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Graphene oxide: a promising material for the germination of melon seeds under salinity stress

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Abstract: It is well-known that salinity is an important adverse environmental factor (abiotic stresses) that limits plant growth, development, and productivity all over the world. Herein, we report for the first time the use of graphene oxide (GO) as a promising material against the salinity stress using melon (*Cucumis melo* L.) seeds as the model crop. GO as a priming material possesses various advantages such as being highly water-soluble, nontoxic, producible on a large scale from graphite, and low-cost over the existing materials. In this study, we identified the effect of different GO doses (0, 0.25, 0.50, 0.75, and 1.0 mg/mL) on seed germination of melon (*Cucumis melo* L. cvs. 'Hasanbey', 'Kırkağaç-589', and 'Kırkağaç-637') seeds under the salinity stress. The effect of GO as a priming material showed variance depending on the GO dose, melon cultivars, and level of salinity stress. It was determined that the germination percentage and the length of the radicle were higher in GO primed seeds compared to the control in all the melon cultivars tested. All cultivars did not germinate in the control at the highest salinity stress (26.20 dS m^{-1}). The germination percentage of the GO-treated seeds changed in the range of 12.25% (0.50 mg mL^{-1} GO) –16.63% (0.25 mg mL^{-1} GO) at the highest salinity stress in 'Kırkağaç-589'. Radicle lengths of 'Hasanbey', 'Kırkağaç-589', and 'Kırkağaç-637' varied from 0.47, 0.57, 1.67 cm to 7.80, 9.47, and 8.80 cm, respectively, as the salinity stress elevated to 15 g L^{-1} (0 to 26.20 dS m^{-1}). We believe that this study will pave the way for the application of GO as a priming material for both melon and different crops for eliminating problems of salinity stress.

Key words: *Cucumis melo* L., graphene oxide, priming, germination, melon, abiotic stress

1. Introduction

Melon (*Cucumis melo* L.), also called muskmelon or cantaloupe, belongs to the family Cucurbitaceae. It is one of the highly produced vegetable grown almost all over the world. World production of melons is about 27 million metric tonnes and the major melon producer countries are Turkey, China, and Iran, respectively (Welbaum, 2015; Sharma et al., 2017). Its primary gene center is Iran (Decoteau, 2000). Although a rich genetic diversity of these species is found in Anatolia, no wild types, or forms of genera, such as *Cucumis*, *Cucurbita*, *Citrullus* and *Lagenaria* have been found in Turkey. However, some researchers reported that the origin of some cucurbits such as melons grow in Ukraine and Russia is Anatolia while European cantaloupes originate from the Van area and eastern Anatolia (Balkaya and Karaağaç, 2006). It was also reported that Anatolia is the secondary center of the genetic diversity of melon (Pitrat et al., 1999). Melon seeds are sown directly in different cultivation areas/regions of Turkey. In order to grow not only melon but also other horticultural crops at the desirable rate, fast and uniform seedling output must be provided. However, the high salinity rate of the soil and high or low temperatures in the region negatively affect the growth of melon at desired yield due to their high sensitivity to the soil salinity and acidity (Mavi and Demir, 2007; Welbaum, 2015).

It is well known that salinity is an important adverse environmental factor, including abiotic stress that limits plant growth, development, and productivity. The biology of salinity stress and different levels of the seed responses have been investigated by many researchers until today (Ebrahimi and Eslami, 2011). These studies revealed that almost every aspect of plant physiology and biochemistry is influenced by salinity stress (Cuartero et al., 2005). For example, the salinity stress has an adverse effect on the seed germination performance and seedling development because the salts influence the functions of cell walls and cell membranes by affecting the water potential of cellular extensibility and cytosol (Tingle and Chandler, 2003; Tobe et al., 2004; Sohrabikertabad et al., 2013). In this regard, a proper solution that can be applied in all geographic origin

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must be developed for the salinity stress considering the fact that the rapid elevation of the world population.

Seed priming is a presowing process leading to a physiological condition that allows the seeds to germinate at a higher rate and enable them to germinate faster and simultaneously (Sivritepe and Dourado, 1995). Additionally, the seed priming allows the seeds to run through the first reversible phase of the germination. However, this process is based on seed imbibition which does not allow radicle protrusion through the seed coat (Lutts et al., 2016). In other words, seed priming covers various applications for the seed before planting to minimize the problems which may occur during the germination and emergence processes, to ensure uniform seedling emergence and strong seedling development in a short time, and to increase resistance to stress conditions. Therefore, researchers have utilized various seed priming materials including PEG, KNO_3, KCl, K_3PO_4, KH_2PO_4, $MgSO_4$, $CaCl_2$, mannitol, and plant growth-promoting rhizobacteria to ensure faster and better seedling development under different abiotic stress conditions (Demir and Mavi 2004; Farooq et al., 2005; Sivritepe et al., 2005; Kaymak et al., 2009; Sohrabikertabad et al., 2013). On the other hand, these priming materials and agents are either not efficient or cost-effective for practical usage considering the large-scale production of crops, which indicates the requirement of new priming materials.

Nanomaterials have created new advanced technologies that had a high impact in various fields including medicine, environmental science, energy, and agriculture, and become more important every day, as they help reduce environmentally harmful substances (Nel et al., 2006, Kah et al., 2014; Meral and Metin, 2014; Huang et al., 2015; Erdogan et al., 2016; Guo et al., 2018; Xie et al., 2019, Altan and Metin, 2021). When comparing to the utilization of nanomaterials in other fields, their usage in agriculture is an emerging field thanks to their effectiveness in plant protection and production. Many studies have recently appeared in the literature revealing the potential of nanomaterials in improving seed germination/growth, plant protection, the detection of the pathogen, and pesticide/herbicide residue (Khot et al., 2012; Peters et al., 2016). The beneficial effects of nanomaterials on the agricultural inputs such as fertilizers, pesticides, and plant hormones can increase their wider use in agriculture (Ghidan et al. 2017). In addition, nanomaterials could decrease the number of hazardous chemicals that have negative effects on the environment (Huang et al., 2015). In previous studies, the effects of nanomaterials on different crops such as tomato, corn, and cucumber were investigated comprehensively (Lopez-Moreno et al., 2010; Raliya et al., 2015). It was pointed out that nanomaterials had a positive effect on not only the seed germination ratio, root performance, and leaf number but also the chlorophyll content and enzyme activity (Siddiqi and Husen, 2017). An exciting report was published on the use of different nanomaterials in agriculture (Zhang et al., 2015). In this report, Zheng et al. (2005) stated that the use of nano-TiO_2 at appropriate doses increases the germination and the viability of aged seeds of spinach. El-Temsah and Joner (2012) also reported that the low concentrations of silver nanoparticles and different concentrations of zero-valent iron nanoparticles inhibit the seed germination of rye, barley, and flax. In another study by Lin and Xing (2007), who investigated the phytotoxicity of multiwalled carbon nanotubes, aluminum, alumina, zinc, and zinc oxide nanoparticles on seed germination and root growth in radish, rape, rye, lettuce, corn, and cucumber. According to their results, only Zn and ZnO particles had phytotoxic effects on seed germination and root growth.

Graphene oxide (GO), which is the oxidized derivative of graphene possessing high-density of oxygenated functional groups (carboxylic acid, hydroxyl, carbonyl, and epoxy), is an emerging nanomaterial and it has been widely studied intensively in different fields in recent years (Dreyer et al., 2010; Xing et al., 2018). GO has received great attention in many daily life applications due to its excellent solubility in water (1 mg/mL), scalability, and low-cost production from graphite (Wang et al., 2011; Chong et al., 2014; Perreault et al., 2015). Moreover, it is a versatile material for multivalent functionalization (Liu et al., 2013) and efficient ion loading (Wan et al., 2016) owing to its oxygenated functional groups. The superior properties of carbon nanomaterials such as graphene, fullerenes, and carbon nanotubes take an unrivalled place in agricultural practices due to their ability to influence seed germination, seedling and plant development of various crops (Zhang et al. 2015). For instance, carbon nanotubes increased seed germination in tomatoes (Khodakovskaya et al., 2009). Likewise, Zhang et al. (2015) reported that graphene had a positive effect on the germination of tomato seeds by facilitating water intake, while it negatively affected the seedling biomass accumulation. Similarly, Nair et al. (2012) indicated that graphene-treated rice seedlings had better root and shoot systems than nontreated groups. To the best of our knowledge, although the effects and mechanisms of many carbon-based nanomaterials on seed germination of different crops have been examined, the effects of GO on seed germination under stress conditions such as salinity stress have not been evaluated yet. We have foreseen that GO has a high potential to promote the seed germination under salinity stress conditions owing to its high solubility in water, two-dimensional layered structure, multifunctionalization property, and high ion loading capacity that eliminates the adverse effects of alkaline ions (Na^+, K^+, Mg^{2+}, and Ca^{2+}) via removing them out of the seed during the germination. Therefore, the present study

was undertaken to determine the effect of graphene oxide on seed germination of melon under salinity stress.

2. Materials and methods

2.1. Materials

Potassium permanganate ($KMnO_4$), hydrogenperoxide (H_2O_2, 30%), sodium nitrate ($NaNO_3$, 99%), sulfuric acid (H_2SO_4, 98%), potassium peroxodisulfate ($K_2S_2O_8$, 99%), and phosphorus pentoxide (P_2O_5, 99%) were purchased from Sigma-Aldrich, Merck. Natural graphite flakes were purchased from Alfa-Aesar.

2.2. Instrumentation

All transmission electron microscope (TEM) images were recorded on a FEI Technai G2 Spirit BiO (TWIN) at 120 kV. The scanning electron microscopy (SEM) images were obtained on a QUANTA FEG 250 SEM instrument. Fourier transform infrared spectroscopy (FTIR) analysis was performed on a Bruker VERTEX 70v instrument. Raman spectrum was recorded on a WITec alpha 300R instrument in the range of 350–1050 nm at room temperature.

2.3. Synthesis of graphene oxide (GO)

GO was synthesized according to the well-established two step procedure, which was described in our earlier studies (Meral and Metin, 2014; Metin et al., 2014). In the first step, the graphite flakes were preoxidized by dispersing 20 g of natural graphite flakes in 50 mL of H_2SO_4 gently. Next, 10 g of $K_2S_2O_8$ and 10 g of P_2O_5 were added into the mixture (Caution: Add P_2O_5 gently because a highly exothermic reaction has occurred) and then the temperature of the mixture was kept at 80 °C for 6 h. Next, the mixture was cooled down to room temperature and 300 mL of distilled water was added to the mixture. After filtering the resultant mixture in vacuum, bright gray preoxidized graphite was yielded.

In the second step of the GO synthesis, preoxidized graphite was converted into graphene oxide via the oxidation reaction. Firstly, 1 g of preoxidized graphite was taken into 250 mL of a flask and dispersed in 50 mL of H_2SO_4 using a magnetic stirrer. Next, 1 g of $NaNO_3$ was added into the dispersion cooled down to 4 °C in an ice bath. After obtaining a homogeneous mixture, 6 g of $KMnO_4$ was added very slowly in the ice bath and the temperature was kept at 3–5 °C. The obtained mixture was stirred in the ice bath for half an hour more. Next, the ice-bath was removed and the temperature of the mixture was gradually adjusted to 35 °C and mixed at this temperature for 3 h. Then, 100 mL of distilled water followed by 8 mL of 30% H_2O_2 were added dropwise into the mixture. The brown mixture turns yellow after the adding of H_2O_2. Following stirring for a while, the resultant mixture was filtered and washed four times with 50 mL of water. The material on the filter paper was carefully dried at 80 °C to obtain graphene oxide (GO). The schematic representation for the synthesis of GO from preoxidized graphite is shown in Figure 1.

2.4. Germination experiments

This study was carried out in growth chambers (BINDER, D-78532, Tuttlingen, Germany) between August 2016 and January 2017, to determine the effect of GO on seed germination of melon seeds under salinity stress. Three melon (*Cucumis melo* L.) cultivars (cvs. 'Hasanbey', 'Kırkağaç-589', and 'Kırkağaç-637') and five GO doses (0, 0.25, 0.50, 0.75, and 1.0 mg mL^{-1}) were used in experiments, and the melon cultivar seeds were supplied by vegetable seed companies (ASGEN and POLTAR Vegetable Seed Corporation, Turkey).

For surface sterilization, seeds of melon cultivars, shaken in 5% sodium hypochlorite solution for 2 min, were immersed in GO solutions and primed in a rotary shaker at 150 rpm in the dark and at room temperature for 24 h. After priming with GO, the seeds were dried at room temperature for 24 h in the laboratory before use. The standard germination test was conducted using the between-paper method with four replicates of 50 seeds from each cultivar in a growth chamber (BINDER, D-78532, Tuttlingen, Germany) where the temperature was 20 °C for a period of 8 days in the dark (ISTA, 2011).

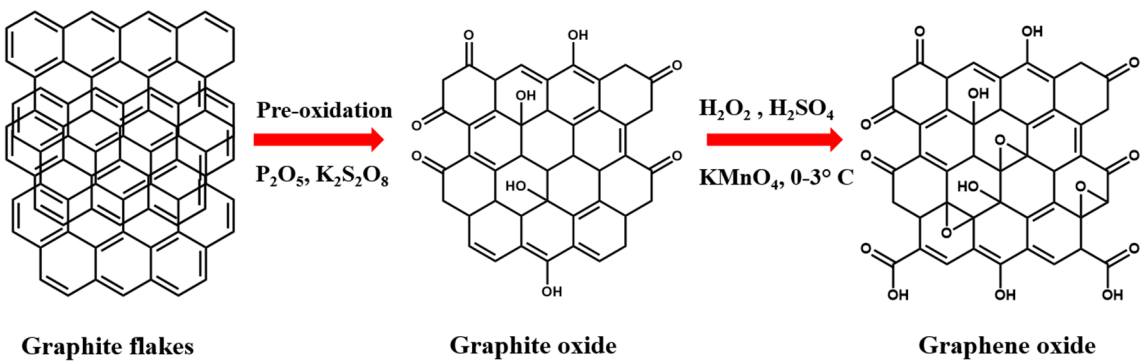

Figure 1. Schematic representation of the synthesis of GO.

To investigate the effect of salt stress and to create salinity stress, the melon seeds were moistened daily with five NaCl solutions prepared with sterile distilled water by adding 0 (control), 5, 10, and 15 g L^{-1} of NaCl (0, 9.45, 18.29, and 26.20 dS m^{-1}, respectively). Seeds only moistened with sterile distilled water and not treated with GO were considered controls.

Visible radicle emerged seeds were accepted germinated (Kaymak et al., 2009). The first count was made on the 4th day of the standard germination test (ISTA, 2011). Germinated seeds were counted at 24-h intervals for 8 days (ISTA, 2011) and the results were recorded as the final germination percentage (%). In addition, radicle length was measured by using a ruler at the end of the germination test and presented as cm.

Experiments were conducted in completely randomized complete block design, with four replicates. The data analysis was done according to the analysis of variance (ANOVA) statistical model and the means were obtained according to the methods described in our previous published study (Kaymak et al., 2009).

3. Results

3.1. Characterization of graphene oxide (GO)

GO was synthesized according to the modified Hummer's method (Kovtyukhova et al., 1999). The morphology of GO was examined by using a scanning electron microscope (SEM) and transmission electron microscope (TEM). Figures 2A and 2B show the SEM images of GO recorded at different magnifications. From the SEM images, it can be concluded that GO has a layered and sheet-like structure folded or continuous at times and including wrinkled areas. Figures 2C and 2D show the TEM images of GO from which two-dimensional (2D), thin-layered graphene oxide flakes are seen clearly. These results, being consistent with the related literature, revealed that thin-layered, 2D GO nanosheets were successfully synthesized by the presented method.

Raman and FTIR spectroscopies, which are indispensable techniques for the characterization of carbon-based materials, were studied to get more insights about the structure of as-synthesized GO. The Raman spectrum of GO (Figure 3A) shows the typical first-order D and G bands at 1359 and 1603 cm^{-1}, respectively (Ferrari et al., 2006). The G band is associated with bond stretching of sp^2 carbon atoms while the D band is attributed to the breathing mode of aromatic rings arising from the structural defects or partially disordered graphitic domains (Tuinstra and Koenig, 1970). The ratio of the intensities of D and G bands (I_D/I_G) is generally used to interpret the structural disorder in GO, where the higher the ratio, the higher the disorderliness in the structure

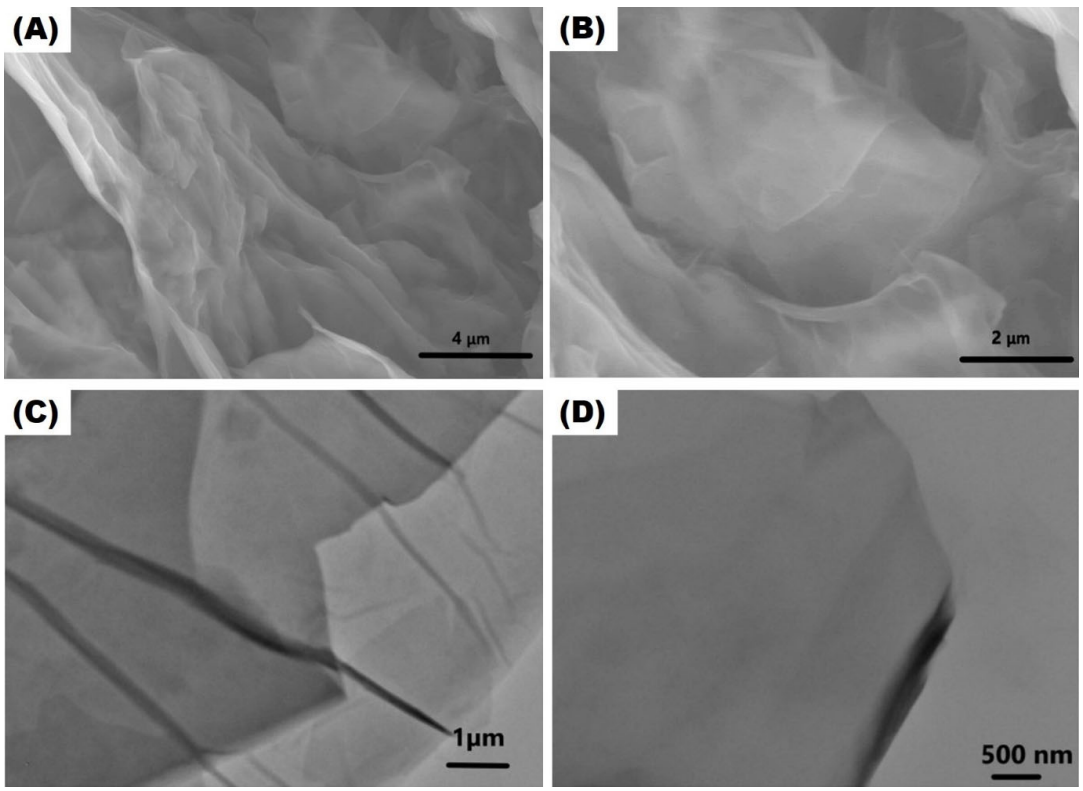

Figure 2. (A, B) SEM and (C,D) TEM images of GO at different magnifications.

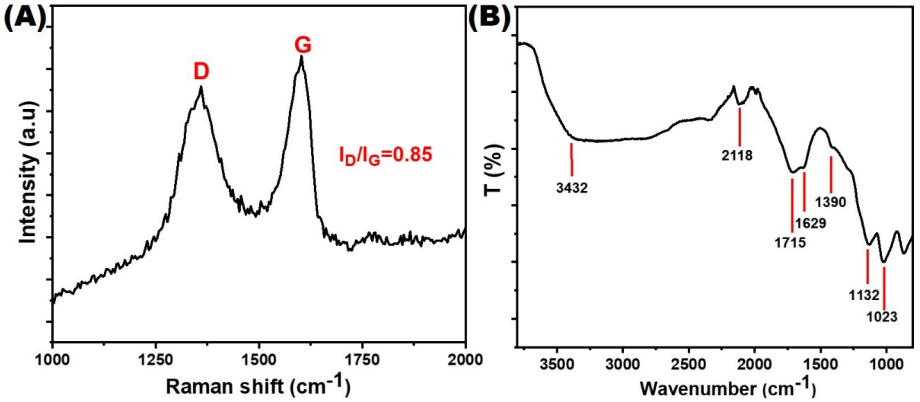

Figure 3. (A) Raman and (B) FT-IR spectrum of as-synthesized GO nanosheets.

(Ferrari and Robertson, 2000). Considering the calculated I_D/I_G ratio of 0.85 for the GO nanosheets presented herein, it can be concluded that they possess significant structural disorders due to the presence of oxide functional groups. The presence of the oxygenated functional groups can be further confirmed by the FTIR spectrum of the GO nanosheets (Figure 3B). The GO nanosheets showed sharp adsorption bands at 3482 cm^{-1} for the hydroxyl O-H, 1715 cm^{-1} for the carbonyl C=O, 1629 cm^{-1} for the aromatic C=C, 1132 and 1390 cm^{-1} for the epoxy C–O, and 1023 cm^{-1} for the alkoxy C–O stretchings (Cao and Zang, 2015). These results clearly indicate that graphite flakes were successfully oxidized to GO.

3.2. Seed germination experiments

The first clear observation of the current study was that the germination percentage of melon seeds decreased by increasing NaCl concentrations in both first and the last count (Tables 1 and 2). For example, increasing NaCl concentration from 0 to 5 g L^{-1} (0 to 9.45 dS m^{-1}) did not affect the germination percentage, while increasing NaCl concentration from 5 to 15 g L^{-1} (9.45 to 26.20 dS m^{-1}) decreased the germination percentage of 'Kırkağaç-589' compared to 'Hasanbey' and 'Kırkağaç-637' cultivars ($p < 0.05$) (Tables 1 and 2). However, comparing GO doses were compared with each other and it was determined that the effect of priming with GO on seed germination under salinity stress varied by the GO doses in both first and last count (Tables 1 and 2). The germination percentage of melon cultivars was higher in primed seeds with the GO than the controls in all treatments. Although the effect of GO priming on seed germination was statistically insignificant in the control group, the germination percentage increased in all GO doses. On the other hand, the effect of priming with GO was clearly observed due to the increase in the salinity stress, the germination percentage in seeds primed with GO was higher than that of control in all GO applications. In addition, it was observed that the effective dose of GO differed according to the cultivars. The highest germination percentage of 'Hasanbey', 'Kırkağaç-589', and 'Kırkağaç-637' at 10 g L^{-1} NaCl was determined as 0.75 mg mL^{-1}, 0.50 mg mL^{-1}, and 0.25 mg mL^{-1} GO, respectively. The seeds of melon cultivars did not germinate under the highest salinity stress (15 g L^{-1} NaCl) in the control. However, GO increased germination percentage significantly ($p < 0.01$) at 15 g L^{-1} NaCl (Table 2). Although there was no germination in the 'Kırkağaç-637' at the highest salinity stress (15 g l^{-1} NaCl), different rates of germination were detected in GO applications in the other two cultivars ('Hasanbey' and 'Kırkağaç-589'). The highest germination percentage of 'Hasanbey' and 'Kırkağaç-589' at the highest salinity stress was determined in 1.00 mg mL^{-1}, and 0.25 mg mL^{-1} GO, respectively (Table 2).

The salinity stress tolerance of different melon cultivars was found significantly different. When considering the mean germination percentage, 'Hasanbey' (57.90%) was found to be more tolerant to the NaCl stress than 'Kırkağaç-637' (47.03%), and 'Kırkağaç-589' (42.93%) (Table 2). Furthermore, the highest germination percentage (17.13%) was determined in the 'Hasanbey' cultivar which priming with 1.0 mg L^{-1} GO at 15 g L^{-1} NaCl, whereas there was no germination in 'Kırkağaç-637' in all GO doses. On the other hand, different rates of germination were observed in all GO doses in 15 g L^{-1} NaCl in 'Kırkağaç-589' cultivar (Table 2).

The increasing NaCl concentration decreased radicle length in all cultivars (Table 3). Radicle lengths of 'Hasanbey', 'Kırkağaç-589', and 'Kırkağaç-637' varied from 0.47, 0.57, 1.67 cm to 7.80, 9.47, and 8.80 cm, respectively, as the salinity stress elevated to 15 g L^{-1} (0 to 26.20 dS m^{-1}) (Table 3). Although the decrease in radicle length was higher on 'Kırkağaç-589' than the other cultivars when NaCl concentrations elevated to 15 g L^{-1} (0 to 26.20 dS m^{-1}), the decrease in radicle length was higher on 'Kırkağaç-637' than the other cultivars when NaCl concentrations increase from 0 to 10 g L^{-1} (0 to 18.29 dS m^{-1}). The effect of priming

Table 1. The effect of priming with different graphene oxide doses on germination percentage of three melon cultivars under increasing NaCl (g L^{-1}) salinity at first count.

			NaCl (g L^{-1})			
Cultivars	Graphene oxide	0	5	10	15	Mean
	Control	83.16 ^{NS}	79.51 ^{NS}	33.52 b*	1.28 ^{NS}	
Hasanbey	0.25 mg ml^{-1}	83.16	83.16	57.10 a	1.28	
	0.50 mg ml^{-1}	85.06	74.37	49.90 a	1.28	52.86 A**
	0.75 mg ml^{-1}	75.85	73.95	55.09 a	1.28	
	1.00 mg ml^{-1}	85.06	79.51	52.34 a	1.28	
	Mean	82.46 A**	78.10 A	49.59 B	1.28 C	
	Control	52.22 ^{NS}	44.90 ^{NS}	25.83 b*	1.28 b**	
	0.25 mg ml^{-1}	61.98	41.84	40.28 a	1.28 b	
Kırkağaç-	0.50 mg ml^{-1}	61.22	51.85	32.37 ab	1.28 b	35.40 C
589	0.75 mg ml^{-1}	59.54	48.82	33.56 ab	1.28 b	
	1.00 mg ml^{-1}	60.18	47.27	30.76 ab	10.16 a	
	Mean	59.03 A**	46.94 B	32.56 C	3.06 D	
	Control	81.41 ^{NS}	58.39 b*	20.34 ^{NS}	1.28 ^{NS}	
Kırkağaç-	0.25 mg ml^{-1}	77.61	79.51 a	23.24	1.28	
637	0.50 mg ml^{-1}	72.05	70.57 ab	20.34	1.28	42.66 B
	0.75 mg ml^{-1}	78.03	70.04 ab	27.90	1.28	
	1.00 mg ml^{-1}	75.85	71.15 ab	20.34	1.28	
	Mean	76.99 A**	69.93 B	22.43 C	1.28 D	
	Control	72.26	60.93	26.56	1.28	40.26 B**
	0.25 mg ml^{-1}	74.25	68.17	40.21	1.28	45.98 A
Mean	0.50 mg ml^{-1}	72.78	65.60	34.20	1.28	43.46 AB
	0.75 mg ml^{-1}	71.14	64.27	38.85	1.28	43.89 AB
	1.00 mg ml^{-1}	73.70	65.97	34.48	4.24	44.60 A
	Mean	72.83 A**	64.99 B	34.86 C	1.87 D	

*Significant at $p < 0.05$, **Significant at $p < 0.01$, NS: Nonsignificant.

with GO on radicle length changed with salinity stress (Table 3). Radicle length in all tested melon cultivars was higher in GO-treated seeds than control. It was clear that priming with GO increased the radicle length according to the control in all tested melon cultivars, but 0.25, 0.50, and 0.75 mg mL^{-1} of GO doses were more effective than 1.0 mg mL^{-1} GO dose.

As a result of statistical analysis, significant interactions were found among melon cultivars, NaCl concentrations, and GO doses for the germination percentage in the first count, final germination percentage, and the radicle length (Table 4). Namely, the CV × NaCl, CV × GO, NaCl × GO, and CV × NaCl × GO interactions were statistically significant for the germination percentage in the first count, final germination percentage, and the radicle length. These interactions studied for the characters revealed that the impact of GO doses ranged noticeably in different salinity stress and cultivars. In short, these interactions also showed that the performance of tested cultivars under different salinity stress was positively influenced by GO treatments for examined parameters.

4. Discussion

The total germination percentage for the control 1.28% in 8 days at the highest salinity stress (26.20 dS m^{-1}) in 'Hasanbey', whereas the germination percentage of the 1.0 mg ml^{-1} GO-treated seeds 17.13%. Moreover, there was no germination in 'Kırkağaç-637' after 8 days at the same

Table 2. The effect of priming with different grapheme oxide doses on germination percentage of three melon cultivars under increasing NaCl (g L^{-1}) salinity at last count.

			NaCl (g L^{-1})			
Cultivars	Graphene oxide	0	5	10	15	Mean
	Control	83.16 ^{NS}	85.06 ^{NS}	40.60 b*	1.28 b**	
Hasanbey	0.25 mg ml^{-1}	88.72	83.16	61.56 a	1.28 b	
	0.50 mg ml^{-1}	85.06	85.06	55.23 ab	1.28 b	57.90 A**
	0.75 mg ml^{-1}	88.72	88.72	61.68 a	1.28 b	
	1.00 mg ml^{-1}	85.06	85.06	58.82 ab	17.13 a	
	Mean	86.15 A**	85.41 A	55.58 B	4.45 C	
	Control	60.34 ^{NS}	51.85 b*	28.05 b**	1.28 b**	
	0.25 mg ml^{-1}	66.00	54.24 ab	47.34 a	16.63 a	
Kırkağaç-	0.50 mg ml^{-1}	64.06	59.34 a	51.06 a	12.25 a	42.93 C
589	0.75 mg ml^{-1}	64.80	59.25 a	40.29 ab	12.25 a	
	1.00 mg ml^{-1}	62.86	56.03 ab	36.93 ab	13.82 a	
	Mean	63.61 A**	56.14 B	40.74 C	11.25 D	
	Control	75.85 ^{NS}	71.00 ^{NS}	27.05 b*	1.28 ^{NS}	
Kırkağaç-	0.25 mg ml^{-1}	83.16	79.51	35.67 a	1.28	
637	0.50 mg ml^{-1}	77.75	75.85	33.52 a	1.28	47.03 B
	0.75 mg ml^{-1}	79.51	71.20	34.74 a	1.28	
	1.00 mg ml^{-1}	79.51	76.13	33.67 a	1.28	
	Mean	79.16 A**	74.74 A	32.93 B	1.28 C	
	Control	73.12	69.30	31.90	1.28	43.90 B**
	0.25 mg ml^{-1}	79.29	72.30	48.19	6.40	51.55 A
Mean	0.50 mg ml^{-1}	75.62	73.42	46.60	4.94	50.15 A
	0.75 mg ml^{-1}	77.67	73.05	45.57	4.94	50.31 A
	1.00 mg ml^{-1}	75.81	72.41	43.14	10.74	50.53 A
	Mean	76.30 A**	72.10 B	43.08 C	5.66 D	

*Significant at $p < 0.05$, **Significant at $p < 0.01$, NS: Nonsignificant.

salinity stress level. However, the germination percentage of the GO-treated seeds changed in the range of 12.25% (0.50 mg mL^{-1} GO) –16.63% (0.25 mg mL^{-1} GO) at the highest salinity stress in 'Kırkağaç-589' (Table 2). Zhang et al. (2015) reported that as GO increases the water intake, germination can be affected positively, and the increase in seed germination may be due to GO layers that can penetrate the seed coat. Moreover, airtight two dimensional sheets not only penetrate the seed coat but also retain higher moisture content than those of control seeds. In this study, GO applications had a positive affect by resulting in the increase in the length of the melon radicle under salt stress in this work (Table 3), which is consistent with the report of Zhang et al. (2015), where the longer root length was determined in graphene-treated tomato seedlings than in control seedlings. Most probably, NPs accelerate the seed metabolism and germination rate by increasing the water and oxygen uptake of seed coat into cells (He et al., 2018). It was also reported that carbon nanotubes stimulate tomato germination by entering the seed coat and increasing the expression of the water channel gene LEAqp2 (Khodakovskaya et al., 2009; Khodakovskaya et al., 2011). In this work, the effect of GO changed according to the doses. Similarly, He et al. (2018) reported that while the GO-50 (µg mL^{-1}) increased the germination of spinach seeds, a lower germination percentage was determined than the control in GO-200. Additionally, Zhang et al. (2015) showed that 40 µg mL^{-1} GO application improved the germination of tomato

Table 3. The effect of priming with different grapheme oxide doses on the radicle length of three melon cultivars under increasing NaCl (g L^{-1}) salinity.

			NaCl (g L^{-1})			
Cultivars	Graphene Oxide	0	5	10	15	Mean
	Control	6.30 ^{NS}	7.80 a*	2.77 ^{NS}	- ^{z}	
Hasanbey	0.25 mg ml^{-1}	5.37	6.93 ab	4.00	-	
	0.50 mg ml^{-1}	6.57	5.80 b	3.40	-	5.14 ^{NS}
	0.75 mg ml^{-1}	6.03	5.40 b	3.73	-	
	1.00 mg ml^{-1}	7.47	7.13 ab	3.03	0.47	
	Mean	6.35 A**	6.61 A	3.39 B	0.47 C	
	Control	6.47 b*	7.57 ^{NS}	2.23 c*	-	
	0.25 mg ml^{-1}	7.93 a	7.47	4.93 ab	0.60 bc*	
Kırkağaç-	0.50 mg ml^{-1}	8.10 a	7.17	5.20 a	1.10 a	5.40
589	0.75 mg ml^{-1}	8.80 a	9.47	4.20 ab	0.57 c	
	1.00 mg ml^{-1}	7.97 a	8.47	3.67 b	0.77 b	
	Mean	7.85 A**	8.03 A	4.05 B	0.76 C	
	Control	7.50 ^{NS}	7.83 a*	2.20 ^{NS}	-	
Kırkağaç-	0.25 mg ml^{-1}	7.30	7.97 a	2.07	-	
637	0.50 mg ml^{-1}	6.63	8.80 a	2.27	-	5.43
	0.75 mg ml^{-1}	7.30	6.93 a	2.60	-	
	1.00 mg ml^{-1}	6.13	4.23 b	1.67	-	
	Mean	6.97 A**	7.15 A	2.16 B	-	
	Control	6.76	7.73	2.40	-	5.63 A**
	0.25 mg ml^{-1}	6.87	7.46	3.67	0.60	5.46 A
Mean	0.50 mg ml^{-1}	7.10	7.26	3.62	1.10	5.50 A
	0.75 mg ml^{-1}	7.38	7.27	3.51	0.57	5.50 A
	1.00 mg ml^{-1}	7.19	6.61	2.79	0.62	4.64 B
	Mean	7.06 A**	7.26 A	3.20 B	0.70 C	

** : Significant at $p < 0.01$, * : Significant at $p < 0.05$, NS: Nonsignificant.
'-' ^{z} indicates that seeds did not germinate, i.e. data could not be collected.

seeds. Moreover, Song et al. (2020) reported that graphene concentrations greater than 100 mg L^{-1} caused adverse effect on roots, shoots, leaves, and enzyme activity etc. in seedlings of forest species. The aforementioned studies explain why seeds treated with GO have a higher germination rate under salinity stress and according to our experience, plants are affected by salinity stress in two ways, osmotic and toxic effects based on the current results. As a general knowledge, the osmotic effect, increasing the amount of salt in the soil increases osmotic pressure and decreases the water uptake potential, causing a kind of drought stress by preventing the roots from taking in water. Seed germination occurs first at a suitable temperature with sufficient moisture and oxygen. Moisture is the most important and vital factor in the environment where the seed is sown. Therefore, Copeland and MacDonald (2001) reported that the first process that occurs in seed germination, called imbibition, which is the absorption of water by the seed. The water uptake of seed during germination can be affected by different factors such as salinity. In previous studies, the germination of melon seeds, other types of vegetables, and different crops were tried to be improved by using different priming materials such as NaCl, polyethylene glycol (PEG), potassium nitrate (KNO_3), mannitol, etc. For example, Demir and Mavi (2004) reported that priming with KNO_3 solutions increased seed germination and seedling growth of watermelon under salt

Table 4. Interactions between cultivars, NaCl (g L^{-1}) concentrations, and graphene oxide treatments.

		Germination percentage at first count	Germination percentage at last count	Radicle length
Source of variation	Degrees of freedom	F values		
Cultivar (CV)	2	123.894**	113.154**	16.861**
NaCl concentration	3	1259.556**	1509.936**	251.034**
Graphene oxide (GO)	4	4.322**	10.626**	1.261*
CV × NaCl	6	39.308**	42.817**	3.186**
CV × GO	8	0.683**	1.819**	4.827**
NaCl × GO	12	1.686*	2.338**	1.35 *
CV × NaCl × GO	24	1.832**	1.188*	2.344**
Error	120	37.266	31.712	1.055
Total	179			

*Significant at p = 0.05% level, ** Significant at p = 0.01% level

stress compared to control. Sivritepe et al. (2003) also found that seed priming with NaCl improved salt tolerance of melon seedlings under salinity stress compared to control. Farhoudi et al. (2011) showed that salt stress negatively affects both germination and seedling growth of melon cultivars, but priming with NaCl reduces the negative effects of salt stress. Similarly, priming with mannitol was found to be a successful method for increasing salt tolerance and improving the seed germination of alfalfa under high salinity conditions (Amooaghaie, 2011). It has been also reported that PEG priming of spinach seeds also increased the germination, resulting in increased stress tolerance (Chen and Arora, 2011).

Whatever material is used to increase germination under the salinity stress conditions, the main purpose is to prepare the seeds for unfavorable conditions. Thus, priming with different materials activates the enzymes that break down the storage materials in the seed and prepares the seed for germination in advance by ensuring the optimum use of the storage materials. The philosophy of priming, especially with different salts, is to overcome the osmotic effect, which is one of the harmful effects of salt stress, by trying to balance the osmosis between the seed and the germination environment. Briefly, it can be said that seed priming is a convenient method used to increase the water intake of seeds and improve germination.

5. Conclusions

In conclusion, we have successfully applied GO nanosheets as a priming material against salinity stress, which is a rising problem affecting germination and seedling development in different soils. The effect of GO as a priming material showed variation among applied GO doses, melon cultivars, and level of salinity stress levels. It was determined that the germination percentage and the length of the radicle were higher in GO primed seeds compared to the control in all the melon cultivars tested. Although there was no germination in the 'Kırkağaç-637' under the highest salinity stress (15 g L^{-1} NaCl), the highest germination percentage was determined in GO dose of 1.00 mg mL^{-1}, and 0.25 mg mL^{-1} in 'Hasanbey' and 'Kırkağaç-589' cultivars, respectively, under the highest salinity stress. GO doses of 0.25, 0.50, and 0.75 mg mL^{-1} for the length of the radicle were found to be more effective than 1.0 mg mL^{-1} GO. The results presented in this study revealed that GO has a high potential to be used as a new priming material against the salinity stress owing to its advantageous properties such as being highly water-soluble, nontoxic, producible on a large scale from graphite, and economical. In this regard, we believe that this study will pave the way for the application of GO as a promising material in different crops for eliminating problems of salinity stress.

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Conflicts of interest

The authors declare no conflicts of interest.

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