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Effect of ash carbon nanofibers on GABA shunt pathway in germinating seeds of tomato (*Lycopersicon esculentum* Mill., c.v. Rohaba.) under salt stress

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Abstract: The aim of the current study was to examine the effect of black ash carbon nanofibers (CNFs) on gamma-aminobutyric acid (GABA) shunt pathway in germinating seeds of tomato (*Lycopersicon esculentum* Mill., c.v. Rohaba) under salt stress. Seed's germination pattern, seed moisture content, GABA shunt metabolite levels (GABA, Glutamate and Alanine), total proteins and total carbohydrates, and the level of oxidative damage in response to sodium chloride (NaCl) treatments were determined. A significant increase of moisture content in CNFs treated seeds associated with significant increases in germination percentage was found. Data also showed a significant increase in GABA shunt metabolites in treating seeds compared to control seeds under different concentrations of NaCl. The total protein and carbohydrate levels significantly increased with positive correlation in control and treated seeds as NaCl concentration increased. A significant increase in MDA level was found in both treated and control seeds under salt stress. However, treated seeds showed lower MDA accumulation compared to control seeds. Our results suggested that the elevation of GABA in CNFs treated seeds was to maintain metabolic stability under salt stress, while, in control seeds, GABA elevation was to mitigate the effect of salt stress. CNFs activated GABA shunt, which might be involved in reduction of MDA accumulation and alleviation of oxidative damage under salt stress. In conclusion, CNFs enhanced tomato seed germination during salt stress.

Key words: Carbon nanofibers (CNFs), Gamma aminobutyric acid (GABA), *Lycopersicon esculentum*, metabolism, tomato, seed germination, salt stress

1. Introduction

Tomato is a vegetable crop that is consumed as fresh, cooked, or processed all over the world (Cuartero and Fernández-Muñoz, 1998). The tomato can adapt to various environmental conditions. It can be grown in most soil types from the tropical to the arctic circle areas (Foolad, 2004). Tomato is rich in phytonutrients (Levy and Sharoni, 2004), a common source for providing vitamins A and C and can reduce prostate cancer risk (Campbell et al., 2004).

Stress in plants in the form of biotic or abiotic stress is defined as external factor(s) that adversely affects plants metabolism, growth or development, crop yield, or the primary adaptation processes (Gerszberg and Hnatuszko-Konka, 2017). Examples of abiotic stress include heat, cold, drought, salinity, waterlogging, wounding, ozone, toxic ions, light intensity, and UV irradiation (Ashraf and Foolad, 2007). Consequently, stress tolerance is determined by the ability of a plant to survive under an extreme condition (Gerszberg and Hnatuszko-Konka, 2017).

Salt stress is a serious agriculture worldwide problem that affects plant physiology, growth, and development (Saito et al., 2008). However, mechanisms of plant growth reduction under salt stress are not well understood. Ionic and osmotic effect, nutritional, production of reactive oxygen species (ROS) and hormonal imbalance are associated with salt stress negative impact on plant biosystems (Ashraf and Foolad, 2007). Generally, soil salinity imposes stress on plants by either saline ions, consequently lowering the soil water potential in both uptake and translocation processes (Amini et al., 2007) or through salt toxicity due to high accumulation of Na^+ and Cl^- ions in cytoplasm (Kafkafi and Bernstein, 1996). Moreover, the plants can be categorized into two groups depending on their survival ability in saline conditions. Glycophytes plants' growth and development are negatively affected by salinity and halophytes plants which are tolerant to high salt concentrations (Parvaiz and Satyawati, 2008). Levels of chemical potency, metabolic activity, and salt concentration are higher than normal in

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plants subjected to salt stress. Thus, plant species and their cultivars differ in their ability to grow positively under salt stress (Porgali, 2001).

Tomato exposure to high salinity is considered as a serious constraint for its growth and production (Foolad, 2004). Salt stress affects tomato at different growth stages; it reduces seed germination (Cuartero and Fernández-Muñoz, 1998) and slows root and shoot growth (Snapp and Shennan, 1992). In addition, salinity leads to the formation and accumulation of ROS, which could cause membrane, protein, and DNA damage and eventually cell death, unless their concentration is regulated (Mittler et al., 2004; Amini and Ehsanpour, 2005).

Tomato can resist salt stress during flowering and fruiting phases (Yin et al., 2010). Salinized tomato produces osmotically active organic substances such as sugars and amino acids to reduce the effect of salinity (Shannon et al., 1987). The high level of total sugars in tomato leads to higher salt tolerance though the high seed germination percentage and better growth (Amini and Ehsanpour, 2005). Therefore, selection of tomato cultivars with enhanced salt tolerance is needed to increase tomato production in saline lands (Foolad, 2004).

Gamma-aminobutyric acid (GABA) is a four-carbon nonprotein amino acid presents in plant tissues (Shelp et al., 1999). GABA level is relatively higher in tomato than other crops (Matumoto et al., 1997). The high GABA level was observed in mature green fruit stage, but when tomato reaches the ripening stage, GABA level rapidly decreased (Akihiro et al., 2008). GABA level was significantly accumulated in tomato fruits exposed to salinity, stored under low CO_2 or low O_2 conditions after harvesting (Mae et al., 2012). In addition to its role in tomato salt tolerance, GABA contributes to tomato seed dispersal during fruit development through fluctuations in the amino acid composition (Yin et al., 2010).

Nanoparticles (NPs) are atoms or molecules that have one dimension range from 1 to 100 nm. NPs have physical and chemical properties that can be altered compared to bulky materials (Nel et al., 2006). NPs are classified into three types based on their source: natural, engineered or synthetic, and incidental (Monthioux et al., 2010). Engineered or synthetic NPs includes nano size polymers, composites, metal-based material (e.g: nano gold, nano zinc, nanoscale metal oxides like TiO_2, ZnO, and Al_2O_3), single-walled carbon nanotubes (SWCNTs), and multiwalled carbon nanotubes (MWCNTs) (Monica and Cremonini, 2009). Nanoparticles are widely used in agriculture to improve plant growth and biomass yields (Srinivasan and Saraswathi, 2010). Due to its small size, SWCNT has the ability to penetrate the plant cell wall and cell membrane of cultivated tobacco cells (*Nicotiana tabacum*) (Liu et al., 2009). Low dose of TiO_2 nanoparticles was able to activate and improve plants growth and photosynthetic efficiency in spinach seedling (Zheng et al., 2005). Although there are several studies on the toxicity of NPs on animals and bacteria, few studies are available on the effect of NPs on higher plants (Monica and Cremonini, 2009).

Carbon is an important element that forms different structures with different properties (Zaytseva and Neumann, 2016). The novel forms of carbon are carbon nanomaterials (CNMs) and engineered nanomaterials (ENMs), which have electrical, thermal, mechanical, and optical properties (Bennett et al., 2013). The CNM family includes carbon nanotubes (CNTs), nano-fibres, nano-beads, nano-diamonds, nano-onions, nano-cones, nano-horns, carbon dots, graphene, and fullerenes (Sharon and Sharon, 2010). CNMs contribute up to 40% of all agriculture nanotechnology by acting as additives, active components or in regulating plant growth (Gogos et al., 2012). The physiological effects of CNMs on plants vary depending on CNMs types and concentration, plant growth conditions, and plant species (Mukherjee et al., 2016). CNTs have unique properties which make them the most commonly used nanomaterial among all the others (Dresselhaus et al., 2004). CNTs increase root growth of some plant species, such as ryegrass and cucumber (Cañas et al., 2008).

Fly ash (carbon nanofibers) is a heterogeneous mixture of crystalline and amorphous phases that generally considered as ferroaluminosilicate (Aggarwal et al., 2009). Fly ash contains essential micronutrients (B, Cu, Co, Fe, Mn, Mo, and Zn), macronutrients (Ca, K, Mg, P and S), and some are rich in heavy metals (Cd and Ni) (Basu et al., 2009). Fly ash has chemical and physical properties with a pH of 8.5 that are useful to neutralize soil acidity and improve plant production (Sims et al., 1995). Therefore, the utilization of fly ash in agriculture may provide a possible alternative for its safe disposal without severe harmful effects (Aggarwal et al., 2009). In tomato, CNTs enhance tomato growth and development, activate genes expression, and stimulate seed germination (Khodakovskaya et al., 2009; Khodakovskaya et al., 2011). Furthermore, fly ash leads to improve growth and yield of many tomato cultivars (Khan and Singh, 2001). Traditionally, local farmers in Jordan preserve tomato (*Lycopersicon esculentum* Mill., c.v. Rohaba.) seeds for the next growing seasons by mixing it with fire carbon ash and keeping it under sunlight until complete drying.

In this study, the effect of ash carbon nanofibers (CNFs) on GABA shunt pathway through the assessment of seed germination pattern, GABA shunt metabolite levels (GABA, Glutamate and Alanine), total proteins and total carbohydrates, and the level of oxidative damage in germinating seeds of tomato (*Lycopersicon esculentum*

Mill., c.v. Rohaba.) under salt stress were determined. The functional role of the GABA shunt pathway, metabolic homeostasis, redox stability, and the strength of the C:N sink during tomato seed germination were also investigated.

2.Materials and methods

2.1. Plant materials and growth conditions

Tomato (*Lycopersicon esculentum* Mill., c.v. Rohaba) seeds (local variety, landrace "Rohaba") used in this study were obtained from local farmers in the Rohaba region, north of Jordan. Treated seeds were prepared by mixing it with the black ash carbon nanofibers and water to form complete seed coating. The nontreated (control) seeds were mixed with water only without the black ash carbon nanofibers. After that, both treated and nontreated (control) seeds were allowed to dry in the air for five days to return to its original moisture content and kept in sealed bag at 4 °C until experimental usage. All experiments on both treated and nontreated (control) seeds were performed in the lab using artificial growth substratum (filter paper) in 12 cm in diameter petri dishes supplemented with sodium chloride (NaCl) 0, 25, 50, 75, 100 and 200 mM, separately. Seeds were grown under continuous illumination provided by cool white fluorescent lamps (40μmol m^{-2} s^{-1}) at 25 °C.

2.2. Seed moisture content

Three replicates of 12 surface sterilized tomato seeds (*Lycopersicon esculentum* Mill., c.v. Rohaba) (treated and control) were placed in 1.5 mL eppendorf tube supplemented with sodium chloride (NaCl) (0, 25, 50, 75, 100, 200 mM). Seed moisture content was measured immediately after imposing seed treatments for 24 h according to the International Seed Testing Association (ISTA) by calculating the difference in seed fresh weight before and after drying them in an oven at 70 °C for 96 h (oven dry weight). The difference was then divided by seed fresh weight and was expressed as a percentage (%) of the wet weight as follows:

Seed moisture content = ((Fresh weight – oven dry weight) / Fresh weight) × 100%

2.3. Seed sensitivity to salt stress assay

Three replicates of 10 surface sterilized tomato seeds (*Lycopersicon esculentum* Mill., c.v. Rohaba) (treated and control) were placed on two filter papers as artificial growth surface in 12 cm in diameter petri dishes supplemented with 0, 25, 50, 75, 100, and 200 mM NaCl for seven days and allowed to germinate at 25 °C incubator. Emergence of radicles from germinating seeds were scored daily for five days. The effect of NaCl on seed germination was calculated.

2.4. Metabolites extraction

Zhang and Bown (1997) protocol was used to extract metabolites with the following adjustments: every day, germinating seeds (treated and control) under each NaCl treatment (0, 25, 50, 75, 100, and 200 mM) for five days separately were grounded in 1.5mL microfuge tubes until a fine powder was obtained. 400μL methanol was added to each tube, and the samples were vortexed for 10 min. Liquids were removed by overnight evaporation. To each tube, 500μL of 70mM lanthanum chloride was added. Tubes were vortexed for 15 min and centrifuged at 15000 rpm for 5 min. Supernatants were transferred to new tubes and mixed with 160μL of 1M KOH. Tubes were vortexed for 10 min and then centrifuged at 15000 rpm for 5 min. The resulting supernatants were transferred to a new tube and used to determine the quantity of GABA shunt metabolites. The average of three replicates were used for each treatment.

2.5. GABA (γ-aminobutyric acid) level determination

GABA level was assayed using Zhang and Bown (1997) protocol with the following adjustments: reaction mixture contained: 19μL of 0.5M potassium pyrophosphate (pH 8.6) ,14μL of 4mM $NADP^+$, 10μL of (2unit/μL) GABASE enzyme (suspended in 0.1M potassium pyrophosphate (pH 7.2) containing 12.5% Glycerol and 5mM β-marcaptoethanol), 50μL of the sample extract, and 10μL of α-ketoglutarate was finally added. Change in spectrophotometric absorbance at 340 nm after addition of α-ketoglutarate was recorded after 90 min incubation at 25 °C using the microplate reader (Multiskan FC, Thermo-Fisher Scientific, Finland). NADPH standard curve was used to calculate GABA level (nmol/mg FW). The average of three replicates was recorded and used for each treatment.

2.6. Alanine level determination

Bergmeyer (1983) protocol was used to measure alanine level with the following modifications: the reaction mixture contained 180μL of 0.05M Na-carbonate buffer (pH 10), 7μL of 30mM β-NAD^+, 1μL of 0.3u/μL alanine dehydrogenase (Sigma–Aldrich) suspension, and 10μL of sample extract was finally added. Change in spectrophotometric absorbance at 340 nm after the addition of alanine dehydrogenase was recorded after 60 min incubation at 25 °C using the microplate reader (Multiskan FC, Thermo-Fisher Scientific, Finland). NADH standard curve was used to calculate alanine level (nmol/mg FW). The average of three replicates was used for each treatment.

2.7. Glutamate Level Determination

Bergmeyer (1983) protocol was used to measure glutamate level with the following modifications: the reaction mixture contained 180μL of 0.1M Tris-HCl (pH 8.3), 8μL of 7.5mM

β-NAD^+, 1µL of 0.8unit/mL glutamate dehydrogenase (Sigma-Aldrich) suspension, and 10µL of sample extract was finally added. Change in spectrophotometric absorbance at 340 nm after the addition of glutamate dehydrogenase was recorded after 60 min incubation at 25 °C using the microplate reader (Multiskan FC, Thermo-Fisher Scientific, Finland). NADH standard curve was used to calculate glutamate level (nmol/mg FW). The average of three replicates was used for each treatment.

2.8. Protein content determination

SMART BCA protein assay kit (Intron Biotechnology, South Korea) was used to determine protein content according to manufacturer directions with the following modifications: every day for five days separately, germinating seeds under each NaCl treatment (0, 25, 50, 75, 100, and 200 mM) were grinded in 1.5mL microfuge tubes by using pestle with 100µL distilled water. 50µL of each treated sample was added to 1 mL of working solution and vortexed for 1min. All samples were incubated at 37 °C for 30 min., and the tubes were kept at room temperature for 5 min before spectrophotometric absorbance reading at 562 nm using the microplate reader (Multiskan FC, Thermo-Fisher Scientific, Finland). BSA standard curve was used to measure the protein concentration. The average of three replicates was used for each treatment.

2.9. Carbohydrate content determination

Total carbohydrate quantification assay kit (catalog number: ab155891, Abcam, USA) was used to measure total carbohydrates content according to manufacturer directions. Every day for five days separately, germinating seeds under each NaCl treatment (0, 25, 50, 75, 100, and 200 mM) were grinded in 1.5mL microfuge tubes by using pestle with 200µL ice cold assay buffer, centrifuged at 15000 rpm for 5 min. In 96 well microplate; 30 µL Supernatant of each treated sample and the standard solution were mixed with 150µL of concentrated H_2SO_4 for 5min on a shaker and incubated at 90°C for 15min. The microplate was kept at room temperature for 15min. After that, 30µL of the developer solution was added to each treated sample and standard solution wells, mixed on shaker for 15min. The spectrophotometric OD was measured at 490 nm using the microplate reader (Multiskan FC, Thermo-Fisher Scientific, Finland). Glucose standard curve was used to measure total carbohydrates concentration. The average of three replicates was used for each treatment.

2.10. Oxidative damage assay

TBARS assay protocol (Heath and Packer, 1968) was used to measure malondialdehyde (MDA) level in treated and control germinating tomato seeds. Germinating seeds (20 mg) were grinded in 1.5 mL microfuge tubes using pestle. 250 µl of 0.5% (w/v) thiobarbituric acid in 20% (w/v) trichloroacetic acid and 250µl of (175 mM NaCl in 50mM Tris-HCl, pH 8) were added. Tubes were incubated at 90°C for 25 min. Tubes were centrifuged at 15000 rpm for 20min and supernatant was collected. Spectrophotometric OD for collected supernatant was measured at 532 nm using the microplate reader (Multiskan FC, Thermo-Fisher Scientific, Finland). The MDA standard curve was used to measure MDA level (nmol/mg FW). The average of three replicates was used for each treatment.

2.11. Experimental design and data analysis

All treatments and assays were repeated three times. Results were expressed as mean and were analyzed by analyses of variance (ANOVA) as a completely randomized design (CRD). Least significant difference (LSD) was used for treatments comparison. Statistical analyses were done using SPSS version 25.0 (IBM Corp., Armonk, NY, USA) software. P-value ≤ 0.05 was considered significant.

3. Results and discussion

3.1. The effect of ash carbon nanofibers (CNFs) on seed moisture content and seed sensitivity under salt stress

Germination percentage of tomato seeds was significantly reduced under all NaCl treatments in control seeds compared to treated seeds as shown in Figure 1. There was no seed germination at day 1 under all treatments for both control and treated seeds. After 5 days, the germination percentages of control and treated seeds were as follows: 20%–100% and 70%–90% at 0 mM NaCl, 20%–90% and 90-100% at 25 mM NaCl, 10%–60% and 60-100% at 50 mM NaCl, 10%–30% and 10-100% at 75 mM NaCl, and 10%–20% and 0%–90% at 100 mM NaCl, respectively. After two days, there was a significant increase with negative correlation in germination percentage when NaCl concentration increased from 0, 25, 50 and 75 mM for treated seeds ($P = 0.050$, $r = -0.801$) compared to control seeds ($P = 0.008$, $r = -0.751$). After three and four days, there was a significant increase in germination percentage for treated seeds ($P = 0.022$, $r = -0.877$) and ($P = 0.007$, $r = -0.929$) respectively, compared to control seeds at all NaCl treatments except for 200 mM, which showed complete germination inhibition in the control seeds. However, treated seeds were able to reach 10% germination percentage at 200 mM NaCl concentration after five days.

Figure 2 shows that the moisture content percentages were: 61.5% and 56.0 % at 0 mM NaCl, 61.5% and 63.6% at 25 mM and 50 mM NaCl, 64.7% and 63.6% at 75 mM NaCl, 76.9% and 91.1% at 100 mM NaCl, and 85.7% and 95.0% at 200 mM NaCl in control and treated seeds, respectively. In general, no significant difference in seed moisture content between control and treated seeds except at 100 and 200 mM NaCl with positive correlation in treated ($P = 0.018$, $r = 0.888$) compared to the control seeds ($P = 0.006$, $r = 0.939$).

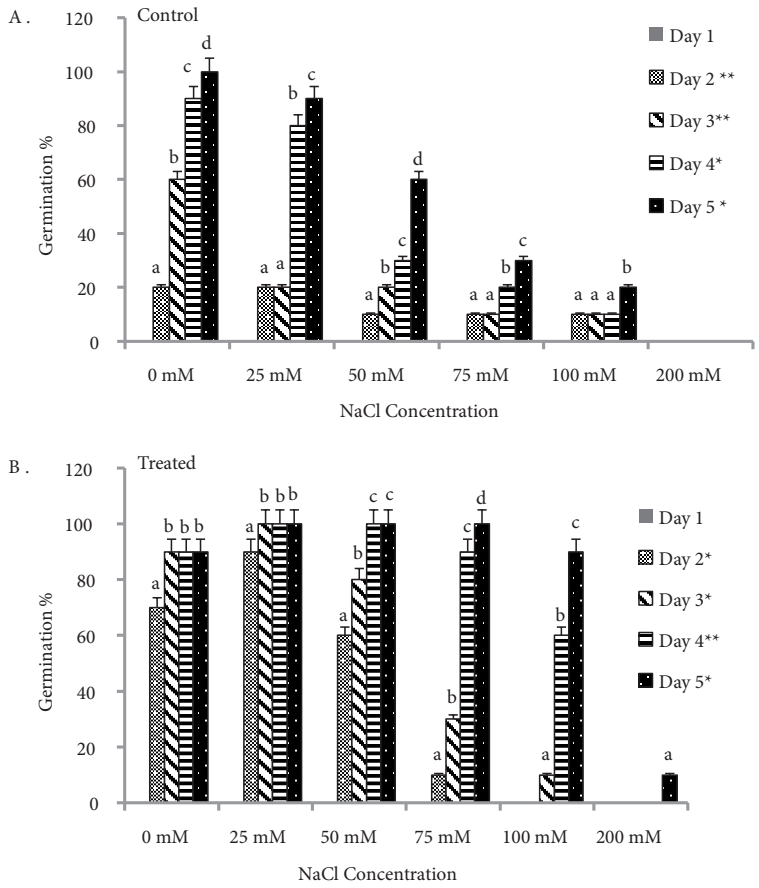

Figure 1. Germination percentage of tomato seeds: control (**A**), treated (**B**) supplemented with (0, 25, 50, 75, 100, and 200) mM of NaCl under continuous light at 25 °C. The Emergences of radical from germinating seeds were scored daily for 5 days. Within each treatment, columns with different letter scripts are statistically different ($P \leq 0.05$). (*) P-value ≤0.05–0.01, (**) P-value <0.01.

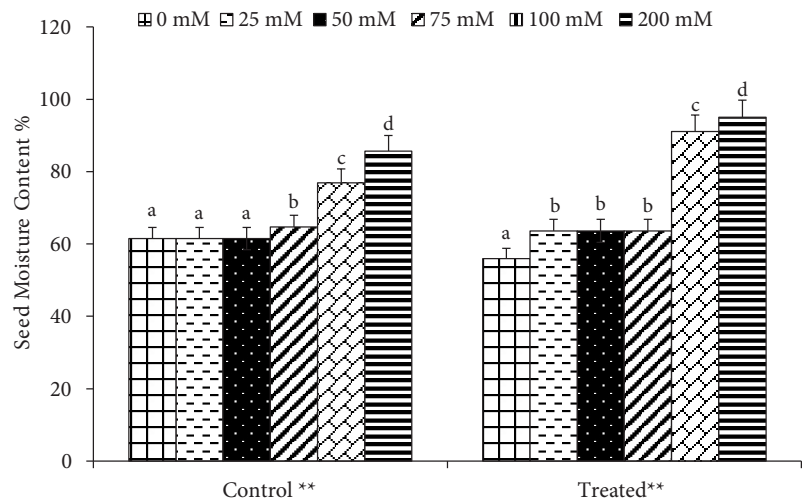

Figure 2. Seed moisture content of tomato seeds immediately after imposing treatments with (0, 25, 50, 75, 100, and 200) mM of NaCl, separately for 24 hrs at 25 °C. Within and among treatments, columns with different letter scripts are statistically different ($P \leq 0.05$). (**) P-value < 0.01.

In this study, data showed that seeds treated with carbon nanofibers (CNFs) resulted in higher improvement of seed moisture contents with the increase in NaCl concentration when compared with control seeds. In addition, our results showed that NaCl treatments caused a significant decline in the germination percentage for control seeds and improved germination in CNFs treated seeds under all salt stress treatments. Furthermore, seed moisture content data indicated that ash carbon nanofibers penetrated tomato thick seed coat and allowed water absorption. Activation of water absorption process could be responsible for the enhanced germination of CNFs treated tomato seeds at different NaCl concentrations. CNFs contain macronutrients, such as phosphorus, potassium, calcium, magnesium and sulfur and micronutrientss such as iron, manganese, zinc, copper, cobalt, boron, and molybdenum, which can be very beneficial as a rich source for plant growth (Panda and Dash, 2020). The increase in germination of CNFs treated tomato seed under salt stress might be due to the presence of these macro- and micronutrients. However, the chemical composition of black ash carbon nanofibers used in this study was not determined.

The mechanism of seed water absorption enhanced by nanoparticles is not well understood. However, previous studies suggested two explanations. The first one was that nanoparticles have the ability to penetrate seed coat by creating new pores resulting in better water permeation which leads to breaking of seed dormancy (Khodakovskaya et al., 2009). The second was that nanoparticles regulate water channels (aquaporins) gating in plant seeds coat (Mondal et al., 2011). Seed germination and embryo growth are critical processes in plant growth and development cycle (Cheng et al., 2018). Salinity delays or lower the percentage of seed germination either by enhancing toxic ions intake, which may change hormonal or enzymatic activities, or by decreasing water absorption which is required for gas exchange, aeriation and cellular activities associated with germination (Kaveh et al., 2011). Cuartero and Fernández-Muñoz (1998) showed that germination of tomato seed was reduced at low NaCl concentrations. In another study, tomato Shirazy cultivar had higher seed germination and better growth than cultivar Isfahani under salt stress (Kaveh et al., 2011). Due to it is small size, nano-materials affect seed germination by penetrating seed coats (Khodakovskaya et al., 2009). In our study, carbon nanofibers (CNFs) enhanced tomato seed germination even at high NaCl concentrations. This finding is in agreement with the results of Haghighi et al (2012), who reported that nano-Silicon (N-Si) treatments enhanced seed germination of tomato that were exposed to different concentration of salt stress. Another study reported that germination of the aged spinach seeds was accelerated by nano-TiO2 treatment in appropriate concentration and lead to an increase in seed vigour (Zheng et al., 2005). Nair et al (2010) observed that MWCNTs and SWCNTs enhanced germination of rice seeds. Khodakovskaya et al (2009) confirmed that tomato seeds exposed to carbon nanotubes increased the germination percentage and enhanced seedlings growth. Additionally, Morla et al (2011) reported that CNTs penetration of tomato seeds coat and activation of seeds water absorption lead to a significantly faster germination rate and higher biomass production for tomato seeds and seedlings.

3.2. The effect of ash carbon nanofibers (CNFs) on GABA shunt metabolism in response to salt stress

GABA shunt metabolites of control and treated seeds were analyzed for five days at different NaCl concentrations. Table 1 shows that GABA level significantly increased and positively correlated with increasing NaCl concentrations in both control and treated tomato seeds. On day 1 of germination and with increasing NaCl concentrations GABA level significantly increased with a positive correlation ($P = 0.005$, $r = 0.987$) in treated seeds compared to control seeds ($P = 0.001$, $r = 0.840$). However, on days 2, 3, 4 and 5 of germination, GABA level significantly increased with a positive correlation in control seeds compared to treated seeds.

Significant changes in alanine and glutamate concentrations were found in control and treated seeds at different NaCl concentrations for five days (Table 1). It was observed that alanine level has the same trend as GABA level for control and treated seeds with increasing NaCl concentrations. On day 1 of germination, alanine level significantly increased with positive correlation in treated seeds ($P = 0.013$, $r = 0.904$) compared to control seeds ($P = 0.002$, $r = 0.961$) at different NaCl concentrations. However, on days 2, 3, 4 and 5, control seeds showed a significant increase with positive correlation in alanine level at different NaCl concentration compared to treated seeds. In addition, our study showed that at different NaCl concentrations glutamate level significantly increased with positive correlation in treated seeds for days 1, 2 and 3 compared to control seeds. Whereas, glutamate level significantly increased with positive correlation for days 4 and 5 in control seeds compared to treated seeds.

To the best of the authors' knowledge, there has been no previous literature available regarding the effects of nanomaterials on GABA metabolism under abiotic stress. For the first time, the findings of this study suggested that CNFs could enhance GABA metabolism during tomato seed germination under different concentrations of NaCl. The significant increase in GABA level in control seeds might be due to the high sensitivity of tomato seeds to salt stress. However, the increase in GABA level in CNFs

Table 1. Level of GABA shunt metabolites GABA (γ-Aminobutyric acid), Ala (alanine) and Glu (glutamate) in control and treated tomato seeds supplemented with (0, 25, 50, 75, 100, and 200) mM of NaCl daily for 5 days under continuous light at 25 °C. Metabolite levels were calculated as nmol/mg FW. P-value≤ 0.05–0.01 was considered significant and P-value≤0.01 was considered highly significant in all metabolite analysis; r = correlation coefficient.

		Day 1			Day 2			Day 3			Day 4			Day 5		
	[NaCl]	GABA	Ala	Glut	GABA	Ala	Glut	GABA	Ala	Glut	GABA	Ala	Glut	GABA	Ala	Glut
Control	0 mM	3.25	0.57	0.72	8.27	2.22	0.87	12.82	2.67	0.90	31.02	2.82	1.47	76.36	3.88	1.70
	25 mM	7.69	0.57	1.25	20.98	2.37	1.47	71.65	2.45	1.70	80.39	2.70	2.15	77.77	4.48	2.22
	50 mM	8.84	1.02	1.32	34.16	2.67	1.92	77.62	2.76	2.15	83.32	2.90	2.37	106.17	4.93	2.82
	75 mM	10.62	1.17	1.62	36.35	2.82	2.07	83.27	2.98	2.60	84.36	3.13	2.82	112.08	5.08	2.97
	100 mM	12.61	1.17	1.77	58.79	2.97	2.67	86.40	2.67	2.82	91.79	3.28	3.13	119.04	5.08	3.43
	200 mM	12.71	1.70	3.50	59.73	3.28	3.73	87.50	3.26	3.97	102.09	3.43	5.08	125.78	6.28	5.98
	P-value	0.001	0.001	0.001	0.001	0.002	0.001	0.002	0.001	0.001	0.001	0.010	0.001	0.001	0.001	0.001
	r*	0.840	0.961	0.984	0.889	0.961	0.987	0.655	0.823	0.975	0.759	0.916	0.996	0.864	0.973	0.991
Treated	0 mM	6.26	0.78	0.91	6.43	1.83	0.91	52.38	1.96	0.97	55.94	2.22	0.98	64.16	2.35	1.10
	25 mM	7.07	0.91	1.30	7.44	2.22	1.50	67.22	2.22	1.70	71.14	2.48	1.85	70.96	2.74	1.98
	50 mM	9.26	1.04	1.50	10.52	2.48	1.84	68.86	2.61	1.99	71.82	2.78	2.01	73.79	3.00	2.48
	75 mM	11.18	1.04	1.56	28.29	2.50	2.74	70.32	2.68	2.87	75.25	2.84	3.14	75.70	3.22	3.43
	100 mM	15.70	1.83	1.89	30.53	2.87	2.74	71.14	3.00	3.40	77.30	3.14	3.54	77.62	3.53	4.18
	200 mM	22.81	1.96	1.96	33.63	3.14	3.40	80.72	3.27	3.46	79.49	3.66	3.66	79.76	3.92	5.23
	P-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	r*	0.987	0.904	0.886	0.863	0.940	0.927	0.881	0.940	0.873	0.781	0.984	0.866	0.868	0.959	0.962

* $\alpha < 0.05$

treated seeds under salt stress may suggest the critical role of GABA in different metabolic pathways such as amino acids synthesis and C:N assimilation rather than alleviation of salt stress. The presence of CNFs probably reduced the effect of salt stress as was shown in a high percentage of germination in treated seeds.

Plants exposed to stressful conditions exhibit various defense mechanisms such as accumulation of osmolytes. Among these, osmoprotectants are gamma-aminobutyric acid (GABA), which is a nonprotein amino acid (Roychoudhury and Chakraborty, 2013). Several studies demonstrated that different abiotic stresses lead to high level of GABA accumulation in plant tissues. Wallace et al (1984) reported that 20- to 40-fold increase in GABA levels in soybean (*Glycine max*) leaves occurred 5 min after exposure to cold shock or mechanical stimulation. AL-Quraan et al (2015) found an increase in GABA level in lentil (*Lens culinaris* Medik) plants after treatment with chemical compounds such as synthetic 1,2,3-thiadiazole. Moreover, many studies demonstrated an increase in GABA levels in *Nicotiana tabacum* tissues treated with 500 mM NaCl (Zhang et al., 2011) and in *Arabidopsis thaliana* shoots and roots under salt stress (Renault et al., 2010).

High GABA accumulation was detected in wheat (AL-Quraan et al., 2013) and *Arabidopsis thaliana* seedling (AL-Quraan and Al-Share, 2016) under both salt and osmotic treatments. In tomato, salt stress promotes GABA accumulation in leaves, roots, and fruits (Bolarín et al., 1995). Harborne (1997) reported that a reduction of growth was observed due to different patterns of GABA accumulation in salt sensitive and resistant tomato cultivars. NaCl stress could enhance GABA accumulation during seed germination by significantly increasing glutamate decarboxylase (GAD) and diamine oxidase (DAO) activities in *Glycine max* L. Merr. roots (Xing et al., 2007) and in foxtail millet (*Setaria italica* L.) (Bai et al., 2009). Alqarawi et al (2016) reported that exogenous application of GABA alleviated reduction of germination percentage from 23.4% to 11.66% of *Cassia italic* seeds under salt stress relative to control.

GABA effectively mitigated salt-caused inhibition of germination in barley (Chung et al., 2009), fava beans (Li et al., 2010) and soybean (Brechenmacher et al., 2010). Exogenous application of GABA significantly increased seed germination percentage of white clover (Cheng et al., 2018) and tomato (Luo et al., 2011) by enhancing catabolism of starch and utilization of amino acids and sugar for growth maintenance, antioxidant defense improvement and osmotic adjustment through increasing Na^+/K^+ transportation. Carbon (C) and nitrogen (N) assimilation is crucial during seed germination (Fait et al., 2011). Thus, GABA is considered as a transient and readily accessible reserve for carbon and nitrogen metabolism where TCA cycle and GABA shunt are functionally linked (Shelp et al., 1999; Fait et al., 2011). In our study, the increase in GABA content in treated seeds was less than that in control seeds, so the function of GABA in treated seeds was to maintain or assimilate carbon and nitrogen and CNFs were responsible for alleviating salt stress.

GABA, along with glutamate, is a major amino acid in tomato (Saito et al., 2008). Forde and Lea (2007) reported that glutamate has a major role in amino acid metabolism and for efficient nitrogen incorporation and it must be maintained at certain levels with tight regulation. Glutamate is a precursor for synthesis of chlorophyll in developing leaves (Yaronskaya et al., 2006). Additionally, it provides link between nitrogen and carbon metabolism, and provides both the carbonic skeleton and alpha-amino group that are involved in the synthesis of GABA and proline that play important role in plant defense mechanism (Galili et al., 2001). Bartyzel et al (2003) found that no significant changes in L-glutamate and L-alanine concentrations in wheat plants under osmotic stress. In our study, glutamate and alanine increased in both treated and control seeds under salt stress, which means that GABA tends to synthesis these two amino acids, which are involved in protein synthesis, through GABA shunt. Our study revealed that the significant increase in GABA accumulation in control seeds alleviated the effect of NaCl treatments possibly by maintaining metabolic homeostasis to overcome the osmotic effect, low water availability, and ion toxicity during seed germination. On the other hand, seeds treated with ash CNFs maintained a steady state of GABA accumulation compared to control seeds, which implicated the significant effect of CNFs on seeds water uptake that in turn reflected the stable metabolic interaction under salt treatments. Improving water uptake as a result of CNFs treatment increased the metabolic efficiency inside the seeds and reduced the stress phenotype even at high NaCl treatments. GABA shunt induction in treated seeds could be involved in C:N assimilation rather than being associated with salt stress effect. The significant increase in alanine and glutamate strongly indicated the usage of GABA shunt outcome in amino acids metabolism and protein synthesis.

3.3. Protein content in tomato seeds treated with ash carbon nanofibers (CNFs) under salt stress

In general, our results showed significant increase with positive correlation in protein content for control seeds compared to treated seeds for five days with the increase in NaCl concentrations (Table 2). On day 1, there was significant increase with positive correlation in protein content for all NaCl concentrations in treated seeds ($P = 0.006$, $r = 0.780$) compared to control seeds ($P = 0.002$, $r = 0.965$). However, protein content significantly

Table 2. Level of total protein in tomato seeds supplemented with (0, 25, 50, 75, 100, and 200) mM of NaCl daily for 5 days under continuous light at 25 °C. Protein level was calculated as µg/mL FW. For each day under different NaCl treatments, columns with different letter scripts are statistically different ($P \leq 0.05$), r = correlation coefficient.

	[NaCl]	Day 1	Day 2	Day 3	Day 4	Day 5
Control	0 mM	.95[a]	1426.03[a]	1671.17[a]	2257.42[a]	2721.75[a]
	25 mM	1123.83[b]	1793.74[b]	1961.70[b]	2520.71[b]	2778.82[b]
	50 mM	1402.68[c]	1864.55[c]	1996.72[c]	2623.83[c]	2822.92[c]
	75 mM	1682.84[d]	1920.19[d]	2271.95[d]	2867.80[d]	2915.14[d]
	100 mM	1727.98[e]	2026.50[e]	2519.42[e]	2903.72[e]	3027.59[e]
	200 mM	2118.64[f]	2415.65[f]	2781.67[f]	3044.63[f]	3404.63[f]
	r*	0.965	0.960	0.957	0.898	0.993
Treated	0 mM	324.86[a]	1036.29[a]	1098.55[a]	1269.75[a]	1671.83[a]
	25 mM	1208.13[b]	1490.23[b]	1517.48[b]	2049.90[b]	2245.10[b]
	50 mM	1451.97[c]	1658.20[c]	1842.37[c]	2229.53[c]	2579.08[c]
	75 mM	1747.69[d]	1694.51[d]	1924.09[d]	2267.80[d]	2593.35[d]
	100 mM	1785.95[e]	2042.76[e]	2122.53[e]	2451.97[e]	2737.32[e]
	200 mM	1906.58[f]	2077.13[f]	2219.81[f]	2497.37[f]	2820.97[f]
	r*	0.780	0.861	0.857	0.754	0.791

* $\alpha < 0.05$

increased with positive correlation with increasing NaCl concentrations in control seeds compared to treated seeds on days 2, 3, 4 and 5.

The majority of organic solutes involved in plant stress are free amino acids and soluble proteins. Salt stress affects protein metabolism and pool composition of free amino acids inside plant tissues (Saneoka et al., 1995). Therefore, the amount of proteins in various plant organs may change with salt stress (Gapińska et al., 2008). High protein contents in *Arabidopsis thaliana* and *Fragaria ananassa* cv. Camarosa in response to high NaCl treatments were observed (El-Baz et al., 2003). The effects of nanomaterials had a significant increase in protein content up to 60 mg/L in *Pelargonium zonale* cultivars leaves when treated with nano-scale silver (Hatami and Ghorbanpour, 2014) and in zinc oxide nanoparticles (ZnONPs) treated cotton (*Gossypium hirsutum* L.) seedlings compered to control (Venkatachalam et al., 2017). In our study, both control and CNFs treated seeds showed significant increase in total protein contents and high germination percentages in treated seeds under salt stress. The increase in protein content might be due to the increase in GABA level, which is involved in protein synthesis through GABA shunt activation in both treated and control seeds. Protein content was higher in *Linum usitatissimum* (Linaceae) plants treated with titanium dioxide nanoparicles (TiO_2-NPs) at 100 mg/L compared to control under drought stress (Aghdam et al., 2016). Salt tolerant cultivars of barley, sunflower, rice and finger millet had high soluble proteins content under salt (Hurkman et al., 1989; Ashraf and Tufail, 1995; Uma et al., 1995; Lutts et al., 1996). Positive correlation between seed protein content with seed size and seedling vigor in cotton (Snider et al., 2016) and wheat and maize (Wen et al., 2018) were found. Total soluble protein content increased at high NaCl concentration and decreased at low concentration in *Pancratium maritimum* plants (Khedr et al., 2003). Total soluble protein content increased in salt tolerant *Phaseolus acutifolius* and *Oryza sativa* cv. Pokkali plants and significantly decreased in salt-sensitive *Phaseolus vulgaris* and *Oryza sativa* cv. IR-28 plants under salt stress (Yurekli et al., 2004; Demiral and Türkan, 2006). Similarly, in tomato, Porgali and Yurekli (2005) observed that salt tolerant *Lycopersicon pennellii* plants showed higher protein content under salt stress compared to control, while the total amount of protein in salt-sensitive *Lycopersicon esculentum* plants decreased with salinity. Soluble proteins increased in stem and leaf of cv. Isfahani but decreased in cv. Shiraz tomato plants (Amini and Ehsanpour, 2005). However, total soluble protein content in *Lupinus angustifolius* plant was not affected by salt stress (Doganlar et al., 2010). Similarly, Qasim et al (2003) showed that salt tolerant and sensitive canola cultivars total soluble protein content was not affected under salt stress. In addition, there was no change observed in soluble protein content in tomato seedling roots under salt stress (Debouba et al., 2006).

Proteins accumulation as a nitrogen source can be re-utilized under stressful conditions and involved in osmotic adjustment under salt stress (Amini and Ehsanpour, 2005). In our study, the increase in protein content in control seeds could provide osmotic adjustment and mitigate the effect of salt stress. This increase in protein content might also act as a storage reservoir for nitrogen and carbon that could rapidly mobilized during seed germination. The significant increase of seed germination in treated seeds compared to control seeds might be due to the ability of CNFs to increase seed moisture content through enhancement of water uptake. In addition, the increase in protein content was correlated with the significant increase in GABA level because of GABA shunt activation. Amino acids such as proline accumulated in tomato under salt stress (Shannon et al., 1987). Bai et al (2009) detected a reduction in soluble protein content in millet under salt stress; however, it enhanced free amino acid content, which could serve as a substrate for GABA shunt induction. Our results showed that protein content was increased as salinity increased in treated and control seeds. The increase in protein content in treated seeds supports that CNFs has the ability to tolerate salt stress by enhancing water uptake and increasing protein content which resulted in the increased in seed germination.

3.4. Carbohydrates level in tomato seeds treated with ash carbon nanofibers (CNFs) under salt stress

Table 3 shows significant increase with positive correlation in total carbohydrates level in treated and control seeds as NaCl concentration increased. There was significant increase in carbohydrate content when NaCl concentration increase for both control and treated seeds during all days of NaCl treatments.

Carbohydrates produced from starch catabolism is necessary for seeds germination and growth (Cheng et al., 2018). Decreased rice seeds germination under salt stress resulted from inhibition of starch catabolism (Kim et al., 2006). Soluble sugars were essential for cell turgor maintenance in white clover seeds under salt stress (Li et al., 2014). Total soluble sugars content increased significantly with salinity in five sunflowers cultivars (*Helianthus annum* L.) (Ashraf and Tufail, 1995). In our current study, significant increase in carbohydrate level was observed during seed germination in both CNFs treated and control tomato seeds under salt stress. This might suggest that GABA shunt induction in both treated and control seeds is involved in carbohydrate level increase.

Few studies are available about the effect of nanomaterials on carbohydrate content under abiotic stress. An increase in carbohydrate content occurred in aquatic plant water hyacinth *Eichhornia crassipes* (Mart) Solms when treated with biological silver nanoparticles (B-AgNPs), while synthesized silver nanoparticles (S-AgNPs) treated leaf extracts showed no significant change in carbohydrate level (Rani et al., 2016). Carbohydrate content decreased with increased in silica nanoparticles (SNPs) in hawthorn seedlings under drought stress (Ashkavand et al., 2015). The soluble sugar content in root and bud of oat plant increased as NaCl level increased (El-Tayeb, 2005). In addition, soluble carbohydrate increased in roots of *Prosopis alba* under salt stress (Meloni et al., 2004). In contrast, sugar level decreased in soybean with salinity (El-Samad and Shaddad, 1997). Amini and Ehsanpour (2005) suggested that the increase in total sugars in tomato cv. Shirazy is responsible for salt tolerance.

High carbohydrate concentration played an important role in reducing water potential, maintenance of protein structure during water shortage and increasing cell membrane selectivity to ions entry such as sodium and chloride (Zahra et al., 2011). Exogenous GABA application improved the ability of barley to resist salinity and oxidative stress through increased proline and soluble sugar contents (Chen et al., 2007). An increase in soluble sugars content and proline level were observed in moderately and severely salt-stressed maize seedlings with exogenous GABA treatment. However, a better response to GABA regulation occurred in plants exposed to moderate salt stress by accumulating more soluble sugar

Table 3. Level of total carbohydrates in tomato seeds supplemented with (0, 25, 50, 75, 100, and 200) mM of NaCl daily for 5 days under continuous light at 25 °C. Total carbohydrates level was calculated as µg/mg FW. For each day under different NaCl treatments, columns with different letter scripts are statistically different ($P \leq 0.05$), r = correlation coefficient.

	[NaCl]	Day 1	Day 2	Day 3	Day 4	Day 5
Control	0 mM	8.56^{a}	11.03^{a}	26.61^{a}	28.71^{a}	30.82^{a}
	25 mM	24.48^{b}	26.35^{b}	27.79^{b}	29.31^{b}	30.37^{a}
	50 mM	28.48^{c}	29.79^{c}	30.64^{c}	33.28^{c}	34.76^{b}
	75 mM	29.29^{c}	30.39^{d}	32.42^{d}	35.44^{d}	37.67^{c}
	100 mM	30.45^{d}	33.44^{e}	36.22^{e}	38.19^{e}	40.22^{d}
	200 mM	34.21^{e}	35.63^{f}	38.26^{f}	40.33^{f}	45.33^{e}
	r*	0.768	0.772	0.945	0.932	0.968
Treated	0 mM	8.26^{a}	24.23^{a}	26.83^{a}	27.15^{a}	34.97^{a}
	25 mM	22.26^{b}	28.06^{b}	28.25^{b}	35.85^{b}	36.79^{b}
	50 mM	24.54^{c}	28.47^{c}	29.83^{c}	35.83^{c}	36.97^{bc}
	75 mM	28.64^{d}	30.28^{d}	33.92^{d}	36.98^{d}	37.35^{d}
	100 mM	30.55^{e}	31.13^{e}	35.66^{e}	37.11^{de}	39.38^{e}
	200 mM	34.23^{f}	37.52^{f}	38.23^{f}	39.31^{f}	41.71^{f}
	r*	0.835	0.985	0.942	0.748	0.976

* $\alpha < 0.05$

and proline than severely salt-stressed plants. Both proline and soluble sugars indirectly provide citric acid cycle with intermediates products such as succinic acid and alpha-ketoglutarate which both are also generated by GABA oxidation (Wang et al., 2017).

Our results suggested that an increase in GABA shunt occurred in CNFs treated tomato seeds under salt stress. This caused an increase in carbohydrate content and was indirectly involved in the citric acid cycle that is the main metabolic route to produce energy, thus led to an enhancement of seed germination. An increase in proline and soluble sugars in cultivated tomato was associated with an increased in GABA and its role in salt tolerance (Harborne, 1997). Our data showed that CNFs enhanced the capacity of seed to stand salt stress by increasing water uptake and maintaining the osmotic potential inside CNFs treated seeds. In addition, GABA shunt is involved in the synthesis of both carbohydrate and protein to maintain C:N balance which was observed through the increase in GABA in both control and treated seeds under salt stress.

3.5. Oxidative damage in tomato seeds treated with ash carbon nanofibers (CNFs) under salt stress

The measured MDA level in this study reflected the oxidative damage that occurred in tomato seeds under salt stress. A significant increase with positive correlation in MDA level was observed in control and treated seeds as concentrations of NaCl increased for five days (Table 4). At 100 and 200 mM NaCl treatments, a spike increase in MDA level occurred in control and treated seeds starting from day 2 until day 5. In addition, control seeds accumulated more MDA than CNFs treated seeds at these two NaCl concentrations. Generally, a decrease in MDA level with increase in GABA level was observed (Tables 1 and 4).

In response to stress, plants accumulate MDA which is the product and biomarker for lipid peroxidation (Chakrabarty et al., 2007). Production of ROS is associated with lipid peroxidation and membrane damage during salt stress (Mittler et al., 2004). Under salt stress, an increase in MDA level with reduction in plant growth was reported in maize (Wang et al., 2017), tomato (Al Hassan et al., 2015) and sesame seedlings (Koca et al., 2007). In addition, an increased in membrane lipids oxidation due to salinity was reported in *Cicer arietinum* (Rasool et al., 2013) and *Panicum turgidum* (Hashem et al., 2015).

Previous studies detected the effect of nanomaterials on MDA content. Titanium dioxide nanoparticles (TiO_2-NPs) caused a decrease in H_2O_2 and MDA levels in two chickpea (*Cicer arietimun* L.) genotypes (tolerant Sel11439 and sensitive ILC533) treated seedlings compared to control under cold stress (Mohammadi et al., 2014) and in *Linum usitatissimum* (Linaceae) under drought stress (Aghdam et al., 2016). Also, zinc oxide nanoparticles (ZnONPs) caused a significant reduction in MDA level in treated cotton (*Gossypium hirsutum* L.) leaves (Venkatachalam et al., 2017) and chickpea seedlings (*Cicer arietinum* L.) compared to control (Burman et al., 2013). In addition, zinc oxide nanoparticles (ZnO-NPs) improved the antioxidant systems to scavenge the excessive ROS in tomato (Faizan et al., 2018). Silicon oxide nanoparticles (SiO_2-NPs) enhanced the antioxidant system and improved tomato seed germination under salt stress (Haghighi et al., 2012) and reduced salt stress effects on *Cucurbita pepo* L. (Siddiqui et al., 2014). Furthermore, reduction in the total lipid content of *Cassia italica* under salt stress caused mainly by lipid peroxidation (Alqarawi et al., 2016).

GABA has an important role in ROS scavenging in plants (Carillo, 2018). Abnormal accumulation of ROS and hypersensitivity to environmental stresses caused necrotic cell death in *SSADH* knockout mutants in *Arabidopsis thaliana* seedlings (Fait et al., 2011). Therefore, plants treated with GABA reduced ROS production to maintain cell membrane stability (Alqarawi et al., 2016).

GABA effectively alleviate oxidative damage by improving the activity of multiple antioxidant enzymes in rice (*Oryza sativa*) seedlings under heat stress (Nayyar et al., 2014), black pepper (*Piper nigrum*) seedlings under osmotic stress (Vijayakumari and Puthur, 2016), perennial ryegrass (*Lolium perenne*) under drought stress (Krishnan et al., 2013) and tomato seedlings (Luo et al.,

Table 4. Level of Malondialdehyde (MDA) in tomato seeds supplemented with (0, 25, 50, 75, 100, and 200) mM of NaCl daily for 5 days under continuous light at 25 °C. MDA level was calculated as nmol/mg FW. For each day under different NaCl treatments, columns with different letter scripts are statistically different ($P \leq 0.05$), r = correlation coefficient.

	[NaCl]	Day 1	Day 2	Day 3	Day 4	Day 5
Control	0 mM	23.61^a	27.75^a	31.89^a	36.02^a	44.30^a
	25 mM	29.75^b	31.89^b	40.16^b	46.32^b	46.82^b
	50 mM	34.64^c	37.40^c	40.16^c	48.43^c	51.19^c
	75 mM	42.01^d	42.92^d	44.30^d	52.57^d	60.84^d
	100 mM	45.68^e	64.98^e	67.74^e	74.55^e	80.12^e
	200 mM	59.46^f	89.80^f	93.93^f	103.58^f	110.48^f
	r*	0.987	0.978	0.970	0.981	0.982
Treated	0 mM	27.67^a	32.48^a	37.29^a	38.50^a	42.11^a
	25 mM	31.28^b	39.70^b	40.11^b	42.11^b	51.73^b
	50 mM	34.89^c	42.11^c	44.51^c	49.53^c	54.14^c
	75 mM	38.50^d	43.00^d	45.21^d	51.23^d	56.54^d
	100 mM	51.73^e	53.35^e	56.54^e	60.23^e	68.57^e
	200 mM	70.98^f	74.59^f	78.20^f	85.04^f	90.28^f
	r*	0.986	0.987	0.984	0.994	0.989

* $\alpha < 0.05$

2011) and white clover (Cheng et al., 2018) under salt stress. Moreover, GABA treatment reduced MDA content and maintained membrane integrity under chilling stress in tomato seedlings (Malekzadeh et al., 2014). Our study found that MDA content significantly increased for both treated and control tomato seeds as NaCl concentration increased. However, treated seeds showed less increase in MDA level due to the CNFs treatments that reduced the effect of salt stress by increasing in GABA level that was accompanied by less lipid peroxidation and ROS production. Consequently, the findings of our study suggested that CNFs has the ability to reduce oxidative damage and to enhance tomato seed germination under salinity.

In conclusion, this study demonstrated that carbon nanofibers (CNFs) can be used to reduce salt stress effect during tomato seed germination. CNFs increased tomato seed moisture content by enhancing seeds water absorption, which in turn led to enhanced seed germination. In addition, CNFs increased GABA accumulation and their metabolites, alanine and glutamate, which may be involved in protein synthesis. In addition, CNFs caused an increase in protein and carbohydrate levels and activation of GABA shunt, which contributed to protein and carbohydrate production and C:N balance. Activation of the GABA shunt could be also involved in reduction of MDA accumulation and oxidative damage under salt stress. Further studies are needed to explore the effects of CNFs on different developmental stages of plant species under different abiotic stresses.

Abbreviations

GABA: gamma-aminobutyric acid
GAD: Glutamate decarboxylase
NAD+: Nicotinamide adenine dinucleotide
NADP+: Nicotinamide adenine dinucleotide phosphate
MDA: Malondialdehyde
ROS: Reactive oxygen species
TBARS: Thiobarbiturate reactive substances

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Authors' contribution

Nisreen A. AL-Quraan: study design, statistical analysis, results interpretation, writing, and revision of the manuscript. Mohammed-Ali H. AL-Akhras: study conceptualization, methodology assistance, and data analysis. Ala'a A. Ayoub: experimental work, data collection, and writing the first draft of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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