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Araştırma Makalesi/Research Article

Seed Deterioration in Barley Seed Under Accelerated Aging Test

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Abstract

Seed deterioration is considered to begin at physiological maturity and continues during harvesting and storage. It's influenced by genetic, production and environmental factors. Genetic differences are between cultivars for the ability to sustain good seed quality in nonsuitable environments. The study aim was to determine the seed deterioration of *Hordeum vulgare* L. seed lots with accelerated aging tests in three different periods. Seven different proprietary barley varieties used in the study were obtained from Aegean Agricultural Research Institute (İzmir/Turkey). Our methods which used for this purpose; at 30 °C (7 day) germination and seedling test, accelerated aging test with 24-48 and 72 hours at 45 °C and EC test at 20 °C in 24-48-72 hours were made. Observations made at the end of the treatments; seed moisture content (%), normal-abnormal germination rate (%), mean germination time (day). The germination rates of varieties are close to each other and very high. However, at the end of the vigor test of the aged cultivars, significant differences were observed especially in the mean germination time and seedling emergence performance. In EC measurement results, the best value was taken from Sancak variety.

Keywords: *Hordeum vulgare*, germination rate, accelerated aging test, electrical conductivity, genotypes

Hızlı Yaşlandırma Testi Koşulları Altında Arpa Tohumlarında Bozulma

Öz

Tohumda bozulma fizyolojik olgunlukta başlar, hasat ve depolama sırasında devam etmektedir. Bu durum genetik, üretim ve çevresel faktörlerden etkilenir. Uygun olmayan ortamlarda iyi tohum kalitesini sürdürebilmek, çeşitler arasındaki genetik farklılıklar ile mümkündür. Bu çalışmanın amacı, üç farklı periyotta hızlandırılmış yaşlanma testleriyle *Hordeum vulgare* L. tohum lotlarının tohum bozulma durumlarını belirlemektir. Araştırmada kullanılan yedi farklı tescilli arpa çeşidi İzmir Ege Tarımsal Araştırma Enstitüsü'nden temin edilmiştir. Bu amaçla kullanılan yöntemlerimiz; 30 °C'de (7 gün) çimlenme ve fide testinde, 24-48 ve 72 saatte 45 °C'de yaşlanma testi ve 20 °C'de 24-48-72 saatte EC testi yapılmıştır. Çalışmamızın sonunda yapılan gözlemler; tohum nem içeriği (%), normal anormal çimlenme oranı (%), ortalama çimlenme süresi (gün) ve elektriksel kondaktivite değeri (µS/cm) dir. Çeşitlerin çimlenme oranları birbirine yakın ve çok yüksektir. Bununla birlikte, güç testi sonucunda yaşlandırılmış çeşitlerin, özellikle ortalama çimlenme süreleri ve fide çıkış performanslarında önemli farklılıklar gözlenmiştir. EC ölçüm sonuçlarında en iyi değer Sancak çeşidinden alınmıştır.

Anahtar Kelimeler: *Hordeum vulgare,* çimlenme oranı, hızlı yaşlandırma testi, elektriksel kondaktivite, genotipler

Introduction

Cereals, which are the most important source of human and animal nutrition, is the product group which is mostly cultivated and produced most in the world. Most of the foods consumed by people in their daily diets are composed of vegetable-based foods. Almost 66% of these are composed of cereals (Ergutay and Elgün, 1995). Barley, which is the main plants cultivated in the world history, has been used in human nutrition for thousands of years. The findings uncovered the archaeological excavations done in the early 1900s have revealed that barley was grown also in 3000s B.C. It is known that its culture is very old also in Anatolia territory and its homeland is the geographical region named as "Fertile Crescent" including the countries such as Israel, Jordan, Palestine, Syria, Iraq, and Iran and covering also Turkey (Harlan and Zoharry, 1966; Gökgöl, 1969; Nesbitt and Samuel, 1995). Barley is one of the plants that was first cultivated by people in order to meet the basic food needs and has a high adaption capability (Allard and Bradshaw, 1964). Barley, that takes the second place after wheat among the cool climate cereals with the cultivation area of 2.8 ha and the production of 8

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million tons in Turkey, is mostly consumed as animal feed and the raw material of malt industry. Barley production areas have decreased 19.1% in the last 23 years in Turkey, the increase in yield specified in the other products could not be achieved (TÜİK, 2015). In the world, the barley production area was 55 million hectares in 2001 but decreased to 46 million hectares in 2016. The worldwide barley production amount was 140 million tons in 2001 and was 141 million tons in 2016 (FAO, 2018).

Barley takes the 4th place after wheat, maize, and paddy, that are mostly produced cereals in the world and takes the second place among the cool climate cereals. In Turkey, the barley cultivation area was about 36 million decares in 2001; however, this area deceased to 24 million decares in 2017. The production amount was approximately 8 million tones in 2001 and it was 7 million in 2017 (TÜİK, 2018).

Today, 65% of the barley produced is used in feed industry, 33% in beer industry and biodiesel production, 2% in the food industry. It is used for producing bread, biscuits, cracker, tea, and infant formula. Due to its high digestible fiber rate and high ß-glucan content, the use of barley as barley meal has increased in some countries and it has gained importance in human nutrition (Baik and Ulrich, 2008; Ergun et al., 2012). It meets raw material needed in the malt industry including animal feed and beer and it gives the opportunity to cultivate the second crop as it is harvested earlier than wheat. Its use in the food and beer industries is lower compared to animal husbandry and it increases each year regularly (Demir, 1983; Kün, 1988; Sencar and Gökmen 1997; Forster et al., 2000; Schulte et al., 2009).

Seed vigor is described as "general total of the seed characteristics that determine the activity and performance of a seed during germination and seedling emergence". The losses in the seed vigor is related with the decrease in fulfilling all the physiological functions of the seed. This process called as physiological aging starts before harvest and continues during harvest, processing and storage. The ending point of this deterioration is the death of the seed, that is, the complete loss of germination. However, before seeds lose their germination ability, they lose their vigor. Therefore, the physiological age difference of the seed lots with similar high germination values (degree of deterioration) and thus the differences in seed vigor occur. These seed vigor differences are observed in the seed lots of the species of garden plants, field crops and forest plants (Sivritepe, 2012). Seed deterioration may be described as the vigor, viability and quality loss due to the effect of negative environmental factors or aging.

The changes in enzyme activity and the decrease of seedling growth rate are the results of seed deterioration. Accelerated aging techniques have a great potential in understanding the relationship between the aging and the deterioration mechanisms of seeds (McDonald, 1999). In this study, some accelerated aging methods were used in barley (*Hordeum vulgare* L.) cultivars and their performances were revealed.

Material and Method

Seven barley (*Hordeum vulgare* L.) genotypes (Troya, Akhisar, Bayrak, Hilal, Börgüt, Sancak, EgeBeyi) obtained from Aegean Agricultural Research Institute (İzmir/Turkey) were used in this study. Accelerated aging vigor test, electrical conductivity test, protein analysis and germination-emergence tests were performed in different genotypes.

Image1. Seeds of barley used in the experiment

Moisture content calculation:

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Seed moisture of each lot was determined according to the rules of ISTA (International Seed Test Association) (1995). Initial weights of 1g of 2 replicated seed samples were weighed. Then the seeds were kept at 130ºC for 1 hour to determine the final weight of the seeds. At the end of this period, the seeds were removed from the oven and kept in the desiccator for half an hour with their mouths closed in order to cool the seeds and their final weights were weighed (ISTA, 1995).

Germination and emergence tests:

In this study, 3 * 25 seeds / repeats were subjected to petri germination for 7 days at 30 ^{0}C and viol output tests for 21 days (ISTA, 1995).

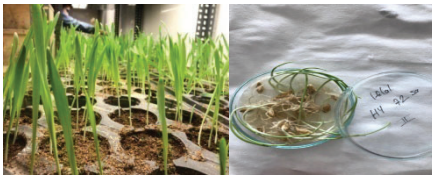

Image 2. Germination and emergence tests

Accelerated aging vigor test:

In this study, 3 * 25 seeds / repetitions were subjected to aging at 45 ^{0}C for 24, 48 and 72 hours. Then it was taken to petri germination at 25 ^{0}C for 15 days.

Electrical conductivity (EC)

3 * 100 seeds / repeat were measured at EC meter (µS / cm) at 20 ^{0}C for 24, 48 and 72 hours.

Mean germination time (MGT)
Mean germination time calculation:

$$MGT = \frac{\Sigma\, n.D}{\Sigma\, n}$$

In formula;
MGT: Mean germination time
n: D. day germinated seed number
D: It refers to the day since the beginning of germination.

Protein analysis

The crude protein (CP) values of the seed lots was determined using Kjeldahl method for total N as stated in AOAC (1998).

a. Wet digestion:

0.5 of the seed sample, ground to sift out from a sieve of 1 mm, was weighed and put in kjeldahl flask and after adding 15 ml of 98% sulfuric acid and 1 kjeldahl tablet, the flasks were placed in the wet digestion part of kjeldahl device. The heater of the device was operated and it was heated up to 410 ^{0}C by increasing gradually and the heating process was continued at a constant temperature of 410 ^{0}C until the content of the flask acquired a clear greenish color. The vacuum system was also operatedsynchronously with operation of the heater in order to remove H_2SO_4, that evaporated during boiling. After acquiring the wanted clear greenish color, the device was turned off and the flasks was left to cool.

b. Distillation:

The flasks cooled after wet digestionwas diluted with 50-60 ml of distilled waterand transferred into the big flasks of the distillation device and placed into the distillation device. Distillation was performed in a fully-automated device. 60 ml of 40% NaOH was used. It was placed in the collecting part of the erlenmeyer device which contained 25 ml of 4% boric acid and in which the distillate was gathered and the distillation process was continued until the distillate became 150 ml.

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c. Titration:

Titration was performed with 0.1 N HCl.The HCl amount used in titration was stated in ml. Calculation was performed as follows:

$$\% \text{ Crude protein: } \frac{6.25 \times 14.01 \times 0.1 \times (a-b)}{c} \times 100$$

a:HCI amount consumed in titration, ml
b:HCI amount consumedin titration in blank trial, ml
c:HCI concentration used (N)

Image 3. Protein analyses

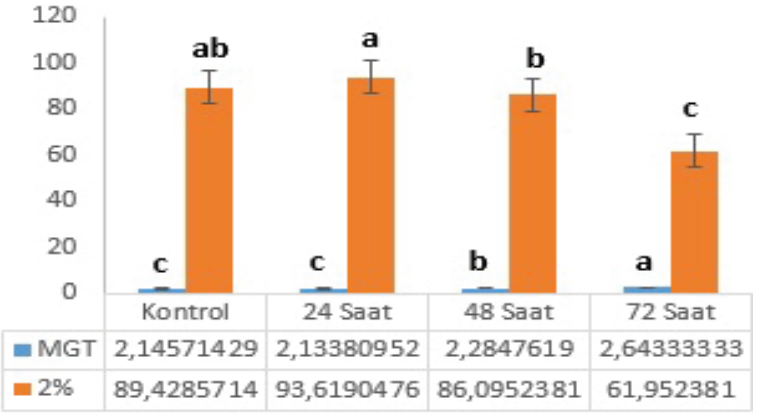
Results

- Moisture Contents of the Cultivars

The moisture values of the genotypes were found to be between 7.6-10.2%.

- Findings of Germination Test

The findings obtained in Petri tests were subjected to analysis of variance and a very significant difference ($p>0.01$) was determined among Time, Cultivar,and Interactions (time*cultivar).The treatments with the fastest emergence in MGT (Mean Germination Time) parameter after the Duncan's multiple comparisons were control and 24-hour treatments and they were included in "c" group. Control and 24-hour treatmentsalso had the highest rate in terms of % germination parameters of 2nd day and they were included in "a-ab" groups (graph 1).



Graph 1. MGT and 2.day Germination of Barley Seeds According to Aging Time (%)

In Duncan's multiple comparison, when the cultivars were compared, Sancak (d) cultivar had the lowest value in terms of MGT, which was followed by Troya cultivar and they were included in "bc" group. Hilal cultivar had the highest value and was included in "a" group.Sancak cultivar also was the first in % germination parameter of 2nd day and it was included in "a" group and Hilal cultivar had the lowest rate and was included in "d" group (graph 2).

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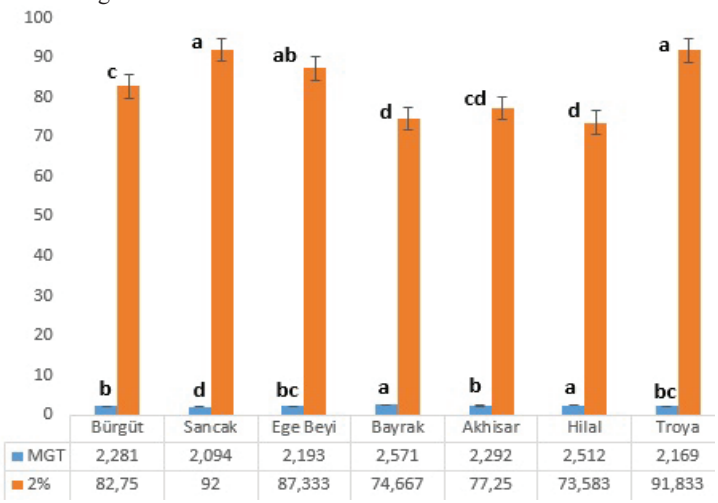
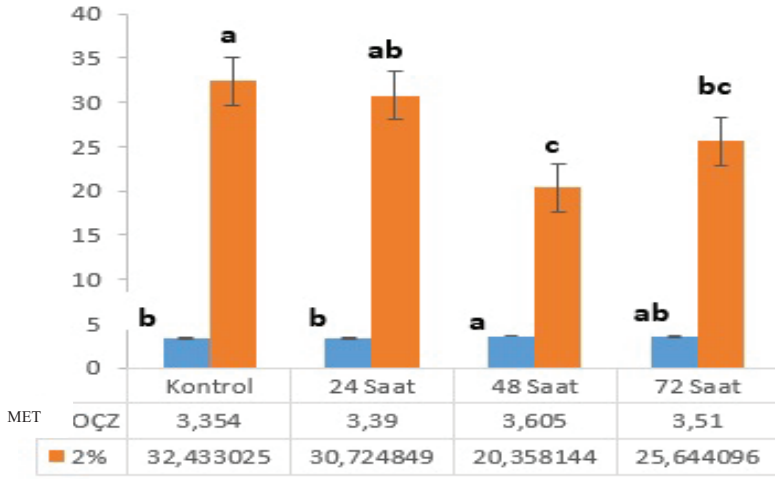


Graph 2. MGT and 2.day Germination rates according to barley varieties (%)

- Findings of Viol Test

Findings of viol tests were subjected to analysis of variance and a very significant statistical difference ($p>0.01$) was determined among time applications in % germination parameter of 2nd day and no significant difference was determined in mean emergence time (MET) parameter ($p>0.05$).When the cultivars were examined, a very significant difference ($p>0.01$) was determined among cultivars in terms of % germination and MET parameters of 2nd day.

After the Duncan's multiple comparison performed between time applications, Control Time application had the best result in % germination parameterof 2nd day and it was included in "a" group.48- and 78- hour treatments had the worst result among the treatments. In MET parameter, Control and 24-hour treatments had the lowest values as expected and they were included in "b" group (graph 3).



Graph 3. Barley Seeds MET and 2. day Emergence Rates according to Aging Time%

When the cultivars were compared, Börgüt and Troya cultivars had good performances in % germination parameter of 2nd day than Bayrak and Hilal cultivars. When MET was assessed, Börgüt and Sancak cultivars had the best expected values. As no data was obtained from Hilalcultivar, the transformed MET data made a difference in grouping (graph 4).

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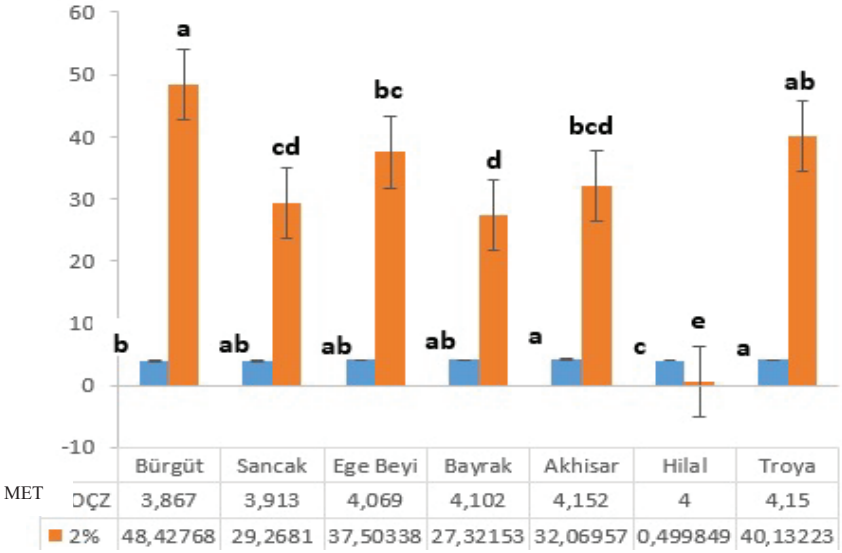
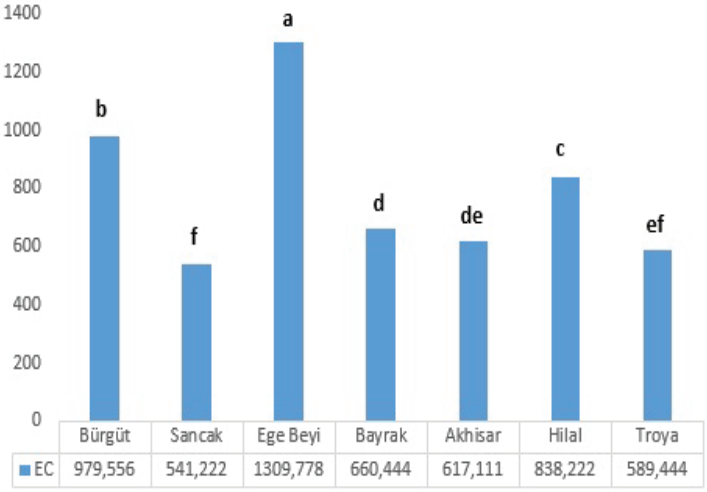


Graph 4. MET and 2. day emergence rates according to barley varieties (%)

- Electrical Conductivity (EC*)*

EC test, one of the vigor tests, was applied for the cultivars and a very significant difference ($p>0.01$) was determined between the cultivars after the analysis of variance. As the seed viability and EC values were inversely proportional, Sancak cultivar having the lowest mean value as a result of the statistical analysis (Duncan) was included in "f" group and it had the best value. Ege Beyi cultivar, with the highest mean value, had the highest EC value and was included in "a" group (graph 5).



Graph 5. EC (µS / cm) Values Among Barley Types

Although EC values and the petri test results supported each other, they were partially different from the viol test results. We considered that this was associated with the germination environment difference.

- Protein Contents of the Cultivars

The cultivars were compared before the test in terms of protein contents and no significant difference was determined as a result of the analysis of variance (graph 6).The fact that the protein rates did not make a difference among the cultivars indicated that the differences in the parameters examined were not caused by protein rates.

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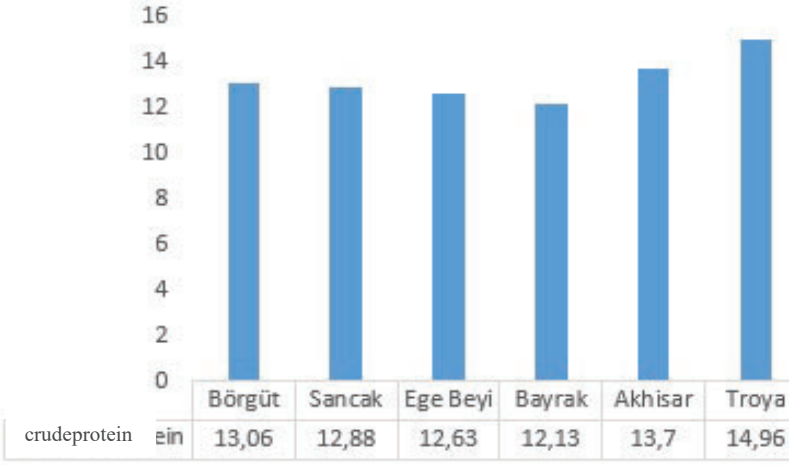

Graph 6. Crude Protein Ratios of Cultivars (%)

Discussion

Deterioration in seed is defined as the quality and viability loss due to the effect of negative environmental factors. In both conditions, the increase in the moisture content and storage temperature of seed is directly proportional with the deterioration rate (Ellis et al., 1985). In the present study, the moisture values of the genotypes were found to be between 7.6-10.2%.The seed deterioration process, storage or viability potential can be determined by the accelerated aging test. Such artificial aging tests demonstrate that this process of seeds is related with the moisture level and temperature. The physiological and biochemical changes during the seed aging are revealed with the seed tests formed for different conditions (Islam et. al, 1973; McDonald, 1999). According to reports by Kapoor et al. (2010) from other researchers, standard procedures about the accelerated aging have been developed for Brassicaceae (*Brassica* spp.), Maize (*Zea mays* L.*),* Lentil (*Lens culinaris* L.*),* Chickpea (*Cicer arietinum* L.), Green Pea (*Pisum sativum* L.), Cajan Pea (*Cajanus cajan* L.), Soybean (*Gylicine max* L.), Mung Bean (*Phaseolus mungo* L.) and some weeds (Kapoor et. al, 2010).

Soluble protein contents may change in germinated seeds based on the proteinase enzyme activity of barley. In the present study, the crude protein amounts of the genotypes varied between 12.1-14.9% (Plot 6).Similarly, the estimated starch content in the germinated seeds varied. The quality criteria such as seed size and seed nitrogen (N) concentration affect seedling production and grain yield of cereals (Ries and Everson, 1973). Lowe et al. (1972) and Ries (1971) found that seed protein concentration was positively associated with seedling vigor and yield. The studies on the effect of P concentration in seeds on plant performance have been conducted recently. Berezkin et al., (1984) stated that higher P concentration in winter wheat seeds increased seedling growth, but P was less effective in barley.

Pandey (1990) has reported that the accelerated aging technique is an instrument commonly used to test the seed quality. The accelerated aging test has been suggested as a method to assess the seed storability in the beginning and this test is fast, cheap, simple, and useful for all the species (Copeland and Donald, 2001; Younesi and Moradi, 2009). As the aging process increases in seed, the germination decreases and similar condition was also determined in rapeseed by Ghassemi-Golezani et al. (1996) and in soybean seed by Saha and Sultana (2008). Also, the previous reports (Bailly, 2004; Goel and Sheoran, 2002; McDonald, 2004), indicated that there were negative effects on seed performance, germination percentage and seedling index depending on aging. In our study, seed germination vigor decreased by 10% during the aging process (graph 3). This result caused differences between genotypes. In aged seeds, the mean germination times had later emergence and germination compared to the control group. (graph 3,4). As a result, Sancak and Troya varieties came to the forefront in terms of germination vigor compared to other varieties.

Conclusion

The performance in the use of high quality seed; Firstly, the percentage of green seedlings produced from high quality seeds are more than the low quality seeds and they may be useful to reach the intensity aimed in this field. Secondly, vigorous seeds have higher seedling growth rate and the heterogeneous conditions may be minimized in the germination period (Ghasemi-Golezani,1966). Seed aging is accepted with some parameters such as delay in germination and emergence, slow growth and the increase of the susceptibility to environmental stresses (Walters, 1998).

In this study, the seed deterioration conditions of the seed lots of *Hordeum vulgare* L. genotypes in three different periods with accelerated aging tests were tried to be determined. As the germination rates of the genotypes were generally high, the comparison with germination rates and times of 2ndday was preferred. Thus, the vigor differences were revealed more clearly.

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