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Impacts of nano-TiO_2 on the initial development stages of barley seedlings under salinity

Nano-TiO_2'in tuzlulukta arpa fidelerinin ilk gelişim aşamaları üzerine etkileri

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ABSTRACT

The most important development period in cereal plants is the initial stage, that is, seed germination and early seedling development. Even if the barley is thought to be a partially salt-tolerant plant, it may be severely affected when exposed to salinity at initial developmental periods. Pre-treatment and preparation of seeds before sowing have an important in agriculture. Nano-seed priming treatment is a new approach used to increase germination, emergence and seedling growth recently. In this study, the effects of nano-TiO_2 (0, 100, 200 mg L^{-1} n-TiO_2) pre-application and ongoing/combination application under salinity (0, 100, 200, 300 mM NaCl) on germination and early seedling growth of barley plants were investigated. Root lengths (RL, mm), germination rates (GR, %), radicle emerging (RE, %), number of coleoptiles (CN) were measured depending on the day (1, 2, 3 days). At the end of the third day, seedling fresh and dry weights (FW,DW mg) were measured. The relative growth index (RGI) of root and mean germination time (MGT) were calculated. It was determined that the application of 100 mg L^{-1} n-TiO_2 increased root length and RGI compared to control groups. It was observed that the application of 100 mg L^{-1} n-TiO_2 significantly increased the germination percentage, biomass and root length especially in 100 mM salt conditions. Also, 100 mg L^{-1} n-TiO_2 increased the RE too in 100 mM salt conditions (1st day). In this study, it was determined that 300 mM NaCl was inhibitory dose, and also germination remained below 20% in 200 mM NaCl in all groups.

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ÖZ

Tahıl bitkilerindeki en önemli dönem, başlangıç aşaması, yani tohum çimlenmesi ve erken fide gelişimidir. Arpanın kısmen tuza toleranslı olduğu düşünülse bile, belirli gelişim dönemlerinde tuzluluğa maruz kaldığında ciddi şekilde etkilenebilir. Ekim öncesi tohumların ön işlemden geçirilmesi ve hazırlanması tarımda önemli bir yere sahiptir. Nano-tohum hazırlama uygulaması son zamanlarda çimlenme, ortaya çıkış ve fide büyümesini arttırmak için kullanılan yeni bir yaklaşımdır. Bu nedenle, bu araştırmada, tuzluluk durumunda (0, 100, 200, 300 mM NaCl) nano-TiO_2 (0, 100, 200 mg L^{-1} TiO_2) ön uygulaması ve devam eden/kombinasyon uygulamalar ile çimlenme ve erken fide büyümesi üzerindeki etkilerini araştıran bir çalışma tasarlanmıştır. Çalışmada güne bağlı olarak kök uzunlukları (mm), çimlenme oranları (%), radikula çıkışı (%) koleoptil sayısı ölçüldü (1, 2, 3. gün). Üçüncü günün sonunda taze ve kuru ağırlık (mg) belirlendi. Kökün bağıl büyüme indeksi ve ortalama çimlenme süresi hesaplandı. 100 mg L^{-1} n-TiO_2 uygulamasının kontrol gruplarına kıyasla kök uzunluğunu ve kökün bağıl büyüme indeksini arttırdığı belirlenmiştir. 100 mg L^{-1} n-TiO_2 uygulamasının çimlenme yüzdesini, biyokütleyi ve kök uzunluğunu özellikle 100 mM tuz koşullarında önemli ölçüde arttırdığı gözlenmiştir. Ayrıca, 100 mg L^{-1} n-TiO_2, 100 mM tuz koşullarında (1. gün) radikula çıkışını da arttırdı. Bu çalışmada, 300 mM NaCl'nin inhibitör doz olduğu ve ayrıca çimlenmenin 200 mM NaCl içinde tüm gruplarda %20'nin altında kaldığı belirlenmiştir.

1. Introduction

Cereal seeds cultivated in the field are exposed to severe environmental stress factors, especially during early germination, emergence and beginning seedling development (Bennett et al. 1992). Aridity and saltiness come first among these undesirable environmental stress conditions (Panuccio et al. 2014). Barley (*Hordeum vulgare* L.), is a wanted cereal crop cultivated in very large areas in Turkey and in the world. Barley is considered a partially salt-tolerant cereal crop and it was reported that the "Kral 97" genotype used in this study was of moderate tolerance (Bağcı et al. 2003; Mahmood 2011).

Pre-treatment and preparation of seeds before sowing in order to improve quality and increase germination energy in agricultural production has been an application area that has been ongoing for a long time and has been popular recently. This method of application may mostly use to increase the germination rate, total germination and seedling power under unfavorable environmental conditions. This technique, called seed priming, which is used especially for cereal and vegetable seeds, stimulates metabolic processes related to seed germination and early seedling development, and as an outcome of these processes, seeds resistant to abiotic stress conditions, like water scarcity develop (Korkmaz and Pill 2003; Armin et al. 2010; Theerakulpisut et al. 2016).

A wide variety of seed priming agents can be used to increase the effectiveness of seeds and reduce the impact of environmental stresses. These seed priming agents may have osmo-, hydro-,chem-, bio-, hormo-, halo-priming properties. New priming materials have been added to the existing seed priming agents with the developing technologies (such as nanotechnology) recently. Nano-seed priming treatment is a new approach used to increase germination, emergence and seedling growth. (Lutts et al. 2016; Mahakham et al. 2017; Acharya et al. 2020). Metallic nanoparticles (zinc, titanium, and silver) can be used as protective agents against biotic and abiotic stress factors (do Espirito Santo Pereira et al. 2021).

TiO_2 nanoparticles (NPs) occur in 3 different forms as anatase, rutile and brookite (Macwan et al. 2011). TiO_2 nanoparticles have been one of the 10 most used nanoparticle types in the world and it has a wide range of usage in different sectors from cosmetics to batteries, paint to construction industry and from the food industry to the pharmaceutical industry (Piccinno et al. 2012; Gogos et al. 2012, Liu and Cohen 2014). In addition to, TiO_2 nanoparticle is included in the list of nanoparticles that should be examined primarily by the Organization for Economic Development and Cooperation (OECD 2010).

Titanium is also a quite widely used chemical element in agricultural research. Although titanium is not included in the essential list of macro and microelements among plant nutrients, it has been widely used in plant nutrition lately (Bacilieri et al. 2017) and farther it is thought to be a benefical element when used correctly in crop production (Lyu et al. 2017). There are many studies reporting that this element has a positive effect on plants under stress conditions, especially germination, root development and vegetative growth (Feizi et al. 2012; Dehkourdi and Mosavi 2013; Haghighi and Silva 2014; Mutlu et al. 2018).

Overall, researchers suggest that the application of TiO_2 NPs in salt stress, drought stress, and heavy metal stress situations can be a promising approach to prevent their negative effects on seed germination and early growth. There is a huge gap still waiting to be investigated regarding the use of nanoparticles in seed preparation. In this study, it has been tried to find answers to the question of whether nano-TiO_2 be one of these new priming agents? The application time of the nanoparticles, the way of application, the concentration to be applied, and how it will be applied in which stress situation, how it will work, is a highly interesting subject. Therefore, in this research, a study that investigates the effects on germination rate, root length, biomass and coleoptile with TiO_2 nanoparticle pre-application and ongoing application under salt stress is designed.

2. Materials and Methods

2.1. Seed samples

The seeds of *Hordeum vulgare* L. (barley), which are widely cultivated, were used in this study. Barley seeds were subjected to surface sterilization prior put to use. After the seeds were kept in 5% sodium hypochlorite for 5 minutes, they were washed five times with pure water and dried at room temperature on filter papers and used in the study.

2.2. Preparation and pre-application of TiO_2 nanoparticle suspensions

The commercial form of TiO_2 nanoparticles (32 nm) was used in the study (Titanium (IV) oxide, NanoArc, anatase, nanopowder, 99.9% metals basis). Suspensions of TiO_2 NPs were freshly prepared by dissolving directly in deionized water and dispersed by ultrasonic vibration for 30 min (100 W, 40 KHz). The suspensions were stirred before use to avoid the aggregation of nanoparticles (Garcia-Lopez et al. 2018). Surface sterilized seeds were kept in aerated solution containing different concentrations of nano-TiO_2 (0, 100, 200 mg L^{-1} TiO_2) for 24 h before salinity application. At the end of this pretreatment, seeds for each application were put between two layers of filter papers in square petri dishes. 25 seeds were placed in each petri dish and the study was carried out with 4 repetitions.

2.3. TiO_2 nanoparticle and salinity applications

Nanoparticle applications were made as pre-applications before switching to salt and nanoparticle combined applications. Solutions containing nanoparticle (0, 100, 200 mg L^{-1} TiO_2) and salt (0, 100, 200, 300 mM NaCl) in different concentrations were added in equal amounts on the seeds found in petri dishes in the combinations given in Table 1. The same amount of deionized water was added to the control groups. There were 12 application groups in total and 100 seeds were used in each application group (Table 1).

2.4. Germination of barley seeds and growth

The application groups in petri dishes were germinated at 24:18°C day:night temperature, 16:8 day:night light period, 150 µmol m^{-2} s^{-1} light intensity and 60±5% humidity conditions for 3 days under controlled conditions in the climate room.

2.5. Assays

In the study, root lengths (RL, mm), germination rates (%), radicle emerging (RE, %), number of coleoptiles (CN) were measured depending on the day (1, 2, 3 days). At the end of the third day, seed fresh and dry weight (at 80°C for 24 h) (mg) were measured. Seed weights were given for 100 seeds. In

Table 1. Experimental application groups.

Groups	NaCl (mM)	TiO2 (mg L^{-1})
1	0	0
2	0	100
3	0	200
4	100	0
5	100	100
6	100	200
7	200	0
8	200	100
9	200	200
10	300	0
11	300	100
12	300	200

addition, plants were photographed daily for three days. The relative growth index (RGI) of root and mean germination time (MGT) were calculated.

Seeds were considered germinated when the radicles were ≥2 mm long and radicle emergence was defined as the radicles were <2 mm long (Kaya et al. 2006; Gao et al. 2018). The number of seeds that emerged and germinated was recorded every 24 h for 3 days. Radicle emergence and seed germination percentages were calculated using the following formulas (Koksal et al. 2015; Gao et al. 2018).

Radicle Emergence (%)= Number of emerged seeds / Total number of seeds X 100 [1]

Seed Germination (%)= Number of germinated seeds / Total number of seeds X 100 [2]

Root length was measured daily with digital caliper. Based on the daily measurement relative growth index (RGI) of root was calculated with following formula:

$$RGI\ (mm\ day^{-1}) = (RL2-RL1)/(t2-t1) \quad [3]$$

The RGI formula was modified from Acosta-Motos et al. (2017) and Ren et al. (2016).

Where, RL2 - RL1, root length for seed at the beginning and at the end of experiment; t2 - t1 was the time duration for the treatment.

2.6. Statistical analysis

The experiment was conducted using a completely randomized experimental design with two factors (NaCl and n-TiO_2 concentrations). Treatments had four replications with 25 seeds each. All quantitative data expressed as percentages were subjected to arcsine transformation. Data were subjected to ANOVA and the means were separated using the LSD multiple range test at $p \le 0.05$. All statistical analyses were performed using the JMP8 software package.

3. Results and Discussion

This study was carried out to evaluate the effects of nanoparticles and salinity at the initial stage of plant development in nano TiO_2-primed barley seeds. Different concentrations of primed nano-TiO_2 (0, 100, 200 mg L^{-1} TiO_2) seeds were germinated under different concentrations of NaCl (0, 100, 200 and 300 mM), nano-TiO_2 (0, 100, 200 mg L^{-1} TiO_2) and NaCl/n-TiO_2 combinations. The effects of Nano-seed priming, nanoparticles and salt applications on the root lengths, germination rates, coleoptile and biomass were comparatively investigated daily for 3 days. In addition, RGI and MGT were calculated based on with these data. Phenotypic images of barley seedlings in different concentrations of TiO_2 NPs/NaCl treatments depending on the days are given in Figure 1 (a, b, c). The effects of nano-titanium and salinity were found significant in terms of germination and early seedling parameters on barley plants, statistically.

The findings in this study showed that the germination and growth parameters of barley were negatively affected due to the increased salt concentration (Figure 1a, b, c). In many studies, including this study, salinity in barley plants has been shown to reduce seed germination, radicle emergence and inhibit root elongation depending on the concentration (Ayers 1953; Bağcı et al. 2003; Katerji et al. 2006; Mahmood 2011; Askari et al. 2016; Demiroğlu Topçu and Özkan 2017). Germination times were lengthen out as the salt concentration increased. It was found that germination did not occur at a salt concentration of 300mM, indicating that this concentration was an overly high dose for the "Barley Kral 97". So what differences have been observed with the application of titanium nanoparticles?

Strikingly, on the first day of application (Figure 1a), the highest RE (20%) ratio was obtained at 100 mg L^{-1} nano-TiO_2+100mM salt application compared to all groups. This rise was exactly twice of 100 mM salt applications (%10) (Figure 2a). It was observed that germination rates dramatically decreased significantly with the increase in salinity (Figure 2b). On the 3rd day of treatment (Figure 1c), the highest values of germination rates were obtained from control (87%) and 100 mg L^{-1} nano-TiO_2 (83%). When the salt concentration was 100 mM, the highest germination rates were found in 100 mg L^{-1} nano-TiO_2 (70%) (Table 2). The protective effect of TiO_2 was seen when the salinity level was 100 mM.

As seen in Figure 2c, when salinity and TiO_2 levels increased, coleoptile numbers drastically decreased (Table 2). The highest values of coleoptile numbers were obtained from control (78%) and 100 mg L^{-1} nano-TiO_2 (76%). Coleoptile numbers decreased at 200 mg L^{-1} nano-TiO_2 (59%). Titanium could not positively effective of coleoptile numbers under saline conditions (Table 3).

Treatment effects in terms of root length on daily observations were shown in Figure 3. The effects of salinity and nano-TiO_2 on root length were presented in Tables 2 and 3. When salinity increased, root length dramatically decreased. The highest values of root length were obtained from control (16.69 mm) and 100 mg L^{-1} Nano-TiO_2 (17.6 mm). Root length decreased at 200 mg L^{-1} nano-TiO_2 (11.0 mm). Although there were no statistical differences among control and 100 mg L^{-1} nano-TiO_2 in terms of root length, the highest root length values were obtained 100 mg L^{-1} nano-TiO_2 levels (5.75 mm). When the salt concentration was 100 mM, the most root length was found in the plants treated with 100 mg L^{-1} nano-TiO_2 levels (4.99 mm). When the salt level was up to 100 mM, root length decreased, drastically (Table 3).

The effects of salinity and titanium on the fresh weight (FW) of seeds were presented in Tables 2 and 3. As salinity increased, fresh weight decreased. The differences between fresh weights in terms of salt treatments were found important, statistically. Nano-TiO_2 did not affect the fresh weights of plants (Table 2). It was not seen statistically significant differences in terms of barley seeds dry weights (DW) under salinity and titanium treatments (Tables 2 and 3).

The root relative growth index (RGI) results clearly demonstrated the negative effects of salt stress on the radicle development stage of the seeds (Table 2). The highest RGI was obtained from control (7.37) and 100 mg L^{-1} nano-TiO_2 (7.68). The mean germination time (MGT) increased with 200 mM NaCI+n-TiO_2 application.

Gohari et al. (2000) showed that 50 and 100 mM NaCl negatively affect the agronomic properties (plant height, shoot and leaf fresh and dry weights and leaf number) of the *Dracocephalum moldavica*, but the application of 100 mg L^{-1} TiO_2 NPs reduces these negative effects, as in the results of this study. Also, Feizi et al. (2012) reported that nano-TiO_2 in a suitable concentration could promote the seed germination of wheat in comparison to bulk TiO_2. Haghighi and Silva (2014) reported that nano-TiO_2 application had a positive effects on germination studies on tomato, onion and radish seeds that 200, 100 and less than 100 mg L^{-1} TiO_2, respectively, were appropriate concentrations and suggested that nano-TiO_2 may function as a seed priming agent for horticultural crops. However, they especially emphasized in their studies that need for more experiments should be done on this subject. Dehkourdi and Mosavi (2013) showed that the application of nano-anatase TiO_2 at a concentration of 30 mg ml^{-1} caused a significant increase in germination, germination rate index, root and shoot length, fresh weight, viability index and chlorophyll content. Mutlu et al. (2018) reported that nano-TiO_2 treatments (0.1%, 0.2% and 0.3%) were ineffective on germination percentage in maize cultivar, regardless of concentration. In the case of stress (300 mM NaCl), they indicated that nano-TiO_2 treatments caused significant increases in root-stem length and fresh-dry weights. Further, notified that the application of salt stress in maize plants inhibits seed germination and seedling growth. Doğaroğlu and Köleli (2016) reported that especially in 80 and 100 mg L^{-1} nano-TiO_2 concentration, number of seed germination increased compared to control in lettuce. Younes et al. (2020) pointed out the extraordinary effect on germination characteristics and seedling growth by treating seeds of eggplant, pepper and tomato plants with gel-coated TiO_2 nanomaterials (0, 50, 100 and 150 mg L^{-1}). They reported that the maximum transplants lengths, fresh and dry weight were recorded at the level 100 mg L^{-1} nano-TiO_2 whatever the crop plant used, in line with the results in this study. Also, in the same study it has been shown that in the solanaceae family that the germinability increased and the mean germination time decreased by gel-coating the seeds with 100 mg L^{-1} nano-TiO_2 (Younes et al. 2020). Besides, It has been suggested that different nanoparticle seed preparation practices under salt stress in cotton and cucumber seeds are a sustainable, practical and scalable tool to improve crop tolerance to stress (An et al. 2020; Mahdy et al. 2020). As seen in the studies, there are differences in the response to nanoparticles between concentration, application method and plant types.

Taken together all of these literature, similar results also reported by other researchers on the different plant species To summarize the study, it was observed that 100 mg L^{-1} nano-TiO_2 application increased root length and RGI compared to control groups. It was determined that 100 mg L^{-1} nano-TiO_2 application significantly increased RE, germination percentage, biomass and root length, especially under 100 mM salt conditions.

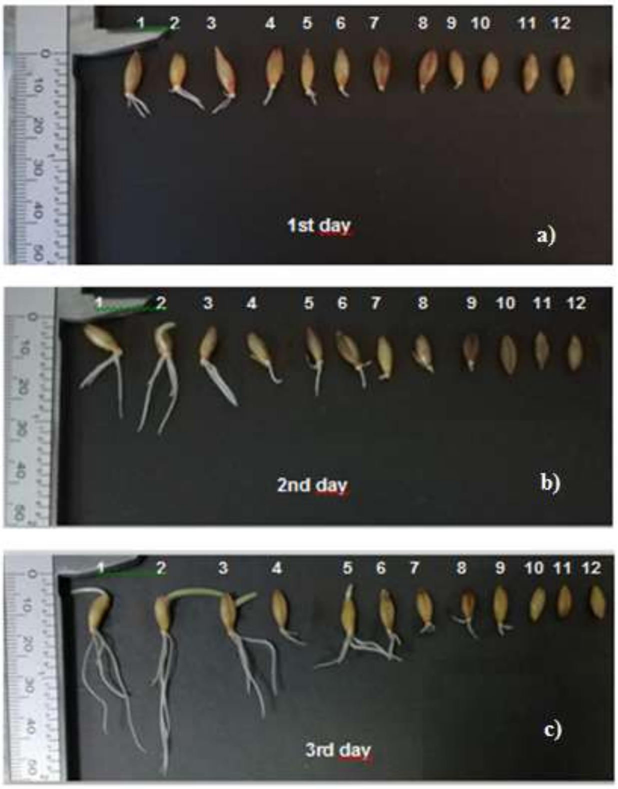

Figure 1. Phenotypic images of barley seedlings in different concentrations of TiO_2 NPs/NaCl treatments depending on the days [a) 1st day, b) 2nd day, c) 3rd day].

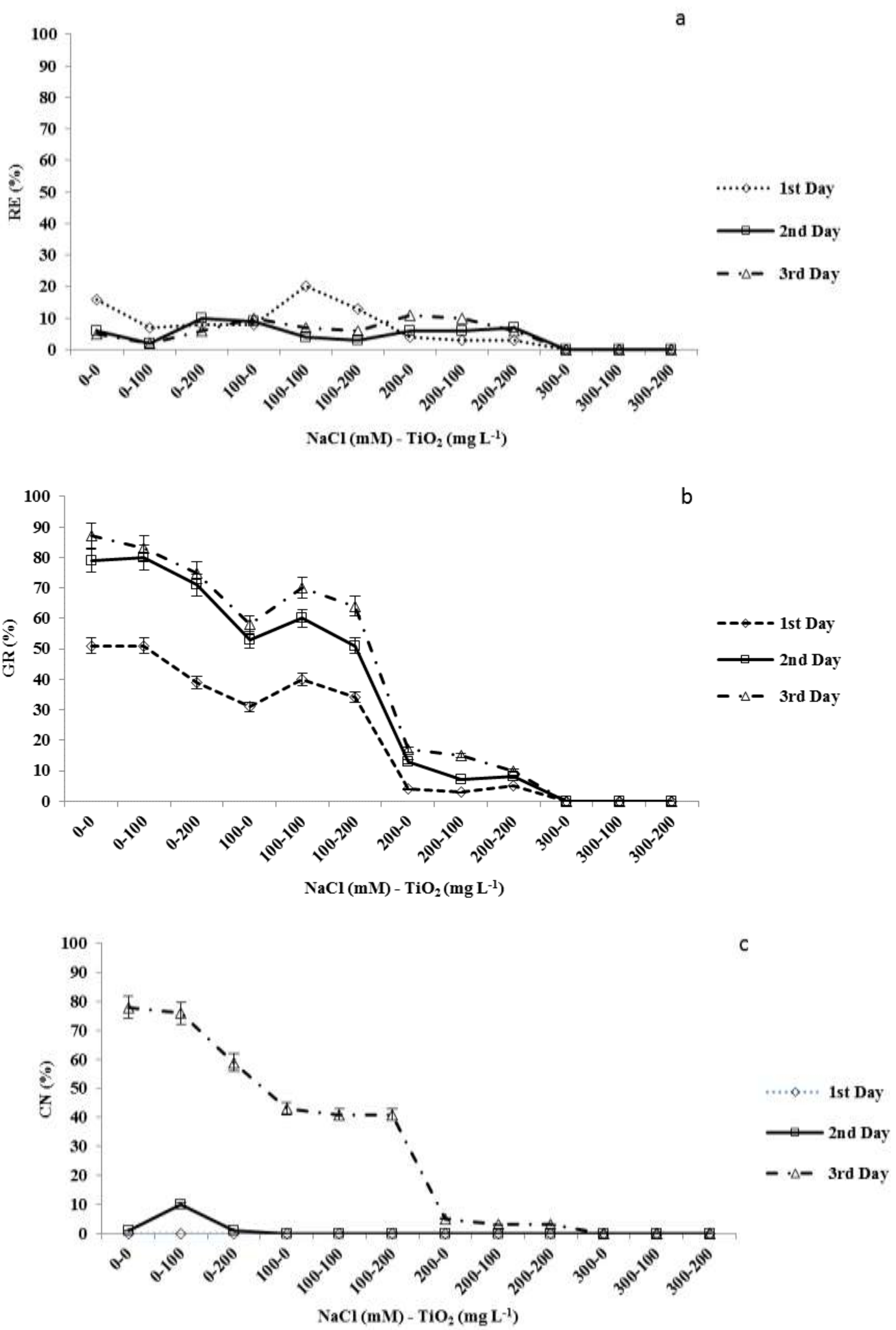

Figure 2. a) Radicle emergence percentage (RE-%), b) Germination percentage (GR-%), c) Coleoptile number (CN-%) of barley seedlings in different concentrations of TiO_2 NPs/NaCl treatments, depending on the days.

Table 2. Effects of n-TiO_2 and salinity interactions on MGT, RE, GR, CN, RL, FW, DW, RGI on thr 3rd day.

Salinity (mM)	n-TiO_2 (mg L^{-1})	Mean Germination Time	Radicle Emerging (%)	Germination rate (%)	Coleoptile number (%)	Root length (mm)	Fresh Weight (g)	Dry Weight (g)	RGI mm day^{-1}
0	**0**	1.48c	5 (12)c	87(69)a	78 (62)a	16.69a	2.90	1.05	7.37a
	100	1.42c	2 (7)d	83(66)a	76 (61)a	17.6a	2.95	1.05	7.68a
	200	1.53c	6 (14)c	75(60)b	59 (50)c	11.0b	2.55	1.06	4.83b
100	**0**	1.55c	10 (18)ab	58(50)d	43 (41)c	3.80c	2.31	1.16	1.56c
	100	1.57c	7 (15) bc	70(57)bc	41 (40)c	4.99c	2.21	1.11	2.12c
	200	1.67c	6 (14)c	64(53)cd	41 (40)c	3.92c	2.14	1.10	1.65c
200	**0**	1.96b	11 (20)a	17(24)e	5 (12)d	0.45d	1.93	1.10	0.22d
	100	2.33a	10 (18)ab	15(23)e	3 (10)e	0.42d	1.89	1.09	0.20d
	200	1.63c	6 (14)c	10(18)f	3 (10)e	0.26d	1.96	1.13	0.12d
300	**0**	0d	0e	0g	0f	0d	1.86	1.13	0d
	100	0d	0e	0g	0f	0d	1.91	1.16	0d
	200	0d	0e	0g	0f	0d	1.77	1.16	0d
LSD		*0.261***	3.609**	*3.856****	*2.552****	*2.080****	---^{NS}	---^{NS}	*0.980****

Table 3. Effects of n-TiO_2 and salinity levels on MGT, RE, GR, CN, RL, FW, DW, RGI on thr 3rd day.

NaCl (mM)	MGT	RE(%)	GR(%)	CN (%)	RL(mm)	FW(g)	DW (g)	RGI
0	1.48b	4 (11)b	82 (65)a	71 (57)a	15.1a	2.80a	1.05	6.62a
100	1.59b	7 (15)a	64 (53)b	42 (40)b	4.2b	2.21b	1.12	1.77b
200	1.97a	8 (17)a	14 (22)c	3 (11)c	0.4c	1.93bc	1.11	0.18c
300	0c	0c	0d	0d	0c	1.85c	1.15	0c
LSD	*0.151****	*2.084****	*2.226****	*1.474****	*1.201****	*0.369****	---^{NS}	*0.566****
n-TiO_2 (mg L^{-1})								
0	1.25	6 (13)a	41 (36)a	31(29)a	5.24a	2.25	1.11	2.29a
100	1.32	5 (10)b	42 (36)a	30 (28)b	5.75a	2.24	1.10	2.50a
200	1.21	4 (10)b	37 (33)b	26 (25)c	3.80b	2.11	1.11	1.65b
LSD	---^{NS}	*1.804**	*1.928***	1.276***	*0.977***	---^{NS}	---^{NS}	*0.489***

*p<0.05, **p<0.01, ***p<0.001, ^{NS}: Nonsignificant. Figures in parentheses are arcsine transformed values of percentages.

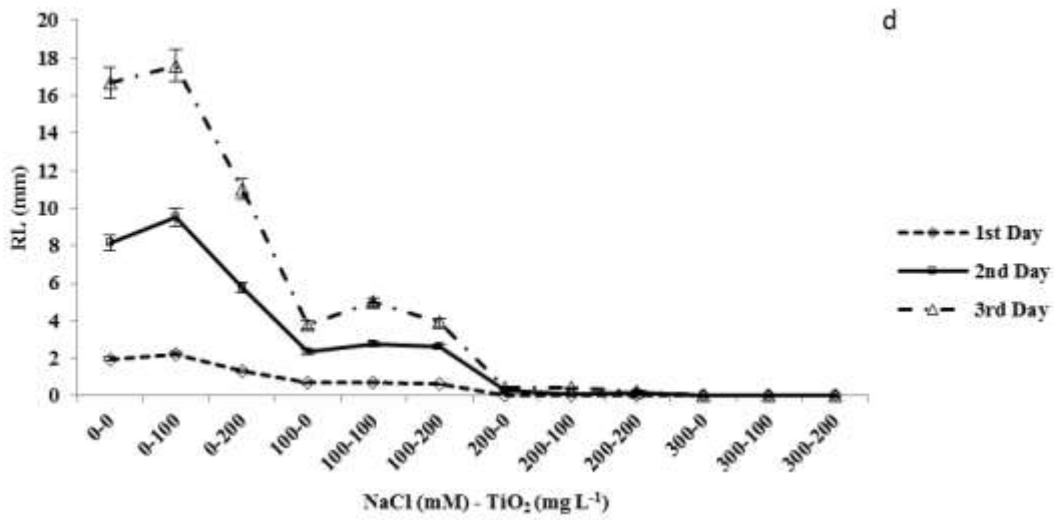

Figure 3. Root length (RL, mm) of barley seedlings in different concentrations of TiO_2 NPs/NaCl treatments depending on the days.

4. Conclusion

This experimental design showed that n-TiO_2 seed priming application at 100 mg L^{-1} improved the seedling growth compared to control, and also alleviated the negative effects of salt stress (100 mM NaCl) by improving growth. The results also show that the effects of nanoparticles may vary depending on concentration. The effects of nanoparticles vary depending on the period of plant development and the duration of application. Therefore, it should be studied in more detail. If the strong clues obtained are supported by observing the advanced development stages of the plants and making field studies, more final results can be reached.

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