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Determination of the Ethyl Methanesulfonate-Induced Resistance in Potato to *Rhizoctonia solani*

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Abstract: There are many pests and diseases that cause yield and quality loss which limits potato production in Turkey and in the world. One of diseases is *Rhizoctonia solani* (telemorph: *Thanatephorus cucumeris*) which gives rise to black scurf and stem canker of potato. In this experiment, potato cv. Alanso plants were treated with Ethyl methanesulfonate (EMS) at different concentrations (0, 20mM, 50mM, 75mM, 100mM) and different pre-soaking times (0, 10, 20 min.) under *in vitro* conditions. EMS treated plants *in vitro* were inoculated with *Rhizoctonia solani* after four consecutive subcultures. The survival plant rates were established after 15 days. Some plant parameters (plant nodes number, plant size, leaf number etc.) were recorded and plant parameters were compared with the negative control. The highest survival rate of the plants (32%) was determined in the 50 mM EMS for 10 min. application, while the lowest survival rate of the plants (10%) was determined at 75 mM for 20 min. application of EMS. The survival rate of positive control plants determined as 4% ($P \leq 0.05$). The highest plant size was determinated at 50 mM dose for 20 minute, the highest number of plant root was determinatedat 75mM dose for 20 minute. The EMS mutagenic applications in potato plants have been effective in improving the tolerance of plants to *R. solani*. These resistant mutants against *R. solani* can be used in a hybridization program to produce better recombinants.

Keywords: Potatoes, *Rhizoctonia solani*, EMS, Mutagen, cv. Alanso, *In vitro*

Etil Metansülfonat Tarafından İndüklenen Mutant Patatesin *Rhizoctonia solani*'ye Karşı Dayanıklılığının Belirlenmesi

Öz: Dünyada ve Türkiye'de patates üretimini sınırlayan, verim ve kalite kaybına neden olan birçok hastalık ve zararlı bulunmaktadır. Bunlardan birisi de kök boğazı nekrozu ve siyah kabukluluk hastalığı [*Rhizoctonia solani* (telemorph: *Thanatephorus cucumeris*)]'dır. Yapılan bu çalışmada Alanso patates çeşidine ait *in vitro* bitkilere farklı dozlarda (0, 20mM, 50mM, 75mM, 100mM) ve farklı uygulama zamanlarında (0, 10, 20 dk) kimyasal mutajen etil matansülfonat (EMS) uygulanmıştır. *İn vitro* koşullarında EMS uygulanmış bitkiler dört kez altkültüre alınmış ve daha sonra bu bitkilere *R. solani* inokulasyonu yapılmıştır. Uygulamadan 15 gün sonra i*n-vitro* denemelerinde, *R. solani* inokulasyonu sonrasında bitkilerin canlı kalma oranları belirlenmiş ve canlı kalan bitkilerin yaprak boyları, yeşil yaprak sayısı, bitki boğum sayısı, kök sayısı ve ortalama yaprak boyu belirlenmiş ve negatif kontrol ile karşılaştırılmıştır. En yüksek canlı kalma oranı (%32) 50mM dozunun 10 dk uygulama süresinde, en düşük ise (%10) 75 mM dozunun 20 dk uygulama süresinde belirlenmiştir. Pozitif kontrol bitkilerinde canlı kalma oranı %4 oranında tespit edilmiştir ($P \leq 0.05$). En yüksek bitki boyu 50 mM dozunun 20 dakika uygulamasında, en yüksek sayıda bitki kökleri 75 mM dozunun 20 dakika uygulamasında belirlenmiştir. EMS mutajen uygulaması patates bitkilerinde *R. solaniye* karş ıtoleransı iyileştirmede eykili olmuştur. *R. solani*'ye karşı bu dirençli mutantlar, daha iyi rekombinantlar üretmek için bir hibridizasyon programında kullanılabilir.

Anahtar Kelimeler: Patates, *Rhizoctonia solani*, EMS, Mutajen, cv. Alanso, *In vitro*

1. Introduction

Potatoes (*Solanum tuberosum* L.) are produced at large quantities in Turkey and the World. According to the 2016 data, potato production in the world was 441 million tons in 1.02 million acres (Anonymous, 2016). Potato production in

Turkey was 5.82 million tons in an area of 1.885.290 decares in 2016 (Anonymous, 2017). *Rhizoctonia solani* Kühn is an important fungal pathogen that causes black scurf and stem canker in potato. *R. solani* causes reduction in quality and quantity of potato tubers. Stem canker consists of stem lesions that may lose tuber yield by reducing the transport of nutrients throughout the plant. Black scurf is the formation of sclerotia, the durable resting structure of the fungus, on newly formed tubers. *R. solani* has widespread host range and can cause damage on more than 200 plant species (Lehtonen et al., 2008; Tsror, 2010). Until today, 14 different anastomosis groups [AG-1 -13 and BI (bridging isolate)] of *R. solani* have been defined based on hyphal anastomosis reactions (Taheri and Pourmahdi, 2013). The current chemical and cultural controls are not perfectly effective, because they are initiated by seedborne or soilborne inoculum and inoculum of *R. solani* is mainly transmitted via infected seed tubers. (Lehtonen et al., 2008). Therefore, resistant varieties to *R. solani* should be developed. Today, mutagenic radiation and chemical mutagen applications are performed to develop disease resistant plants against diseases and pests, in addition to classical breeding.

Mutations are defined as hereditary changes in the DNA sequence (Van Harten 1998; Zia et al., 2018). Mutations may be spontaneous (natural) or induced (artificial, with the aid of agents). Chemical mutagens often cause point mutations (Acquaah G., 2007; Hastings et al., 2009; Haque and Morshad, 2014). There are various chemical mutagens used for generating mutations, such as sodium azide, ethyl methanesulphonate (EMS) and methyl methanesulphonate (MMS). Among the chemical mutagens, EMS is the most commonly used chemical that can induce mutations in plants EMS is a monofunctional ethylating agent that has been known to be mutagenic in a wide variety of genetic testing systems. EMS can produce both AT to GC and GC to AT transition. A lot of literature has confirmed its utility in forward genetic screens in a variety of organisms (Jabeen and Mirza, 2004; Talebi et al., 2012; Ahmed et al., 2017; Zia et al., 2018).

When chemical mutagen applications are carried out at the appropriate time and dose, certain changes can be accomplished in the quality, yield, earliness, adaptability and resistance to plant diseases (Chen et al., 2013; Altındal, 2014; Ge et al., 2015; Zia et al., 2018). There are currently very few studies about the disease resistance of *R. solani* in potato cv. Alanso. In this study, induction of mutation for *R. solani* resistance using EMS in potato was performed.

2. Materials and Methods

This study was carried out between 2014-2016 in the laboratory and climate rooms of the Isparta University of Applied Sciences, Faculty of Agriculture, Department of Plant Protection.

2.1.Plant aaterials

The disease susceptible potato cv. Alanso were used in this experiment.

2.2. Isolation of pathogen

Rhizoctonia solani was isolated from infected potato tubers bearing sclerotia. Pieces of potato tubers with sclerotia were treated with 70% ethyl alcohol for 30 seconds and then with 3% sodium hypochlorite solution for 2 minutes. The tuber pieces were then dipped three times in sterile distilled water and dried on sterile filter papers. After the surface sterilization, sclerotia were excused from potato tubers, transferred on PDA medium (Potato dextrose agar) and were incubated at 24°C ±1 until sclerotia germinated. Isolates *R. solani* were characterized as AG-3 (Anastomosis Groups-3) (Parmeter et al., 1969; Balali et al., 1995).

2.3. Micropropagation

MS+ 0.5 mg/ L GA_3 medium used to cultivate potato plants cv. Alanso *in vitro* was prepared and adjusted to pH 5.7 (Murashige and Skoog, 1962).

The culture media were sterilized by autoclaving at 1.2 atm., 121°C for 20 min. The explants of potato cv. Alanso were surface sterilized by treating with 70% ethanol for 30 seconds, followed for 15 minutes with 7.5% sodium hypochlorite solution. Surface sterilized explants were dipped three times in sterile distilled water and dried on sterile filter papers. The explants were cultured on MS+ 0.5 mg/ L GA_3 and incubated in a growth chamber for one month at 24°C±1 with a 16-hour photoperiod. Plants were sub-cultured for 4 times and enough number of plantlets were obtained. Afterwards, plantlets were treated with different doses of EMS and different pre-soaking time (Altındal, 2014).

2.4 Ethyl methanesulfonate (EMS) treatment and inoculaton of *Rhizoctonia solani*

In the preliminary studies, different concentration of EMS (0, 100, 200 and 300 mM) was applied to potato explants for 30 min *in vitro* (Altındal, 2014). However, these doses and the application time caused toxic effects in the explants (Figure 1).

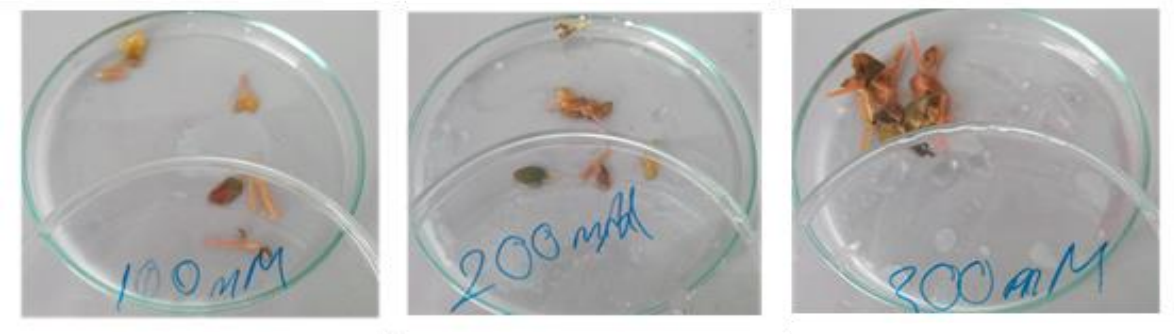

Figure 1. Toxic effects of high dose EMS (100, 200, 300 mM for 30 minutes) on potato cv Alanso explants.
Şekil 1. *Yüksek doz EMS'nin (30 dakika boyunca 100, 200, 300 mM) patates cv Alanso eksplantları üzerindeki toksik etkileri.*

Preliminary studies doses below 100 mM were considered non-toxic. According this study, potato cv. Alanso were treated with different concentrations EMS (0, 20mM, 50mM, 75mM, 100mM) and different pre-soaking time (0, 10, 20 min.). Subsequently, EMS-treated potato plants were rinsed four times with sterile distilled water (Figure 2). EMS treated plants were subcultured four times in magenta boxes on MS + 0,5 mg/l GA3 at 24 °C±1, until a sufficient number of plants were obtained. EMS treated potato plants in each magenta box were inoculated with *Rhizoctonia solani* (5 mm mycelial disc). *R. solani* was cultured on PDA medium for 7 days in petri dishes. A 5 mm mycelial discs were taken from *R. solani* with a cork borer and 5 pieces were placed in each magenta box with EMS treated potato plants. The plantlets were evaluated 2 weeks after the application. The experiment was carried out in 10 replicates, per dose and each time and 10 plants per replicate, according to randomized trial design. The survival rates and the plant parameters were recorded after two weeks after post-inoculation. In addition, plant length, leaf length and plant leaf and root number have been inscribed and compared with the negative control group. All trials were repeated at least three times.

2.5. Statistical analysis

All analysis was performed with SPSS 16.0 version for Windows 8.1 (SPSS INC. USA). All data was transformed and compared by ANOVA using Tukey's multiple range tests (Duncan 1957) and Dunnet's tests ($P \leq 0.05$). Dunnett Test ($P \leq 0.05$)

was used to compare negative control groups with individual subgroups.

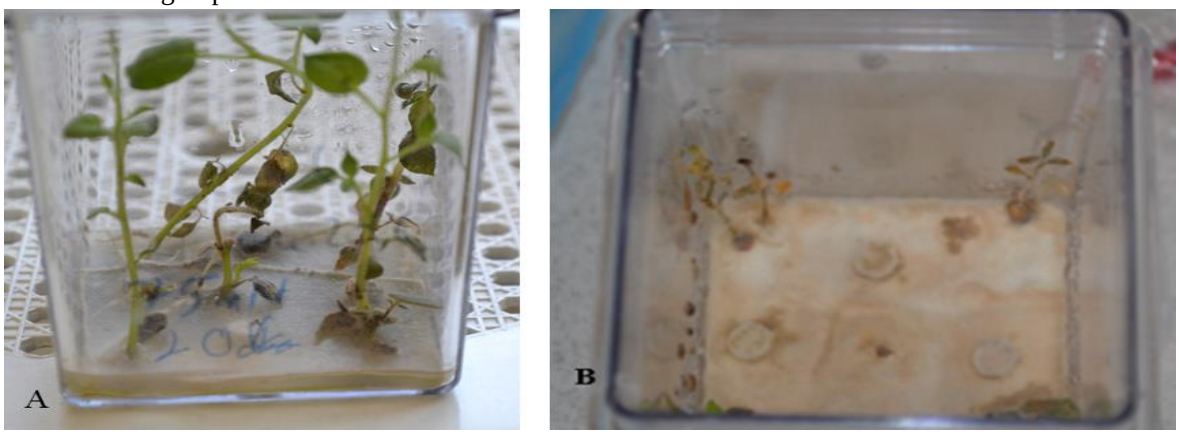

Figure 2. Effect of *R. solani* on EMS mutant potato plant cv. Alanso; A: 75mM EMS-20 min, B: Positive control (non-EMS application plant with inoculated *R. solani*)
Şekil 2. *R. solani'nin EMS mutant patates bitkisi cv. Alanso'ya etkisi; A: 75mM EMS-20 dak, B: Pozitif kontrol (EMS uygulanmamış- R. solani ile inokule edilmş)*

3. Results and Discussion

3.1. The Percentage of survival of EMS-applied plants after *R. solani* inoculation

In this study, potato cv. Alanso was treated with different concentrations EMS (20, 50, 75, 100 mM) and three different pre-soaking time (0, 10, 20 minutes) *in vitro*. In our study when the plants with EMS were compared to the positive control group (inoculated *Rhizoctonia solani* and non-EMS treated plants), a statistically significant difference was found between the mean of the positive control group and the mean of all subgroups. The results are given in Table 1.

The highest survival rate of the plants (32%) was determined in 50 mM EMS for 10 minutes pre-soaking time application, while the lowest survival rate of the plants (10%) was determined in 75 mM EMS for 20 minutes of pre-soaking time treatment ($P \leq 0.05$). The survival rate of positive control plants was determined as 4% (Figure 2). As a result, 50 mM EMS for 10 min. pre-soaking time appeared to be the most appropriate treatment to increase resistance to *R. solani*. It is concluded that the application of EMS may be a useful tool for the improvement of a resistant potato to *R. solani* by mutagenesis *in vitro*. According to the results, it can be said that EMS application may increase resistance to *R. solani* and cause mutagen in potato plants. In this case, it depends on the dose and duration of application of EMS.

Table 1. Survival percentage of plants treated with EMS and non-treated control plants after R. solani application
Çizelge 1. *R. solani uygulamasından sonra EMS uygulanan bitkiler ile uygulama yapılmayan konrol bitkilerin canlı kalma oranı*

Doses	Period of time (minute)	Survival rate (%)
20 mM	10 min	14 d
	20 min	30 b
50 mM	10 min	32 b
	20 min	18 d
75 mM	10 min	16 d
	20 min	10 e
100 mM	10 min	18 d
	20 min	24 c
C(+)		4 f
C(-)		100 a

*There was no statistical difference between the groups and the columns containing the same letter.

New varieties have been developed using mutation breeding techniques. Chemical and physical mutagens are valuable for improving the

occurrence of mutations and differences in potato properties. Chemical mutagens cause gene mutation and chromosomal changes, physical mutagens such as a gamma irradiation cause chromosomal changes rather than gene mutation (Mike and Donnini, 1993; Chahal and Gosal, 2003). Chemical mutagens such as EMS applied to develop disease resistant plants against diseases and pests, in addition to classical breeding (Raina and Danish., 2018). This research was conducted to assess effect of EMS on potato cv. Alanso and to attain new potato genotype with resistance to *R. solani*. In this study, 50 mM EMS for 10 min. pre-soaking time appeared to be the most appropriate treatment to increase resistance to *R. solani*. Many studies have been conducted to increase resistance to diseases and pests in plants for chemical mutagen EMS and resistant and tolerant plants were obtained against to different diseases and pests in different plant. For example, shoot tips of *in vitro* grown banana cv. Highgate were treated with different doses of the sodium azide, EMS, diethyl sulphate, and produced variants of tolerance to *Fusarium oxysporum* f. sp. *cubense*. Twelve weeks after *F. oxysporum* f. sp. *cubense* inoculation, plants were tested and were determined that vascular symptoms of the disease less than 10% and this plants were accepted as tolerant (Bhagwat and Duncan, 1998). Ekanayaka et al. (2016) derived calli from seeds of the glyphosate-susceptible rice variety (Bg250) and exhibited to four different doses of EMS (0.1%, 0.2%, 0.3% and 0.4%) Thereafter mutant calli were put on glyphosate (0.2%) and the tetrazolium test (1% TTC) was performed to determine cell viability in calli. According to the results, the highest mutation callus was found at EMS concentration range of 0.1 – 0.2%. Bg250 calli showed resistance against glyphosate at 0.2%. Shah et al. (2009) developed on Aug-424 chickpea varieties by applying gamma irradiation and EMS to resistance to Fusarium wilt disease. All the 4 parent genotypes showed a highly susceptible reaction to Fusarium wilt. It was reported that 75 mutants exhibited a highly resistant reaction, 31 mutants resistant, 34 mutants moderately resistant/tolerant, 35 mutants susceptible and 75 mutants were highly susceptible. Badawi et al. (2009) used at different concentrations (0, 1, 2 and3 mM/L) of EMS to induce genetic variability in three cultivars of potato, Atlas, Nicola and Simon, for resistance against early blight. EMS treatments induced genetic variation in the potato genome, which lead to new genotypes with high tolerance levels to *Alternaria solani*. Most of the selected clones were induced from 2mM/L EMS treatment. Chen et al. (2013) integrated EMS–induced mutagenesis to investigate Fusarium wilt-resistant lines of Brazil banana (*Musa* spp., AAA). As a result, the optimal EMS concentration and duration were 300 mm EMS and 60 min. One hundred regenerated plantlets were screened for Fusarium wilt resistant lines. Five of the regenerated plants were detected as fusarium wilt-resistant lines. It has been deduced that that induced mutation in banana by EMS was potentially useful to Fusarium wilt disease in banana plant. Çalış et al. (2013) treated the tomato seeds of susceptible to *Clavibacter michiganensis* subsp. *michiganensis* bacterial canker pathogen (EBR3) with 0.5% EMS for 12 hours. Mutant resistant M3-15 and M3-9 mutant tomatoes from EBR3 tomato line were provided against *C. michiganensis* subsp. *michiganensis* isolate 2 (*Cmm2*). Ge et al., (2015) introduced by treating the cell suspension of embryogenic callus from the Sweet orange *[Citrus sinensis* (L.) with 1.5 % of EMS for the selection of sweet orange somaclones tolerant to citrus canker disease. The results showed that the treatment of the EMS to callus of sweet orange was effective and selection of the somaclones tolerant to canker disease by the pathogen *in vitro*.

3.2. Plant parameters applied ethyl methane sulfonate (EMS) treatment

The mean performance of the potato plant parameters after EMS treatments is reported in Table 2. When the subgroup means were compared

with EMS and negative control plants *in vitro* conditions, significant differences were not found in plant roots number, plant nodes number and leaf number. As a result of Dunnett Test, it was found that the differences between the control group and the applications of EMS were significant in the plant length, and leaf length ($P \leq 0.05$). It was determined that dose X time interaction had a significant effect on plant length, number of plant nodes and number of leaves in potato plants treated with EMS. The highest mean of plant length was determined at 50 mM EMS for 20 min pre-soaking time (9.34 cm) and 100 mM EMS for10 min pre-soaking time (9.29 cm), respectively. The lowest mean was noticed for negative control (5.23 cm) and 20 mM EMS for 10 min pre-soaking time (7.54 cm). In this study, it was observed that the numbers of plant nodes were increased with increasing EMS concentration. It was determined that the mutant plants had higher the number of plant nodes (2.57-2.85) than the negative control plants (2. 25). The highest number of plant nodes was determined at 75 mM EMS in 20 min pre-soaking time (2.85). The highest leaf length average was at 50 mM EMS in 20 min pre-soaking time (0.97). The highest mean of leaf number per plant was obtained from the treatment of 50 mM EMS for 20 min (2.30) followed by 100 mm EMS with 10 and 20 min pre-soaking time (2.14). The lowest mean was found for 20 mm EMS for 20 min pre-soaking time (1.83) (Figure 3). No abnormality was found in the physiological development of plants after EMS application. Some positive changes have been observed in the parameters of plants treated with EMS. While the survival rate of the positive control plant was 4%, this survival rate was 32% in the plant in which EMS was used (50 mM EMS for 10 min) It is believed that the plants are mutated. Changes in plant parameters can be phenolic compounds such as anthocyanin and chlorogenic acid. Mutagenesis induced by chemical mutagens has advantages over placement methods because the mutagenes make random changes across the genome and can produce various mutations in a single plant. Molecular analysis is required to determine the condition of the mutation.

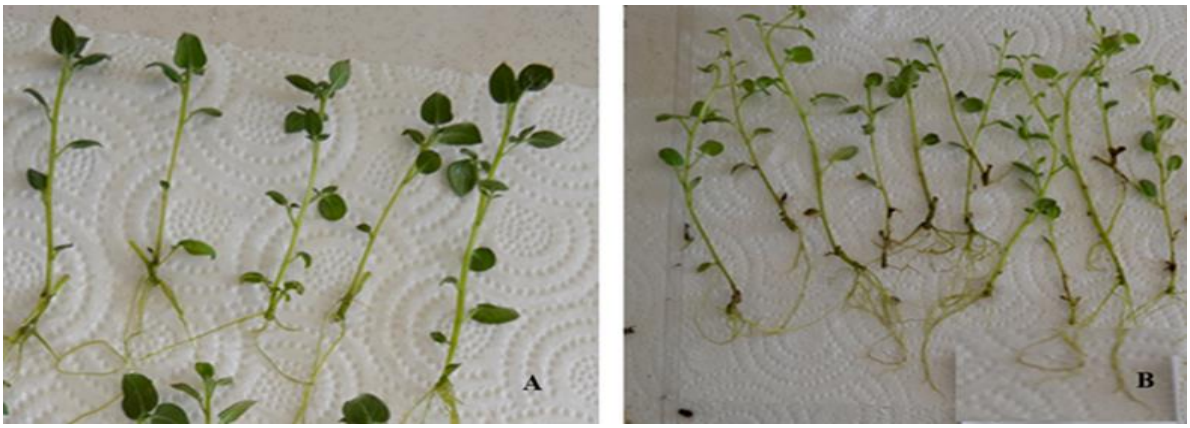

Figure 3. Negative control potato plants (A); the potato plants treated with 50 mM EMS for 20 min. (B)
Şekil 3. *Negatif control patates bitkileri (A), 20 dakika 50 mM EMS uygulanmış patates bitkileri*

In our preliminary studies doses below 100 mM were considered non-toxic. It was established by the literature that the determination would be influenced by index, rate, and percent of germination; seedling height, root length, plant length and root length with increasing EMS. Some researchers reported EMS belonging to the group of the alkylating agents had as a very effective and efficient mutagen to generate somaclonal variation in the crop plants (Kodym and Afza, 2003; Latado et al., 2004; Hofmann et al., 2004; Luan et al., 2007; Khawale et al., 2007; Berenschot et al., 2008,

Altındal, 2014; Yadav et al., 2016; Arıcı et al., 2017; Zia et al., 2018). In addition, Nicoll et al. (2003) applied four different dose EMS (1mM, 3mM, 10mM and 30mM) to somatic embryogenic suspension cultures of soybeans. It was reported that, the average surviving embryonic culture rate was 43-73% and decreased at 30 mM dose EMS application. Luan et al. (2007) performed 0.5% EMS at 0, 1, 1.5, 2, 2.5 and 3 hours, with calli of sweet potatoes from leaf explants initiated to induce a mutation for salt tolerance. After multiplication, mutant plants were found more tolerant to salt than control plants in the 0.5% EMS at 2 and 2.5 hours. Ahmed et al. (2017) investigated the chemical mutagen (EMS) on the tomato plant to affect the plant growth. Tomato seeds were treated with 0.07% EMS. It was reported that EMS proved to be a positive mutagen and helped to speed the growth rate and overall plant size with green leaves.

Table 2. Plant parameters after *R. solani* inoculation on EMS treated plants
Çizelge 2. *R. solani uygulamasından sonra EMS uygulanmış bitkilerde bitki parametreleri*

Doses	Period of time	Plant length (cm)	Plant roots number	Plant nodes number	Leaf number	Leaf length (cm)*
20 mM	10 min	7.54±2.72 b	2.28±0.71	2.57±0.64 a	2.04±0.31	0,72±0,21 a
	20 min	7.72±2.47 a	2.21±0.49	2.56±0.47 a	1.83±0.43	0,85±0,27 a
50 mM	10 min	9.15±2.42 a	2.31±0.56	2.77±0.44 b	2.10±0.34	0,77±0,23 a
	20 min	9.34±2.35 a	2.19±0.62	2.84±0.39 b	2.30±0.40	0,97±0,22 b
75 mM	10 min	7.75±2.95 a	2.23±0.39	2.60±0.50 a	2.22±0.28	0,72±0,18 a
	20 min	9.08±2.31 a	2.86±0.50	2.85±0.26 b	2.11±0.37	0,70±0,31 a
100 mM	10 min	9.29±3.05 a	2.47±0.57	2.81±0.50 b	2.14±0.63	0,95±0,18 a
	20 min	8.43±2.62 a	2.34±0.64	2.84±0.51 b	2.14±0.70	0,79±0,32 a
Negative control		5.23±1.45 b	2.34±0.52	2.25±0.36 a	2.38±0.33	0.77±0.25 a

*There was no statistical difference between the groups and the columns containing the same letter.

Sagel et al. (2017) carried out to determine the effect of chemical mutagen (EMS) on seedling height of TAEK A-3 and TAEK C-10 soybean varieties. Five different EMS doses (0, 0,025, 0,050, 0,075 and 1,0 M) and 3 different pre-soaking time (0, 6 and 18) were applied. After chemical mutagen application in the green house, the effect of EMS dose and pre-soaking time was determined at the germination percentage and seedling height. As a result, seedling height was reduced as the EMS concentration increased. The average germination percentage of both soybean varieties increased with increasing pre-soaking time. Somalraju et al (2018) investigated to induce mutagenesis in diploid potato using EMS. The potato seeds were treated with 1.2% EMS for 4–6 hours. A large variation in the plant parameters (germination rate, tuber phenotype, flower etc.) was determinated in EMS-treated plants. A slightly higher than 50% germination rate was observed in seed treatment with 1.2% EMS for 4-6 hours and these conditions could be used to generate the mutated population.

The effect of EMS on plant growth *in-vitro* depends on the variety and duration of the application. The plant growth is decreasing while dosage and period are increasing. However, the mutagen dose could be either high or low causing mutation frequency. High amounts of EMS led gene mutations and low amounts of EMS cause chromosomal abnormalities (Van Harten, 1998; Jain, 2010). The present results seemed to agree with some researchers. Talebi et al. (2012) applied the different EMS concentrations (0.25%, 0.50%, 0.75%, 1%, 1.25%, 1.5% and 2%) in seeds of *Oryza sativa* cv. Indica. It was determined the sensitivity to EMS by different assessment on the M1 generation. When compared with M1 generation and the non-treatment control, germination, seedling height, root length, emergence, plant height and root length were decreased as the concentration of treatment EMS increased. Badawi et al. (2015) investigated the efficiency of different concentrations (20, 30 and 40 mM) of EMS mutagen on three potato cultivars. It was reported

that the increase of EMS concentrations decreased significantly in survival percentage, number of stems/plant and growth vigour. The highest concentration of EMS (40 mM) showed the most inhibitory effect on the measured vegetative traits. In addition, the significant variations between EMS treatment concentrations within each cultivar and between cultivars were detected in this experiment. Altındal (2014) applied EMS treatment to three potato cultivars (Agria, Marfona and Lady Olimpia) with EMS in two stage. In first stage, shoot tips of potato cultivars were treated four doses of EMS (0, 100, 200 and 300 mM) with different times (30, 60 and 90 min). These shoot tips were cultured on a modified MS medium. In the other stage of the study, tuber eyes of potato cultivars were treated with 0.0, 0.1, 0.2, 0.4, 0.6 % doses and 60, 120, 150 and 180 min time periods of EMS. Seedlings from shoot primordia planted into viols were transferred to field and morphological observations were recorded. As a result, the reaction of Lady Olimpia cultivar *in vitro* was the best in plantlets from shoot tips in the plantlet height, the number of internode, leaf, root and sub branch. 100 mM EMS for 30 min EMS was the most positive effective in the morphological observations *in* vitro. Generally, treatment of increasing EMS doses on tuber eyes led to declining plant growth. Shah et al. (2015) tested several different EMS combinations [(0, 0.5, 1.0, 1.5, 2, 3% v/v) with post-treatment water and 0.1 M $Na_2S_2O_3$ EMS doses (0, 0.5, 1.0, 1.5% v/v) for 8, 16, 24 h; and EMS doses (0, 0.5, 1.0, 1.5% v/v) at 20 and 28°C to create a mutant cucumber cv. Chinese long (9930). In this experiment, index, rate, and percent of germination were decreased with increasing EMS concentration. The germination rate (56.25%) and index (4.13) at 20 °C were more affected than at 28°C (91.31% and 7.68).

4. Conclusions

As a result, potato cv. Alanso showed resistance against *R solani* at 50 mM EMS for 10 min. *in vitro.* The optimal EMS concentration and duration were 50 mM EMS and 10 minute. They showed significantly reduced incidences of disease compared to regenerated plantlets of potato (control). The results showed that the potato cv. Alanso with EMS treatment were tolerant to *R .solani.* It is concluded that the application of EMS for *R. solani* resistant potato may be a useful tool in the study of *in vitro* mutagenesis for potato improvement. Nowadays, besides the efforts to obtain plant resistant plants with classical breeding program, developing varieties resistant to plant diseases with mutagenic chemicals has gained great importance. Ethyl methane sulfonate (EMS) mutagenesis, one of the chemical mutagens used in plants, is the most widely used chemical mutagen technique. EMS has high mutagenicity and low plant mortality and can be used in any laboratory. The chemical mutagenic applications in potato plants have been effective in producing morphological mutations as well as in improving the tolerance of plants to *R. solani*. These resistant mutants against *R. solani* can be used in a hybridization program to transfer resistance genes to elite cultivars with high yield/ produce better recombinants.

5. Acknowledgement

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