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Research Article/Araştırma Makalesi

The effect of sodium chloride salinity on coated and uncoated alfalfa seeds germination

Sodyum klorür tuzluluğunun kaplamalı ve kaplamasız yonca tohumlarının çimlenmesi üzerine etkisi

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ABSTRACT

In this study, a pod experiment was conducted to evaluate the effect of sodium chloride salinity on germination of coated and uncoated seeds of alfalfa seeds under controlled greenhouse conditions at Konya in May 2013. The experimental design was a split-split plot with salinity treatments as main plots, coated and uncoated seeds subplots. Pots were irrigated with tap water (0.3 dS m^{-1}) or (3.0, 6.0 or 9.0 dS m^{-1}). As result of study, seed germination was significantly affected by irrigation water salinity at the initial stage of germination. For coated and uncoated seed, the final seed germination exceeded 79.00% except at the salt concentrations higher than 3.0 dSm^{-1} where germination percentage statically decreased down. The highest germination rate index (GRI, 14.29%), germination index (GI, 998.75), and coefficient of velocity of germination (CVG, 16.97) values were obtained from the coated seeds irrigated with the tap water while the lowest GRI (1.79%), GI (138.75), and CVG (12.57) values were obtained from the uncoated seeds irrigated with salt concentration of electrical conductivity (EC) of 12.0 dS m^{-1}. Earlier germinations were recorded for coated seeds as indicated by lower value of mean germination time (MGT) and time of 50% germination (T_{50}).

Key Words: *Medicago sativa*, Emergence, Chlorinity, Lime

ÖZ

Bu çalışma, Mayıs 2013 tarihinde Konya'da kontrollü sera şartlarında sodyum klorür tuzluluğunun kaplanmış ve kaplanmamış yonca tohumları çimlenmesi üzerine etkisinin araştırılması amacıyla yürütülmüştür. Saksı denemesi olarak kurulan deneme, tuzluluk ana muamele, kaplanmış ve kaplanmamış yonca tohumları alt muamele olarak bölünmüş parsel deneme planına göre tasarlanmıştır. Saksılar musluk suyu (0.3 dS m^{-1}) veya elektriksel iletkenlikleri 3.0, 6.0 ve 9.0 dS m^{-1} olan tuzlu sular ile sulanmıştır. Araştırmanın sonucunda, tuzluluğun çimlenmenin başlangıç aşamasında tohumların çimlenmesini önemli derecede etkilediği, kaplanmış ve kaplanmamış tohumlarda, toplam çimlenme yüzdesinin, 3.0 dS m^{-1} değerinden daha yüksek tuzluluk seviyeleri hariç olmak üzere %79'un üzerinde olduğu, 3.0 dS m^{-1} değerinden daha yüksek sulama suyu tuzluluk değerlerinde çimlenmenin istatistiki olarak farklı olduğu, en yüksek çimlenme oran indeksi (%14.29), çimlenme indeksi (998.75), ve çimlenme hızı katsayı (16.97) değerlerinin musluk suyu ile sulanan kaplanmış tohumlardan, en düşük çimlenme oran indeksi (%1.79), çimlenme indeksi (138.75) ve çimlenme hızı katsayısı (12.57) değerlerinin ise elektriksel iletkenliği 12.0 dS m^{-1} olan suyla sulanan kaplanmamış tohumlardan elde edildiği tespit edilmiştir. Erken çimlenme değerlerinin düşük ortalama çimlenme zamanı (MGT) ve %50 çimlenme zamanı (T_{50}) değerlerinin elde edildiği kaplanmış tohumlarda görüldüğü bulunmuştur.

Anahtar Kelimeler: *Medicago sativa*, Çıkış, Tuzluluk, Kireç

Introduction

Alfalfa (*Medicago sativa* L.) is high quality perennial legume forages for livestock all over the world. It is defined as queen of the forages because of its high nutritional quality. Alfalfa evolved in an area that has a pronounced continental climate with cold winters and hot, dry summers. A late spring and short summer characterize these regions (Hanson and Kehr, 1972). In Turkey, alfalfa cultivated land has increased from 539.000 ha (2007) to 659.000 ha (2017) over period of ten years in Turkey. It is cultivated on over 659.000 ha in solid stand, which is about 3% of total agricultural land and 12% of total irrigated agricultural land in Turkey (TUİK, 2018). Although it is cultivated for many years, there is lack of alfalfa varieties improved in Turkey. So alfalfa seeds are being imported in large quantities to Turkey.

Increasing world population requires greater food production in future. One way to meet this need is to bring marginally productive and presently non-arable land, much of which affected by salinity, under crop production (Allen et al., 1985). The large areas of saline soils and brackish water resources are under utilized worldwide for crop production. The Konya basin where the experiment conducted has a semi arid climate. In this areas where rainfall is insufficient to leach salts from the root zone and evaporation tends to exceed rainfall, salinity remains a major problem facing agriculture.

Although some crops are moderately tolerant of saline conditions many crops are adversely affected by even levels of salt (Greenway and Munns, 1980). In saline environment of plants to salinity during germination and early seedling stages is crucial for the establishment of species. Seedlings are the most vulnerable stage in the life cycle of plants and germination determines when and where seedling growth begins (Gutterman, 2012).

Although alfalfa is a crop that is considered moderately sensitive to salinity (Maas and Hoffman, 1977), (Lehman et al., 1979), it is regarded as susceptible to salts at seed germination (Assadian and Miyamoto, 1987). Coated seeds are accepted widely as a standard product for many crops. Alfalfa and tobacco are two agronomic crops that are coated. (Kaufman, 1991). Seed coating is the practice of covering seeds with external materials to improve handling, protection and to a lesser extent, germination enhancement and plant establishment (Pedrini et al., 2017).

The volume of alfalfa seeds being sold as coated seeds has been increasing for several years in Turkey. The coating normally contains Rhizobia inoculant, fungicides and lime based materials. Therefore, it is generally considered that coating of alfalfa seeds can increase plant establishment.

The objective of this study was to evaluate the effects of coating on germination of alfalfa seeds when the different salinity level water used for irrigation.

Materials and Methods

The experiment was carried out in May 2013 in a greenhouse at Selcuk University Agriculture Faculty in Konya, Turkey with two alfalfa (*Medicago sativa* L.) seeds as coated and uncoated. Alfalfa seed cultivar was Magnum V which is registered variety in Turkish Seed Catalogue. The greenhouse where the experiment conducted was glass covered, 250 m^2 and computer controlled. During experiment, maximum temperature, minimum temperature and relative humidity were maintained as 26±2 °C, 16±2 °C and 65%±5 respectively. The Konya has arid to semi arid climate. Annual rainfall is about 322 mm and mean temperature is 11.6 °C with minimum mean temperature of -4.6 °C in January and maximum mean temperature of 30.1 in July. The sample seeds was treated with Apron XL LS a Fungicide, NitrogenGold – an inoculant and coated with a lime based build up (50%) were purchased from Biotek Seeds Company (Konya, Turkey). The mean seed dry weight per 1000 seeds for coated and uncoated seeds were 3.1

gram and 6.0 gram respectively. Seed moisture ranged between 6 and 7%. When the experiment was conducted, the seeds were less than 12 months old and had been previously stored in bags under laboratory conditions. The seeds were hand-sorted from the coated seed lot and non-coated seeds lots. Treatment was developed by washing the coated seeds with water for 5 min, which effectively removed all the seed coating from the seeds then counted into 100 seed lots. The coated and non-coated seeds were planted immediately after washing into the soils on 8 May 2016. Loamy sand soil taken from a field which previously carried a wheat crop was used for the experiment mainly because of its low crusting potential. The soil with pH of 7.75, electrical conductivity of saturation extract (ECe) of 1.40 dSm^{-1}, sodium adsorption ratio (SAR) of 4.25 and field capacity of 21.2% and permanent wilting percentage of 12.2% by weight was air-dried and sieved through a 2 mm mesh and filled to the plastic pods with a mean diameter of 23 cm an height of 21 cm. The bulk density and available water holding capacity of the soil were 1.43 gr cm^{-3} and 1.29 mm/cm respectively. 100 alfalfa seeds were sown in each pot to a depth 1.5 cm. The plant root zone depth was considered same as height of plastic pods.

The experimental design was a split-split plot with salinity treatments as main plots, coated and uncoated seeds subplots. All treatments were replicated four times.

Saline solutions were prepared with NaCl. The electrical conductivity (EC) of the solutions were determined with a conductivity meter (model PCM 3, Jenway, Felstead,UK). The electrical conductivity of the treatments were tap water (0.3 dSm^{-1}) or (3.0, 6.0, 9.0 or 12.0 dSm^{-1}).

Irrigation water was applied to maintain field capacity of soil depending upon irrigation interval ranging 3 days. The amount of irrigation varied between 0,5 to 0,9 liter per pot per irrigation depending upon irrigation interval. The irrigation water did not have any direct contact with plant leaves during irrigation applications. Crop germination was recorded daily till all the seeds were germinated and emerged from the soil surface. A seedling with the hypocotyls or cotyledon showing on the soil surface was regarded as having emerged. To measure the effect of treatments on seed and seedling performance, coefficient of velocity of germination (CVG), germination index (GI), germination rate index (GRI), mean germination time (MGT), time of 50% germination (T_{50}), final germination percentage (FGP), first day o germination (FDG), Last day of germination (LDG), Time spread of germination (TSG), were calculated by following formulas. $CVG = 100 \times \sum Ni / \sum NiTi$, where Ni is the number of germinated seeds for each day, Ti is number of days from seedling (Jones and Sanders, 1987), $GI = 17 \times N_1 + 16 \times N_2 + ... + 1 \times N_{17}$, where N_1, N_2 …… N_{17} of germinated seeds on the first, second and subsequent days until the 17th day, 17,16,…. and 1 are weights given to the number of germinated seeds on first, second and subsequent days, respectively (Benech Arnold et al., 1991), where, G_1 is the germination percentage at first day, G_2 is the germination percentage at second day and so forth (Esechie, 1994), $MGT = \frac{\sum NiT_i}{\sum N_i}$, where N_i is the number of germinated seeds for each day, T_i is number of days counted from the beginning of germination (Orchard, 1977), $FGP = \frac{N_g}{N_t} \times 100$, where, N_g is total number of germinated seeds, N_t is total number of evaluated seeds. The time of 50% germination (T_{50}) was calculated according to the following formula of Coolbear et al. (1984) modified by Farooq et al., (2006). $T_{50} = ti + \frac{[(N/2) - n_i](t_i - t_j)}{n_i - n_j}$, where N is the final number of germination and n_i, n_j cumulative number of seeds germinated by adjacent count at times t_i and t_j when $n_i < N/2 < n_j$

Data of the germination parameters were statistically analyzed one-way analysis of variance (ANOVA) using SPSS version 9.0 (SPSS, 1999). The

analyses of variance were conducted separately within each seed treatments, considering water salinity and seed treatment as fixed factors. When "F" ratios were significant, means were separated by the Duncan's test ($p \leq 0.005$).

Results and Discussion

The first sign of germination was observed after 5 days except treatments irrigated with water EC of 12.0 dSm^{-1} in both coated and uncoated seeds. For the treatments irrigated with the water EC of 12.0 dSm^{-1} first germination was observed on 6^{th} day with %1 and %2.5 germination rate for uncoated and coated seeds respectively.

The final seed germination ranged between 13.50% and 80,50% for uncoated seeds and 20,25% and 82.50% for coated seeds receiving waters of different salinities. The germination of uncoated seeds decreased down to 79.25% with water EC of 3.0dSm^{-1}, 55.75% with water EC 6.0 dSm^{-1}, 43.50% with water EC of 9.0 dSm^{-1}, 13.50% with water EC of 12.0 dSm^{-1} while the germination of coated seeds decreased down to 80,75% with water EC of 3.0 dSm^{-1}, 63.50 % with water EC 6.0 dSm^{-1}, 45.25% with water EC of 9.0 dSm^{-1}, 20,25% with water EC of 12.0 dSm^{-1}. The difference in final germination was not statistically significant between EC of 0,3 dSm^{-1} and EC of 3.0 dSm^{-1} treatments. The final germination percentage showed decreasing trend with increase in water salinity. For coated and uncoated seed, the final seed germination exceeded 79.00% except at the salt concentrations higher than 3.0 dSm^{-1} where germination percentage statically decreased down (Table 1, Figure 1). Earlier studies stated that alfalfa is susceptible to salinity at stage of germination (Assadian and Miyamoto, 1987; James, 1988; Ungar, 1967; Wang et al., 2009). The results showed that fair emergences can be obtained when the seeds are irrigated with water has higher than EC of 3.0 dSm^{-1}. For salt concentration lower than 3.0 dSm^{-1}, when the salt concentration increase ten times more, the final germination percentage for uncoated and coated seeds hasn't decreased more than 2%. But for the salt concentrations higher than 3.0 dSm^{-1}, the final germination percentage decreased dramatically (Fig. 1). Many researches such as Li et al. (2010), Wang et al. (2009), Johnson et al. (1992), and Assadian and Miyamoto (1987) has stated that salt concentration has negative effects on germination of alfalfa seeds. The results obtained from this study are in compliance with previous studies.

Table 1. Effect of irrigation water salinity and seed treatment (UC=Uncoated; C=Coated) Upon FGP(%), GRI, GI And CVG .
Çizelge 1. Sulama suyu tuzluluğu ve tohum uygulamalarının (K: Kaplanmış; NK:Kaplanmamış) ÇY (%), ÇOI, ÇI Ve ÇHK Etkisi

Salinity levels Tuzluluk seviyesi (dSm^{-1})	FGP (%) ÇY (%)			GRI ÇOI			GI ÇI			CVG ÇHK		
	UC K	C NK	Mean Ort.	UC K	C NK	Mean Ort.	UC K	C NK	Mean Ort.	UC K	C NK	Mean Ort.
0,3	80,50	82.50	81.50a	13.20	14.29	13.74a	922.75	998.75	960,75a	15.32	16.97	16.15a
3.0	79.25	80,75	80,00a	12.54	13.53	13.04a	878.25	961.50	919.88a	14.93	16.41	15.67ab
6.0	55.75	63.50	59.63b	8.28	9.89	9.11b	614.50	716.00	667,38b	14.37	14.81	14.69bc
9.0	43.50	45.25	44.38c	6.01	6.81	6.41c	459.00	506.00	482.50c	13.39	14.49	13.94c
12.0	13.50	20,25	16.63d	1.79	2.58	2.19d	138.75	199.00	168.88d	12.57	12.94	12.75d
Mean	54.50a	58.45a		8.36b	9.42a		602.65a	676.25a		14.12a	15.05a	

FGP: First Germination Percentage GRI: Germination Rate Index, GI: Germination Index, CVG: Coefficient of Velocity of Germination
ÇY : Final Çimlenme Yüzdesi ÇOI: Çimlenme Oranı İndeksi, ÇI :Çimlenme İndeksi ÇHK :Çimlenme Hızı Katsayısı

The seed germination exceeded 80% within 7 days for coated seeds and 15 days for uncoated seeds when the seeds were irrigated with tap water. The seed germination for coated and uncoated seeds didn't exceed 80 % on the other salt concentrations except for coated seed irrigated with salt concentration of EC of 3.0 dSm^{-1}. The seed germination exceeded 80% within 9 days on this treatment.

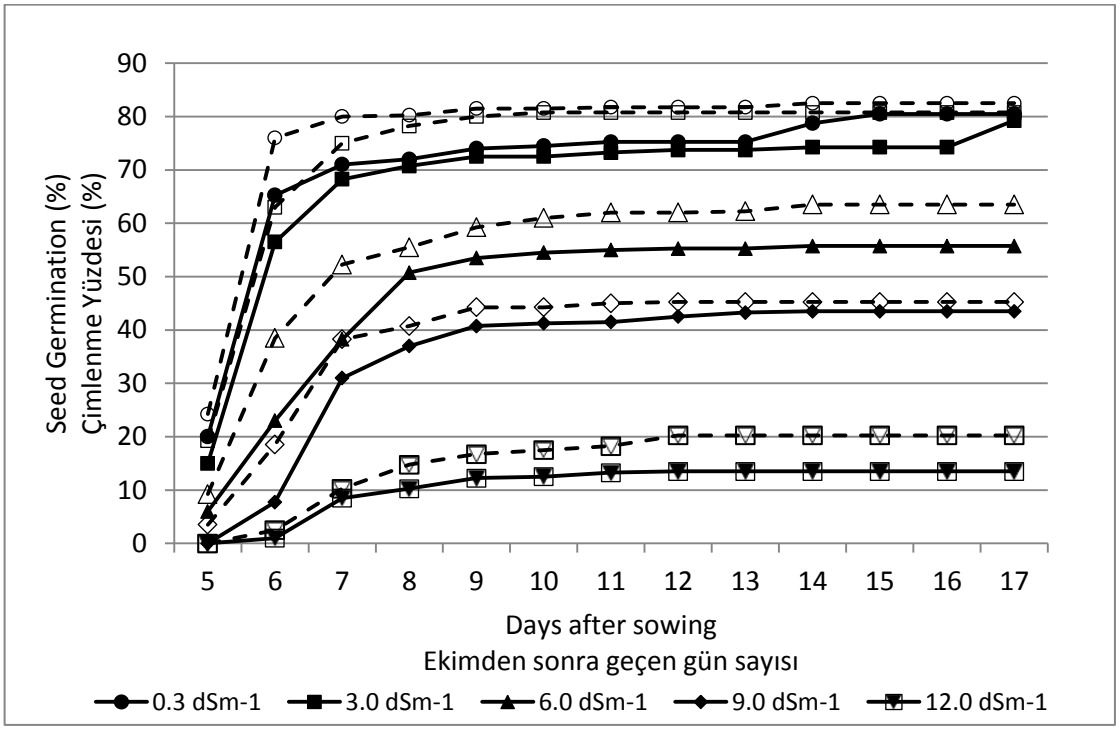


Figure 1. Cumulative seed germination of uncoated (UC, closed symbols) and coated (C, open symbols) alfalfa seeds. Symbols represent the observed percentages with time at each water salinity.

Şekil 1. Kaplamalı (C, açık sembol) ve kaplamasız (UC, kapalı sembol) yonca tohumlarının yığışımlı tohum çimlenmesi

The lower GRI, GI and CVG values were obtained with the increase in water salinity for both coated and uncoated seed. Earlier germinations were recorded for coated seeds as indicated by higher GRI, GI and CVG values (Table 1). The highest GRI (14.29%), GI (998.75), and CVG (16.97) values were obtained from the coated seeds irrigated with the tap water while the lowest GRI (1.79%), GI (138.75), and CVG (12.57) values were obtained from the uncoated seeds irrigated with the with salt concentration of EC of 12.0 dSm^{-1}. Results show that seed coating significantly affected GRI. GRI reflects the percentage of germination on each day of the germination period and basically gives an indication of the percentage of seeds germinating per day of the test run period (Al-Mudaris, 1998). According to results, It has concluded that the salt concentration in the irrigation water has decreased GRI significantly. Accordingly, the coating has increased the percentage of seed germinating per day.

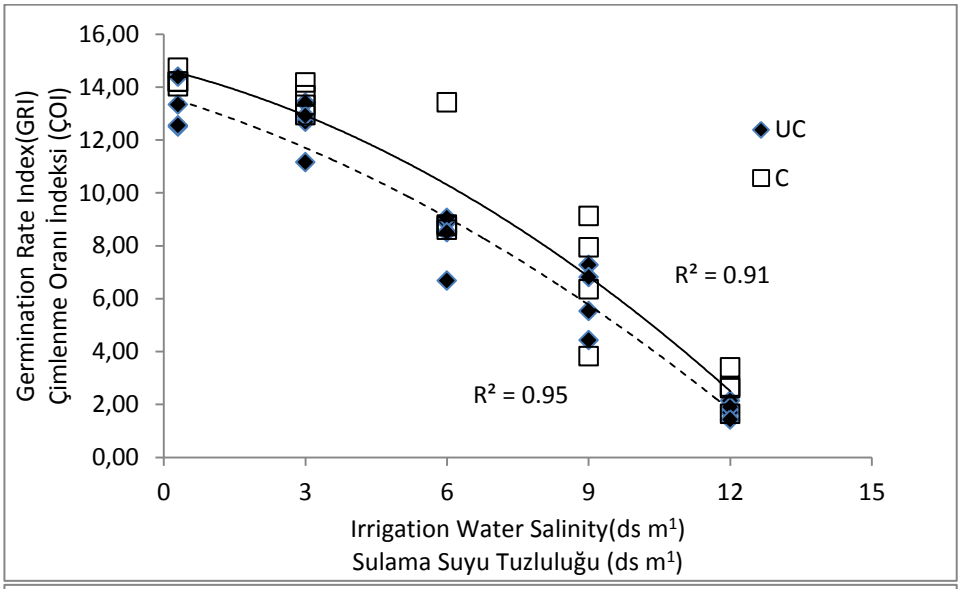
Polynomial regression analysis was used to determine relationship between GRI, GI, CVG and different irrigation salinity level. It was found that strong negative relationship with GRI and GI and irrigation water salinity with coefficient of determination (R^2) ranged from 0.91 to 0.95. R^2 values obtained from coated seeds were higher than uncoated seeds, which means that the relationship between GRI, GI and irrigation water salinity is more strong for coated seeds.

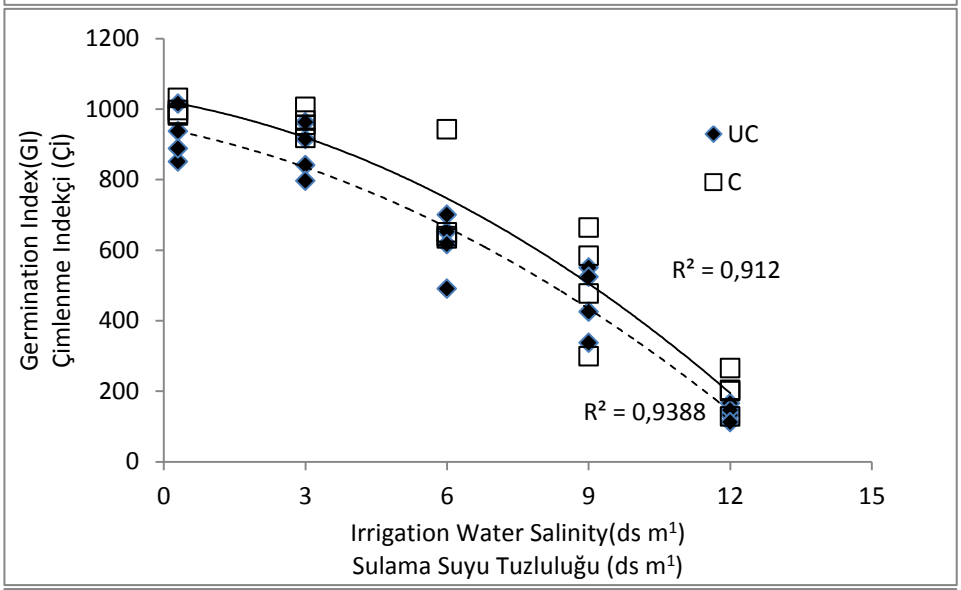
Table 2. Effect of irrigation water salinity and seed treatment (UC=Uncoated; C=Coated) upon MGT (Days) and T50.

Çizelge 2. Sulama suyu tuzluluğunun kaplanmış ve kaplanmamış tohumlarda ortalama çimlenme zamanı (OÇZ) ve %50 çimlenme zamanı (T_{50}) üzerine etkileri

Salinity levels(dS m^{-1}) (Tuz seviyesi)	MGT (Days) OÇZ (Gün)			T_{50} T_{50}		
	UC K	C NK	Mean Ort.	UC K	C NK	Mean Ort.
0,3	6.55	5.89	6.22c	5.44	5.33	5.39d
3.0	6.84	6.09	6.47c	5.60	5.48	5.54d
6.0	6.96	6.76	6.86bc	6.34	5.77	6.08c
9.0	7,50	6.96	7,23b	6.71	6.36	6.53b
12.0	7,73	8.00	7,86a	6.78	7,08	6.93a
Mean Ort.	7,12a	6.74a		6.17a	6.00a	

MGT: Mean Germination Time T_{50}: The time of 50% germination
OÇZ :Ortalama Çimlenme Zamanı T_{50}: %50 Çimlenme Zamanı





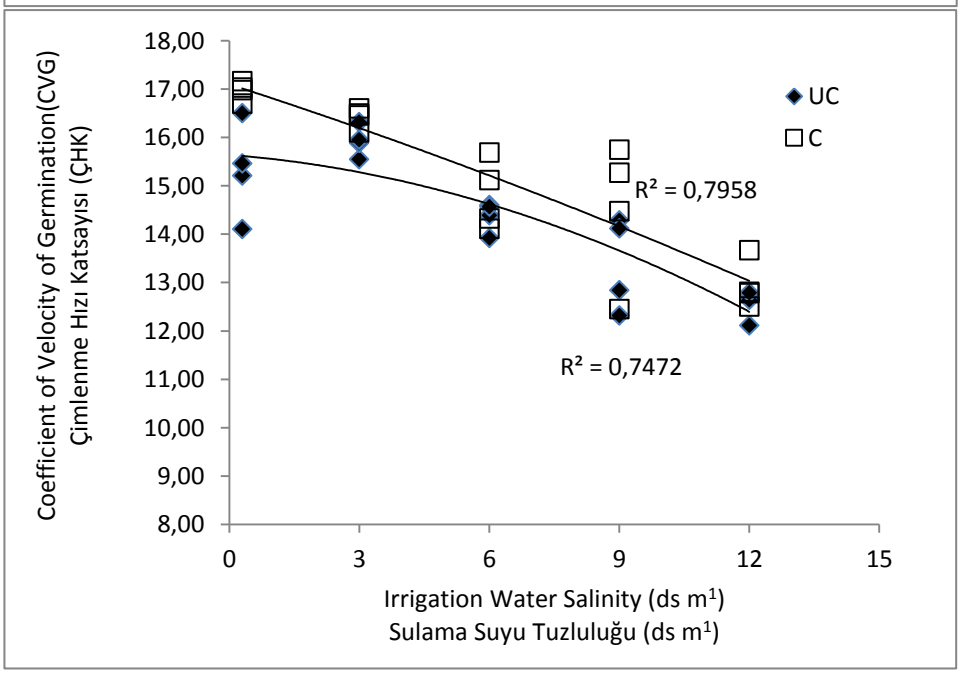


Figure 2. Variation of the germination rate index (GRI), germination index (GI) and coefficient of velocity of germination (CVG) of uncoated and coated alfalfa seed according to different irrigation water salinity. Lines describing the evolution of each parameter were obtained using polynomial regression.

Şekil 2. Farklı sulama suyu tuz seviyelerine göre kaplanmış ve kaplanmamış yonca tohumlarının çimlenme oranı indeksi (ÇOİ), çimlenme indeksi (Çİ) ve çimlenme hızı katsayıları (ÇHK) arasındaki varyasyonlar.

The higher MGT and T_{50} values were obtained with the increase in water salinity for both coated and uncoated seed. Earlier germinations were recorded for coated seeds as indicated by lower value of MGT and T_{50} (Table 2). The coated seeds irrigated with tap water had lowest values of MGT (5.89) and T_{50} (5.33) with no significant difference with uncoated seeds irrigated with tap water. The highest MGT (8.00) and T_{50} (7.08) values was obtained from the coated seeds irrigated with the salt concentration of EC of 12.0 dSm^{-1}. MGT and T_{50} progressively delayed at salt concentration higher than EC of 3.0 dSm^{-1} where the effect of salt concentration on MGT and T_{50} is statistically significant.

Conclusion

The aim of this greenhouse pod experiment was to determine the differences between coated and uncoated seeds in terms of germination. Germination tests showed that the FGP, GRI, GI, CVG have decreased down and MGT, T_{50} have increased up for both coated and uncoated seeds with increasing water salinity. There was found no difference between coated and uncoated seeds in terms of FGP, GI, CVG, MGT and T_{50}. The only difference between coated and uncoated seeds found in GRI values. The higher values have obtained from coated seeds relatively. GRI reflects the percentage of germination on each day of the germination period. It can be concluded that the coating increased the germinated seeds for each day. However, pod experiments may not give precise estimate of seedling emergence in the field. Field experiments should be conducted to understand the effects of seed coating on seedling and growth of alfalfa.

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