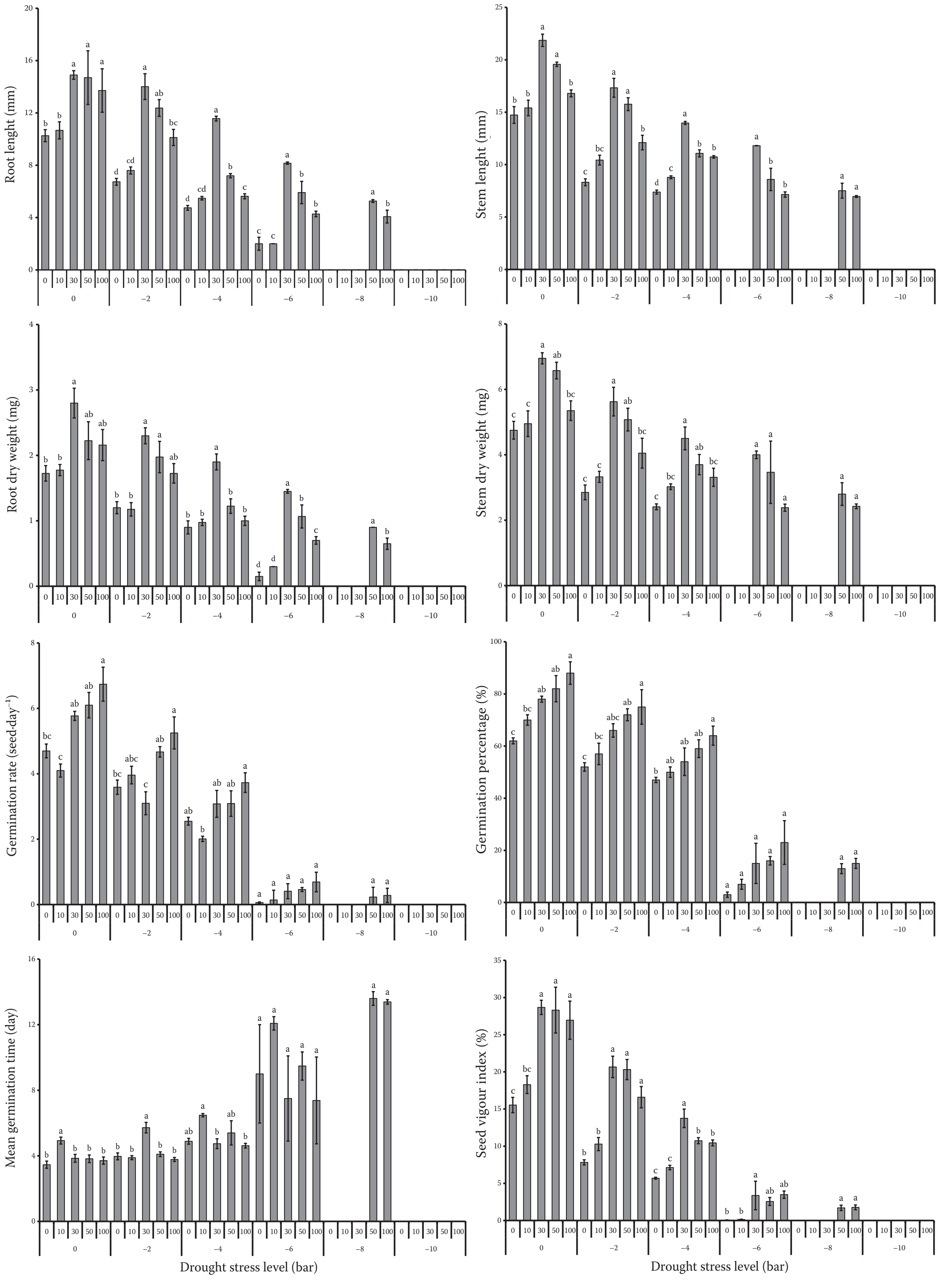

Fig. 2. Effect of nano priming at different concentrations of homogeneous suspensions of multi-walled carbon nanotubes (0, 10, 30, 50, and 100 mg·l^{-1}) on the mean values of *Alnus subcordata* C.A. von Meyer physiological and seed germination traits at varied drought stress levels

Seed vigour index

In terms of the seed vigour index, the highest and the lowest means, at all drought stress levels, pertained to the seeds primed at 30 mg·l^{-1} and the control ones, respectively. As 30 mg·l^{-1} of MWCNTs was applied, the seed vigour index at 0, –2, –4, and –6 bar increased by 84, 164, 141, and 61% over the control, respectively (Fig. 2).

Germination rate

The germination rate of nano primed seeds was higher than that of the unprimed ones (control) (Fig. 2), so that germination rate increased as nano concentration was added. The highest value of germination rate, at all levels of drought stress, was persistently observed upon nano priming at 100 mg·l^{-1}. Furthermore, the efficiency of nano priming on boosting the germination rate was clearly tangible at high levels of drought stress (–6 and –8 bar).

Germination percentage

At all levels of drought stress, the highest and the lowest germination percentages were found at 100 mg·l^{-1} and control treatments, respectively. As 100 mg·l^{-1} of MWCNTs was used, the seed germination percentage at 0, –2, –4, and –6 bar improved by 141, 144, 136, and 766%, respectively, compared to that of the control (Fig. 2). At –8 bar, control seeds as well as those nano primed at 10 and 30 mg·l^{-1} had no germination, whereas 100 and 50 mg·l^{-1} dosages of nano priming showed the germination percentages of 15 and 13%, respectively. On the other hand, germination was terminated in the control as well as in the primed seeds (10 and 30 mg·l^{-1}) at –8 and –10 bar levels. Also, germination was observed in the control and primed (10 mg·l^{-1}) seeds at the stress level of –6 bar, but no shoot formation was detected.

Correlations

The results of Spearman's correlation coefficients between germination traits (Table 5) revealed that germination percentage, germination rate, and seed vigour index showed a significantly positive correlation. Based on the results of this analysis, there were significantly positive correlations of 0.977 and 0.979 between germination percentage and germination rate, and likewise between germination percentage and seed vigour index, respectively. Also, a high correlation (0.948) was observed between germination rate and seed vigour index.

DISCUSSION

Nano priming is a novel method aimed at increasing seedling vigour as well as improving germination percentage and seedling growth (Dehkourdi, Mosavi 2013).

A germination process includes some respective phases, namely physical water absorption, initiation of biochemical processes, and polysaccharide hydrolysis, among which water absorption is known to be the most sensitive. If water absorption is interrupted or conducted slowly, the metabolic activities of seed germination will correspondingly proceed at a slow pace, leading to increased radicle emergence time and reduced germination rate (Bradbeer 1988). The rate of water absorption is dependent on the seed coat permeability and also on the amount of water accessible to the seed (Bradford 1995). Unlike animal cells, the

Table 5. Values of correlation coefficients between germination traits

Root dry weight	Stem dry weight	Root length	Stem length	MGT	SVI	GP	GR	Germination traits
							1	GR
						1	0.977**	GP
					1	0.979**	0.948**	SVI
				1	0.280^{ns}	0.226^{ns}	0.124^{ns}	MGT
			1	0.363^{ns}	0.963**	0.916**	0.878**	stem length
		1	0.948**	0.471^{ns}	0.955**	0.900**	0.875**	root length
	1	0.985**	0.992**	0.445^{ns}	0.944**	0.895**	0.858**	stem dry weight
1	0.982**	0.997**	0.981**	0.502^{ns}	0.957**	0.900**	0.867**	root dry weight

MGT – mean germination time, SVI – seed vigour index, GP – germination percentage, GR – germination rate, **statistically significant at 99% confidence interval, ns – not significant ingress of external entities into plant cells is formidable thanks to their cell walls. The potential of nanoparticles to enter plant cells has been recently demonstrated through some researches (Torney et al. 2007). It is demonstrated that nanoparticles are much smaller than the pore diameter of the cell walls. Thus, they can go through the cell walls (Nair et al. 2010), and in turn, lead to facilitated water permeation and gas exchange, thereby improving the germination rate (Khodakovskaya et al. 2009).

The results obtained from this research confirm the efficiency of MWCNT priming on improving germination traits and increasing the resistance of Caucasian alder seeds against drought stress. As observed in Fig. 2, germination percentage increased as drought stress was reduced. Nevertheless, germination percentage in all the nano primed seeds was higher than that of the control (at all drought stress levels). The highest and the lowest germination percentages at all drought stress levels pertained to nano carbon treatment with 100 mg·l^{-1} and control sample, respectively, so that at 100 mg·l^{-1} nano priming, the stress of –6 bar increased germination from 3 (in control sample) to 23% (766%), and the stress of –8 bar from 0 (in control sample) to 15%. Also, the highest germination rate, at all drought stress levels, belonged to nano priming with concentrations of 100, 50, 30, and 10 mg·l^{-1}, respectively. In principle, as the concentration of CNTs increased, an upward trend was observed in germination rate and likewise percentage (Fig. 2). To note that at high levels of drought stress (–6 and –8 bar), nano priming had a marked effect on the above-mentioned traits. The potential of CNTs to penetrate into the seed coat was reported by Khodakovskaya et al. (2009). They suggested that MWCNTs could improve both growth and germination rates through penetration into the thick seed coat and increase water absorption, thus influencing the biological activities of seeds. The mechanism by which CNTs can maintain the flow of water absorption into the cells is still unknown. Carbon nanotubes can boost water uptake through increasing the number of new pores existing in the seed coat. There also exists a possibility that CNTs can lead to enhanced water permeation into the cells through both influencing water pathways present in the seed coat and regulating their performance (Khodakovskaya et al. 2009). Haghighi and da Silva (2014) stated that the cylindrical shape of CNTs could facilitate water and gas absorption and ease seedling germination and growth processes. It has also been reported in archival publications that CNTs hold the potential to act as nano-transporters and pass through the cell wall (Samaj et al. 2004; Liu et al. 2009).

One of the reasons behind reduced root and shoot lengths under drought stress is attributed to a decrease in and/or termination of nutritive element transfer from seed storage tissues to embryo. In general, the germinated seeds subjected to drought stress possessed shorter root and shoot lengths (Katerji et al. 1994).

The highest mean values of root and shoot lengths and dry weights were pertaining to 30 mg·l^{-1} nanocarbon treatment at all drought stress levels. The control treatment showed the minimal root and shoot lengths and dry weights. At the stress level of –6 bar, the control and primed seeds (10 mg·l^{-1}) germinated, and seedling growth was halted by the emergence of the radicle (2 mm). Similar to the other measured characteristics, the seed vigour index was subjected to CNTs. The highest seed vigour index (SVI) values (at the stress of 0, –2, and –4 bar) corresponded to the treatment with 30 mg·l^{-1}, which decreased at higher concentrations. Yet, the minimal SVI value was still observed in the control treatment. Alder seed priming at 30, 50, and 100 mg·l^{-1} brought about a considerable improvement in SVI, as compared to that of the control.

Researchers demonstrated that MWCNTs, at concentrations ranging between 50 to 500 mg·ml^{-1}, could enhance the growth rate of tobacco cells by 55 to 64%, whereas activated carbon (AC; 5 mg·ml^{-1}) increased the cell growth only by 16%, and higher concentrations of activated carbon (100 to 500 mg·ml^{-1}) led to the dramatically reduced cell growth. From the published literature, the expression of the tobacco aquaporin (NtPIP1) gene, and also production of the NtPIP1 protein, considerably increased in cells subjected to MWCNTs, as compared to the control cells or to those subjected to AC. The expression of marker genes for cell division (CycB) and cell wall extension (NtLRX1) was also upregulated in cells subjected to MWCNTs compared to the control counterparts or those that were exclusively subjected to activated carbon (Khodakovskaya et al. 2012).

The NtLRX1 gene plays a key role in reinforcing cell walls during plant growth and in a response to external signals (Tiré et al. 1994; Merouropoulos et al. 1999). Several reports have indicated that aquaporins are the main components of the plant-water relationship playing a crucial role in water absorption, seed germination, cell elongation, regeneration, and photosynthesis (Kaldenholff, Fischer 2006; Maurel 2007). It was also reported that an increase in the aquaporin gene expression of rice embryo could improve seed germination under water stress conditions (Liu et al. 2007).

Wang et al. (2012) studied the effect of MWCNTs (0, 10, 20, 40, 80, and 160 μg·ml^{-1}) on the physiology and germination of *Triticum aestivum* Linnaeus plants. The obtained results showed that after 7 days of subjecting the seeds to a nanotube culture medium, root growth quickened, and the amount of biomass increased, but no significant difference was reported for the given seed germination rate and shoot length, compared to those of the control. Scanning electron microscopy images showed that MWCNTs could ingress the cell wall and in turn the cytoplasm after absorption by roots. They found out that MWCNTs had the potential to considerably boost root dehydrogenase activity, which in turn led to improved water uptake ability by seedlings. Dehydrogenase activity is a comprehensive evaluation index reflecting the level of metabolic activity and root potential to absorb nutritive elements and water (Taniguchi et al. 2008). The favourable effect of MWCNTs on germination and root growth of plants such as *R. sativus*, *B. napus*, *L. perenne*, *C. sativus*, and *Z. mays* has also been confirmed (Lin, Xing 2007; Larue et al. 2012). Carbon nanotubes hold varied properties and effects on plant biology, for instance Tripathi et al. (2011) revealed that CNTs could improve growth rate in all the organs of *Cicer arietinum* Linnaeus including roots and shoots, and Cañas et al. (2008) reported that single-walled CNTs impeded root elongation in tomato, *Brassica oleracea* Linnaeus, *Daucus carota* Linnaeus, and *Lactuca sativa* Linnaeus during 24 to 48 h, but reduced root length in *C. sativus*. The obtained results demonstrated that the effects of CNTs varied depending on the plant species. Yuan et al. (2011) suggested that single-walled CNTs could stimulate the growth of *Arabidopsis* Heynhold plant mesophyll cells at the nano priming concentration of 50 μg·ml^{-1}. Chai et al. (2013) also stated that the application of CNTs (800 to 2,400 mg·kg^{-1}) reduced the stress resulting from Cd and allowed the recovery of shoot growth, plant height, and water content to their normal level.

CONCLUSIONS

The results obtained from the present research indicated the positive effects of priming with MWCNTs on the improvement of Caucasian alder traits in terms of physiology, germination and tolerance under drought stress conditions. The maximal germination rates (at all drought stress levels) were relative to 50 and 100 mg·l^{-1} concentrations, respectively. The seeds nano primed at 30, 50, and 100 mg·l^{-1} exhibited a marked increase in the seed vigour index, as compared to the control. The root and shoot growth of seedlings was terminated (at the stress level of –8 bar) in all the treatments, except for nanocarbon priming at 100 and 50 mg·l^{-1}. That is, Caucasian alder could germinate at the drought stress level of up to –6 bar without priming treatment, but the growth of control treatment was completely halted at the given level, whereas the seeds primed with CNTs could not only germinate but also grow at the drought stress level of –8 bar.

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Received for publication February 12, 2016
Accepted after corrections May 23, 2016

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