Research Article

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Determination of Gamma Rays Efficiency Against *Rhizoctonia solani* in Potatoes

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Abstract: There are many diseases in potatoes that cause loss of quality and yield in the world. One of them is the *Rhizoctonia* stem canker and black scurf of potato caused by *Rhizoctonia solani* (teleomorph: *Thanetophorus cucumeris*). Disease resistant plants can be generated by irradiated application. By irradiating the plant explants, some radicals and biochemical compounds can be generated in cells. In this study, *in-vitro* cultured explants from potato cv. Alonso were irradiated with gamma rays with five different doses (22, 33, 54, 57 and 109 Gy). After four sub-cultures in MS media, irradiated plants were inoculated with *Rhizoctonia solani* and potato plants were cultured at $24 \pm 1^\circ\text{C}$ in climate room conditions. The survival rates of the plants were determined after 15 days. Some parameters such as plant size, leaf number and number of plant nodes were recorded and compared with control groups. The highest survival rate of the plants (42%) was determined at 22 Gy and 109 Gy doses (42%) and the lowest survival rate of plants (20%) was determined in the positive control group ($P \leq 0.05$). The highest number of plant nodes and number of leaflets were determined at 54 Gy doses and maximum plant size was determined at 109 Gy doses.

Keywords: Potatoes; *Rhizoctonia solani*; Gamma rays; *In vitro*.

1 Introduction

The potato (*Solanum tuberosum* L.) belongs the family of *Solanaceae* and its origin is South America. The potato entered Turkey for the first time in the 19th century through

Russia [1]. Potato tubers contain approximately 20-30% starch, 2% protein, B1, B2, C vitamins and some minerals [2]. The potato has a great economical prescription in the world and Turkey. In 2016, 441 million tons of potatoes were produced using 1.02 million acres in the world, [3]. In Turkey, production included 5.82 million tons in the field of 1.885.290 decares in 2016 [4].

There are many diseases in potatoes that cause loss of quality and yield in the world. One of them is the *Rhizoctonia* stem canker and black scurf of potato caused by *Rhizoctonia solani* (teleomorph: *Thanetophorus cucumeris*). *Rhizoctonia solani* generally has two types of infection. They are black scurf and stem canker. Stem canker infection occurs on growing plants early in the growing season. Black scurf infection occurs on tubers with the formation of sclerotia later in the growing season, and is characterized by the formation of black, irregular sclerotia of various sizes on the tuber [5]. *Rhizoctonia solani* is common in the Central and Eastern Anatolian potato production areas in Turkey [6, 7]. *Rhizoctonia solani* is soil borne and seed pathogen and causes yield losses in the range of 5-34% [8,9,10]. This disease is easily transported by tuber, so it is difficult to control *Rhizoctonia solani* [11]. Therefore it is necessary to develop resistant varieties.

Today, classical breeding methods and mutagenic radiation applications are used to develop resistant plants to diseases and pests. By irradiating the plant explants, some radicals and biochemical compounds can be formed in cells. If radiation is used at the appropriate dose and time, positive changes in yield, resistance, quality, earliness and adaptability of plants can be achieved [12,13,14]. The gamma rays has the potential to enhance plant tolerance to plant diseases. For instance; Gosal et al. [13], irradiated *in-vitro* cultured explants of potato cvs. Kufri Jyoti and Kufri Chandramukhi with two doses gamma rays (20 and 40 Gy). The resulting plants were screened for resistance to late blight (*Phytophthora infestans*), using the detached leaf method. The treatment of 40 Gy dose gave better results in resistance to late blight in both the varieties than the treatment of 20 Gy dose. Lee et al. [15], had developed salt tolerant rice *in vitro* conditions using gamma radiation. Significant increases

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in α -amylase and alcohol dehydrogenase activities were observed in mutant lines when compared with the original. Hassan [16] applied three different doses gamma rays (20, 30, 40 Gy) on potato cvs. Diamant and Spunta to obtain new genotypes resistant to *Ralstonia solanacearum* in potatoes. Mutants were occurred at 30 and 40 Gy in the cv Diamond and at 20 Gy in the cv Spunta, and disease-resistant mutants were obtained.

In this study, the efficacy of gamma-ray applications against *Rhizoctonia solani* in potatoes was investigated.

2 Materials and Methods

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

Isolation of *Rhizoctonia solani*: Potato tubers infected *Rhizoctonia solani* were washed with water and were divided into small parts for surface sterilization. Potato parts were dipped with 70% ethyl alcohol for 30 seconds and 3% NaOCl for 2 minutes, and were then rinsed in sterile distilled water three times. After the surface sterilization, small potato parts were transferred on petri dishes containing PDA media (potato dextrose agar) and petri dishes were incubated at $24^{\circ}\text{C} \pm 1$. 48 hours later, *R. solani* isolates were purified and saved at $+4^{\circ}\text{C}$. *R. solani* isolates were identified as AG-3 (Anastomosis Groups-3).

Clonal propagation of potato plants: The potato cv Alanso, known for being susceptible to disease, was grown in climate room conditions ($24 \pm 1^{\circ}\text{C}$ and $60 \pm 5\%$ humidity) in a 1: 1 ratio of peat and perlite. Seven weeks later, explants were collected from the mother plant of Alanso plants and kept under running tap water for two minutes. Explants were cut into 2 cm segments such that each segment contained one axial bud, which was then dipped in 70% ethanol for 30 seconds and 7,5% NaOCl for 15 minutes. After sterilization, the explants were rinsed three times with sterile distilled water. Experiments were cultured in $\text{MS}+0,5 \text{ mg/l GA}_3$. The pH of the media was adjusted to 5.7 before autoclaving the media at 121°C and 1.2 atm. for 20 min. The cultures were kept in a growth chamber for one month at $24^{\circ}\text{C} \pm 1$ with a 16 hour photoperiod.

Gamma irradiation: Potato plants were cultured in $\text{MS}+0,5 \text{ mg/l GA}_3$ in tubes and were irradiated with 5 different doses (22 Gy, 33 Gy, 54 Gy, 57 Gy, 109 Gy), using gamma rays sources⁶⁰ (Süleyman Demirel University, Faculty Of Arts & Sciences Department of Physic).

Irradiated plants were cultured in $\text{MS}+0,1 \text{ mg/l BAP}$ and the cultures were kept in a growth chamber for one month at $24^{\circ}\text{C} \pm 1$ with a 16 hour photoperiod. Irradiated plants were subcultured 4 times in $\text{MS}+0,5 \text{ mg/l GA}_3$.

Inoculation of irradiated potato plants: Plants that were irradiated and non-irradiated cv Alanso for each treatment were cultured in magenta boxes on $\text{MS}+0,5 \text{ mg/l GA}_3$ at $24^{\circ}\text{C} \pm 1$. After 2 weeks, 5 discs of *R. solani* were put in each magenta box for 2 weeks. Then the survival rates of the plants were determined. A completely randomized experimental design with nine replicates were adopted for each dose and each replicate contained 5 plants. All experiments were repeated three times. Parameters of the plants were determined for living plants and were compared with positive control (non-irradiated plants and inoculated *R. solani*) and negative control (non-irradiated plants and non-inoculated *R. solani*) groups. Plant heights, leaf count, plant root numbers, plant node numbers, and leaf size were recorded.

Statistical analysis: For statistical analysis, all data was transformed and analyzed by ANOVA. The mean values of each parameter measured were analyzed using SPSS 16, and differences between the means were analyzed by Duncan's multiple range tests [17] and Dunnett's tests at the 0.05 level.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussions

Potato plants were irradiated with different 5 doses (22 Gy, 33 Gy, 54 Gy, 57 Gy, 109 Gy), using gamma rays sources⁶⁰. The main purposes of this study were to determine the effect of gamma rays on potato cultivars and to obtain new potato genotypes with enhanced resistance to *R. solani*. Under the selection procedure, the survival percentage of irradiated plants was determined after *R. solani* inoculation and following selection (Figure 1). The results are shown in Table 1.

The highest survival rate of the plants (42%) was determined at 22 Gy and 109 Gy doses and the lowest survival rate of the plants (20%) was determined in the positive control group ($P \leq 0.05$). The study exhibited that the Alanso cultivars were highly susceptible to *R. solani* and severe symptoms were produced on the plants except 22 Gy and 109 Gy. Punja [18] indicated that they were primarily involved in plant defence against pathogens as they catalysed the hydrolysis of β -1,3-glucans which was a major component of the cell wall of most fungi.

Dead plants

Alive plants

Figure 1: Irradiation treatments of potato cultivar (Alonso) after infection with *R. solani*.**Table 1:** Survival percentage of irradiated plants after the *R. solani* inoculation.

Doses	Survival percentage (%)
22 Gy	42
33 Gy	36
54 Gy	40
57 Gy	38
109 Gy	42
Control +	20
Control -	100

In our study, the results suggest that gamma treatment with different doses might cause mutagenesis in improving its resistance against *R. solani*. Mutation breeding in crop plants is an effective tool for plant breeders in crops as they have a narrow genetic base. Mutagenesis has been popular over past decades because it is simple, cheap to perform, applicable to all plant species and usable at small or large scale. By varying mutagen dose, the frequency of induced mutations can be regulated and saturation can be readily achieved [19].

The obtained results are similar to many studies. For instance; *Ralstonia solanacearum*-resistant plants were obtained at 30 and 40 Gy doses in potato cv Diamant and at 20 Gy dose potato cv Spunta [16]. Three different gamma ray doses (25 Gy, 30 Gy, 35 Gy) were applied on potato cvs. Diamant, Draga and Spunta to obtain resistant plants to *Phytophthora infestans*. In the results of the studies; 10 plants from potato cv Draga, 1 plant from potato cv Diamant and, 1 plant from potato cv Spunta were resistant to *Phytophthora infestans* [20]. Some researches suggest

that the use of the irradiation of the plant explants or seeds to generate mutants with desirable properties, phenolic compound, phytoalexins, pathogenesis related proteins such as catalase, glucanase, peroxidase or free radicals can generate in cells. These radicals and biochemical compounds can take an important role as a signal molecule activation of genes of antioxidant enzymes and proline, systemic acquired resistance (SAR) which are defence systems against the most plant pathogens in plant cells [21, 22, 23, 24].

Differences between group mean doses were compared (Table 2). There was no significant difference between the averages of doses for plant size, number of plant nodes and leaf size. But there was a significant difference between 22 Gy and 33 Gy doses for the number of plant roots and there was a significant difference between 54 Gy and 57 Gy doses for the number of leaves.

The obtained data were compared with the positive control group, and the difference between the group means was not significant for the parameter. However, the number of leaves only at the 57 Gy dose was different from the positive control group (Table 3). When the obtained data were compared with negative control groups, the difference between group means was significant for plant size, leaf size and number of leaves (Table 4).

Rasheed et al. [25] investigated to initiate callus and regenerate plants in potato *in vitro* from nodal segments. Cultures of potato were irradiated with 5, 20, 40, and 60 Gy. Doses higher than 20 Gy were lethal to micropropagated plants of potato. Ahmed [26] established the effect of gamma radiation on morphological structure, physical properties and chemical characterization of potato "Alpha cultivar" with doses 0, 10, 20, and 40 Gy. It was determined that the 20 Gy dose gave better potato

Table 2: Differences between the group averages.

Doses	Plant height	Number of plant roots	Number of plant nodes	Number of leaf	Leaf size
22 Gy	6,67±2,51 a	1,94±0,95 b	2,24±0,47 a	1,67±0,38 ab	0,81±0,31 a
33 Gy	6,81±1,88 a	2,66±0,46 a	2,30±0,49 a	1,69±0,61 ab	0,85±0,41 a
54 Gy	6,88±2,54 a	2,33±0,61 ab	2,40±0,44 a	1,60±0,61 b	0,67±0,39 a
57 Gy	7,92±2,57 a	2,35±0,39 ab	2,49±0,36 a	2,09±0,42 a	0,70±0,26 a
109 Gy	7,43±1,89 a	2,39±0,73 ab	2,30±0,27 a	1,90±0,59 ab	0,91±0,42 a

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

Table 3: Comparison of the plant parameters with the positive control groups.

Dose	Plant height	Number of plant roots	Number of plant nodes	Number of leaf	Leaf size
22 Gy	6,67±2,51 a	1,94±0,95 a	2,24±0,47 a	1,67±0,38 a	0,81±0,31 a
33 Gy	6,82±1,88 a	2,66±0,46 a	2,30±0,49 a	1,69±0,61 a	0,85±0,41 a
54 Gy	6,88±2,54 a	2,33±0,61 a	2,40±0,44 a	1,60±0,61 a	0,66±0,39 a
57 Gy	7,92±2,57 a	2,35±0,39 a	2,49±0,36 a	2,10±0,42*	0,70±0,26 a
109 Gy	7,43±1,89 a	2,39±0,73 a	2,30±0,27 a	1,90±0,59 a	0,91±0,42 a
Positive control	7,20±1,94 a	2,27±0,66 a	2,33±0,39 a	1,52±0,60 a	0,72±0,45 a

** There was no statistical difference between the groups and with the same letter in the columns and the positive control groups containing the same letter.

Table 4: Comparison of the plant parameters with the negative control groups.

Dose	Plant size	Number of plant roots	Number of plant nodes	Number of leaf	Leaf size
22 Gy	6,67±2,51	1,94±0,95	2,24±0,47	1,67±0,38	0,81±0,31
33 Gy	6,82±1,88	2,66±0,46 a	2,30±0,49	1,69±0,61	0,85±0,41
54 Gy	6,88±2,54	2,33±0,61 a	2,40±0,44 a	1,60±0,61	0,66±0,39
57 Gy	7,92±2,57	2,35±0,39 a	2,49±0,36 a	2,10±0,42	0,70±0,26
109 Gy	7,43±1,89	2,39±0,73 a	2,30±0,27	1,90±0,59	0,91±0,42
Negatif kontrol	11,50±4,77a	2,99±0,15 a	2,87±0,43 a	2,87±0,43 a	1,38±0,56 a

** There was no statistical difference between the groups and with the same letter in the columns and the negative control groups containing the same letter.

characteristics, longer stem length, root length and higher yield of tubers compared to other irradiated and non-irradiated treatments. Hannapel et al. [27] found that KNOX overexpressers resulted in the reduction of potato plant height by approximately threefold in POTH1 mutant plants relative to the controls. Afrasiab and Iqbal [28] investigated somaclonal variants and induced mutants of potato for desirable characteristics with a special emphasis on yield and yield components in two cultivars of potato cvs. Desiree and Diamant. 10 week

old, well proliferating calli were exposed to 5–50 Gy of gamma irradiation for mutation induction. Regenerated plants were screened on the basis of average shoot height, number of shoots, number of nodes/shoots, average tuber number, tuber size, tuber weight and number of eyes/tuber. Six gamma mutant lines (GM1, GM2, GM3, GM4 GM5 and GM6) were selected on the basis of better yield and other agronomic characteristics. Cheng et al. [29] studied the effects of gamma-rays on the physiological, morphological characteristics and chromosome

aberrations of minitubers of one potato cultivar, 'Shepody' with 8 doses of gamma rays (0, 10, 20, 30, 40, 50, 60, 70 and 80 Gy). Radiation with 20 Gy promoted tuber formation, and the average number and diameter of M1 tubers per plant were significantly increased over the control by 71% and 34%, respectively. With an increase in radiation dose, the emergence percentage, plant height and root length of minituber plants were significantly decreased for 40 and 50 Gy, with about 67% and 31%, respectively. No emergence ability occurred at 60 Gy and higher doses. The morphological variations occurred beginning with the 10 Gy treatment. The peak in variation frequency appeared at 30 Gy (14%) and then decreased. There was no morphological variation at 50 Gy and no survival of plants at 60 Gy and higher doses. It was found that radiation with 10, 20 and 30 Gy had no statistical significant effect on the plant height of minituber plants. Hassan [16] irradiated dry tubers of potato cultivars with different doses of gamma rays (20, 30 and 40 Gy) to study the effect of gamma rays on the vegetative and yield traits. The results showed that there were no significant differences between cultivars for all studied traits except the number of tubers per plant.

4 Conclusion

In the present study, the effect of gamma radiation was investigated (22, 33, 54, 57 and 109 Gy) on the susceptibility of the potato plant to *Rhizoctonia solani*. The best results were found with a dose of 22 Gy. The application of gamma irradiation in this study may offer a new approach for national potato breeders for developing plants resistant to *R. solani*.

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Conflict of interest: Authors declare no conflict of interest.

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