Tarım Bilimleri Araştırma Dergisi 7 (1): 45-48, 2014
ISSN: 1308-3945, E-ISSN: 1308-027X, www.nobel.gen.tr

The Effects of *Trichoderma harzianum* on Germination of Onion (*Allium cepa* L.) Seeds Under Salt Stress Conditions

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Received: April 06, 2014
Accepted: May 18, 2014

Abstract

Although several works have been published on abilities of *Trichoderma harzianum* strains to reduce the effects of some abiotic stress factors, their effects on salinity conditions have not been explained. This study was conducted to investigate the effects of *T. Harzianum* T22 *Rifai* KRL-AG2 on germination of Onion (*Allium cepa* L.) seeds under salinity conditions which prepared using NaCl. The seeds of 'Kantartopu-3' cultivar were treated with different concentrations of *T. harzianum* strain T22. Seeds of onion were tested at germination salinity of 0, 2.4, 4.8 and 9.8 dSm^{-1} in darkness. Germination percentage (%), mean germination time (days), shoot length (mm) and seedling fresh weight (mg) were measured at 12 days after sowing. Treatments of *T. Harzianum* strain T22 showed different responses to measured parameters under abiotic stress conditions.

KeyWords: Onion, Abiotic Stress, NaCl, Salinity, Fungi.

*Trichoderma harzianum'*un Tuz Stresi Altında Soğan (*Allium cepa* L.) Tohumlarının Çimlenmesi Üzerine Etkileri

Özet

Trichoderma harzianum ırklarının, bazı abiotik stres faktörlerinin etkilerini azalttığına dair çeşitli çalışmalar yapılmış olmasına rağmen, tuz stresine yönelik etkileri tam olarak açıklanamamıştır. Bu çalışma, *T. Harzianum* T22 *Rifai* KRL-AG2' nin, NaCl kullanılarak oluşturulan tuz stresi altında, soğan (*Allium cepa* L.) tohumlarının çimlenmelerine olan etkilerini araştırmak için yapılmıştır. Kantartopu-3 soğan çeşidine ait tohumlar, farklı dozda *T. Harzianum* T22 *Rifai* KRL-AG2 ile muamele edilmiştir. Tohumlar, 0, 2.4, 4.8 ve 9.8 ds/m tuzluluk koşullarında test edilmiştir. Denemenin 12 gününde çimlenme yüzdesi (%), ortalama çimlenme süresi (gün), sürgün uzunluğu (mm) ve sürgün taze ağırlığı (mg) ölçülmüştür. *T. Harzianum* strain T22 uygulamaları, abiotik stres koşulları altında ölçülen değerler için farklı sonuçlar meydana getirmiştir.

Anahtar Kelimeler: Soğan, Abiotik Stres, NaCl, Tuzluluk, Fungi

INTRODUCTION

Many important crops are glycophytes that show susceptibility to soil salinity. Seed germination and emergence are critical to the survival of plants in salt-affected areas [1]. Among crop species, different threshold tolerances (Electrical conductivities, EC) and different reduction rates of yield are seen and this indicates that there is variation in salt tolerance mechanisms [2]. According to Maas (1977)[3], the onion is very sensitive to EC values as low as 1.2 dSm^{-1}. Onion (*Allium cepa* L.) is an important crop that is now cultivated globally. According to the most recent data of the United Nations Food and Agriculture Organization (FAO), worldwide onion production was approximately 83 million tonnes in 2012 from 4.20 million hectares. According to the FAO, Turkey produces 1.81 million tonnes of onions annually, which is 2.1% of world onion production, and it ranks as the 7th largest onion producer.

There are reports of enhanced plant growth as a result of the association of *Trichoderma* strains with plants but the effects, as with other plant-growth-promoting microbes [4], are greater when plants are under suboptimal conditions or biotic, abiotic, or physiological stresses [5;6;7]. Several recent reports indicate that the fungi enhances tolerance to abiotic stresses during plant growth [6;8;9], in part due to improved root growth, improvement in water-holding capacity of plants, or enhancement in nutrient uptake (i.e., potassium); whereas, in the absence of stress, plant growth may (Bae and al., 2009) or may not be enhanced [5;6;8;9].

The purpose of this study was conducted to investigate the effects of *T. Harzianum* T22 *Rifai* KRL-AG2 on germination of onion seeds under salinity conditions using germination percentage (%), mean germination time (days), shoot length (mm) and seedling fresh weight (mg) parameters.

MATERIAL AND METHODS

The germination of Kantartopu-3 cultivar was tested at different NaCl concentrations. Seeds of 'Kantartopu-3' were obtained from the Atatürk Central Horticultural Research Institute in Yalova. Three salinity treatments and a control were used. For the salinity treatments, NaCl were added to distilled water. The germination potential of seeds was estimated in accordance with the International Seed Testing Association procedures (ISTA, 1985). The seeds were surface sterilized under aseptic conditions with 70 % ethanol for 1 min, followed by 20% commerical Clorox (5.25 % sodium hypochlorite) for 10 min., and then washed 4 times with distilled water. Three replicates of 50 seeds were evenly spaced in 90 mm petri dishes on Whatman no. 1 filter papers moistened with salt solutions. Salinity levels of the solutions at 20 ˚C were adjusted to 2.4, 4.8 and 9.6 dSm^{-1} (deciSiemens m^{-1}) by using different NaCl concentrations [10]. Distilled water served as the control.

The as biocontrol agent, different densities of *Trichoderma harzianum* Rifai KRL-AG2 were used in this study. For spore production, *T. harzianum* was grown on potato dextrose agar medium (PDA) (Merck) for 7 days at 25 ^{o}C. Conidial densities in the suspension were determined by use of a hemocytometer under a light microscope. The 5 ml of a conidial suspension containing 0 (*Trichoderma* free*)*, 1,5x10^6, 2x10^6, 2,5x10^6 conidia of *T.harzianum* per mililiter was added petri dishes. Petri dishes which includes salt solution+*T.harzianum* solution were covered with lids and placed in an incubator maintained at 20 ± 2˚C in darkness. For determination of salt tolerance, germination percentage (%), mean germination time (days), shoot length (cm) and seedling fresh weight (mg) were used as the parameters during the germination stages.

RESULTS AND DISCUSSION

The germination percentage and fresh weight were significant for three factors (densities of conidium, salinity and their interaction). Shoot lenght and mean germination time (T_M) were significant for only salinity (Table 1).

Generally, germination percentages (%) were reduced by increased salt concentration. That effect was more pronounced in *T.harzianum*-free treatment than other treatments. In all treatments of *T.harzianum*, maximum seed germination was obtained in the salt-free condition (Table 2). Generally, fresh weight (mg) value was reduced by increased salt concentration (Table 3). When seeds were incubated with *T.harzianum*, especially at 2.4 dsm^{-1} and 4.8 dsm^{-1} level, the fresh weight (mg) value was higher than *T.harzianum*-free treatment.

Table 1. A two-way analysis of variance

		F Ratio			
Source	DF	GP	SL	Weigh	T_M
Fungi	3	38,69*	2,53ns	6,95*	2,62ns
Salinity	3	124,60*	83,30*	149,29*	11,60*
Fungi x Salinity	9	9,44*	0,85ns	2,69*	1,01ns
C. Total	32				
Error	47				

*significant (p < 0.01), ns: not significant. F: Freedom, DF: Degree of freedom, GP: Germination percentage, SL: Shoot Lenght, T_M: mean germination time, C. Total: Corrected Total

Table 2. Germination percentage of onion seeds in saline solutions (%)

	Salinity (dSm^{-1})				
Densities of conidia (x10^6)	Salt-free	2,4	4,8	9,6	Avr.
Trichoderma-free	90,00 a*A*	74,67 bAB	60,67 bB	38,00 bC	65,84
1,5	91,33 aA	81,33 aB	78,67 aB	64,67 aC	79,00
2	92,00 aA	84,67 aB	79,33 aC	63,33 aD	79,83
2,5	92,00 aA	82,67 aB	79,33 aB	64,00 aC	79,50
Avr.	91,33	80,84	74,50	57,50	

*Means within a column that have a different small letter are significantly different from each other and means within a row that have different capital letter are significantly different from each other (P < 0.01).

Table 3. Fresh weight of onion seedlings in saline solutions (mg)

	Salinity (dSm^{-1})				
Densities of conidia (x10^6)	Salt-free	2,4	4,8	9,6	Avr.
Trichoderma-free	10,67 a*A*	8,33 bB	7,33 bB	4,00 aC	7,58
1,5	10,00 aA	9,87 aA	9,43 aA	5,33 aB	8,66
2	9,67 aA	10,00 aA	9,33 aA	5,17 aB	8,54
2,5	10,00 aA	10,00 aA	9,40 aA	5,00 aB	8,60
Avr.	10,09	9,55	8,87	4,88	

*Means within a column that have a different small letter are significantly different from each other and means within a row that have different capital letter are significantly different from each other (P < 0.01).

Significant differences were obtained for only salinity regarding shoot length (mm) ($p < 0.01$) (Table 4). The longest shoot length was in the salt-free and 1.2 dsm^{-1} salinity conditions. Shoot length declined as salinity increased from 4.8 dsm^{-1} to 9.6 dsm^{-1}. The mean time to germination was also significantly affected by salinity concentration (Table 5). When seeds were incubated with NaCl, especially at 9.6 dSm^{-1} salinity, the mean time to germination was lower than other concentrations of NaCl. For each level of NaCl and *T.harzianum*, germination was observed. In *T.harzianum*-free treatment, with increasing NaCl concentration from 0 to 9.6 dSm^{-1}, germination percentage decreased from 90,00% to 38,00%. This trend was more lower in *T.harzianum* including conditions.

Reduction in germination caused by increased salinity has been described by numerous authors [11;12]. In the present study, significant differences were obtained for salinity regarding germination percentage, shoot length, mean time to germination (T_M) and final fresh weight. Similar results were earlier noted for several crops for example, eggplant and triticale [13;14]. A few recent reports demonstrated that these fungi alleviate abiotic stresses [15]. Our results supports that of these studies. According to results, it is clear that there is a possitive effect of *T. harzianum* on during germination stages of onion (*Allium cepa* L.) seeds under salt stress conditions. This effect is more pronounced for germination percentages. But, differences of densities of *T. harzianum* were not significant for examined traits. In a recent report, squash plants treated with T22 or other beneficial microorganisms were more tolerant of salinity than untreated ones. These data, along with the frequent observation that the greatest advantage of *Trichoderma* treatments to plants occurs when they are under stress, gives credence to the concept that these beneficial fungi ameliorate both biotic and abiotic plant stresses [9]. Björkman and al., (1998) reported that a seed treatment with *T. harzianum* could confer advantages to maize seed with poor vigor caused by genetic manipulations (early varieties of sweet corn with the *sh* gene), increasing both vigor and germination. In another report, it is examined that effects of *Trichoderma* treatment of tomato seed on several physiological and abiotic stresses during imbibition and germination. All stresses examined were alleviated by T22 treatment, including osmotic and salinity stresses, changes in temperature, and loss of seed quality due to storage conditions that give rise to physiological intrinsic stress. According to results, it is suggested that despite significant differences between these stresses, signaling pathways and cellular responses to them share common features [15].

CONCLUSIONS

In conclusion, *Trichoderma harzianum* Rifai KRL-AG2 had a greater effect on germination parameters of onion seeds under salinity stress. In the *T. harzianum*-free treatment, increased salinity levels resulted in decreased germination, inhibition of seedling growth and increased mean germination time. This negative effects of salinity were less in all *T. harzianum* treatments. The differences of densities of *T. harzianum* were not significant for germination percentage (%), mean germination time (days), shoot length (cm) and seedling fresh weight (mg). The possitive effects of *T. harzianum* is pronounced up to 9,6 dsm^{-1} salinity level.

Table 4. Shoot length of seedlings of onion in saline solutions (cm)

	Salinity (dSm^{-1})				
Densities of conidia ($x10^6$)	Salt-free	2,4	4,8	9,6	Avr.
Trichoderma-free	8,22 A*	7,78 B	4,39 C	2,39 D	5,70
1,5	8,19 A	7,81 A	6,28 A	3,90 B	6,55
2	8,28 A	7,78 AB	6,22 B	3,72 C	6,50
2,5	8,40 A	7,73 A	5,67 B	3,42 C	6,31
Avr.	8,27 A	7,78 A	5,64 B	3,36 C	

*Means within a row that have a different capital letter are significantly different from each other ($P < 0.01$)

Table 5. The mean time to germination of onion seeds in saline solutions (days)

	Salinity (dSm^{-1})				
Densities of conidia ($x10^6$)	Salt-free	2,4	4,8	9,6	Avr.
Trichoderma-free	6,67 B	7,33 B	9,00 A	10,00 A	8,25
1,5	6,67 B	6,67 B	7,67 AB	8,67 A	7,42
2	6,67 A*	7,33 A	7,33 A	8,00 A	7,33
2,5	7,00 B	7,67 AB	8,00 AB	8,33 A	7,75
Avr.	6,75 C	7,25 C	8,00 B	8,75 A	

*Means within a row that have a different capital letter are significantly different from each other ($P < 0.01$)

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