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Araştırma
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Mutation induction using Co^{60} in Pembe Çekirdeksiz (*Vitis vinifera* L.) grape cultivar

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Abstract

In the study an optimum dose among Co^{60} treatments that make less physical injury and positive effects comparing the control plants was investigated in Pembe Çekirdeksiz grape cultivar. Related to this aim, vine cuttings were exposed to the 0, 15, 25, 35 and 45 Gy doses of Co^{60}. Buds of radiation treated cuttings were grafted on 17 old vines of Recel grape cultivars (*Vitis vinifera* L.) by chip bud grafting method. Survival rates obtained from 15 and 25 Gy Co^{60} applications were found to be the closest to the control application. 35 and 45 Gy doses of Co^{60} were observed as lethal in this cultivar. It was observed that progressive radiation doses resulted in a variation in shoot length and leaf area values. The highest shoot length and leaf area values were obtained from control and 15 Gy Co^{60} applications. Radiation treatment made bit late in the bud break but it did not retard the ripening time. On the other hand Co^{60} treatment resulted decreasing in cluster and berry size. Stomata numbers decreased with the progressive Co^{60} doses. Although same bit variations in different characteristics were determined by radiation treatments, positive variations for grape breeding were not observed at this stage.

Key words: Grapevine, mutation, gamma irradiation

Pembe Çekirdeksiz (*Vitis vinifera* L.) üzüm çeşidinde Co^{60} kullanılarak mutasyon oluşturulması

Öz

Bu çalışmada Pembe Çekirdeksiz üzüm çeşidinde kontrol bitkileriyle kıyaslandığında en az fiziksel zarara neden olan ve pozitif etkide bulunan optimum Co^{60} dozu araştırılmıştır. Bu amaca yönelik olarak asma çelikleri Co^{60} 'ın 0, 15, 25, 35 ve 45 Gy dozlarına maruz bırakılmıştır. Radyasyon uygulanan çeliklerin gözleri 17 yaşlı Reçel üzüm çeşidi (*Vitis vinifera* L.) asmaları üzerinde yongalı göz aşılaması yapılmıştır. 15 ve 25 Gy Co^{60} uygulamasının canlılık oranları uygulama yapılmayan kontrol grubuna oldukça yakın değerler göstermiştir. Co^{60}'ın 35 ve 45 Gy dozları bu çeşit için öldürücü olmuştur. Yüksek dozlar bu çeşit için sürgün uzunluğu ve yaprak alanı değerlerinde değişime neden olmuştur. En yüksek sürgün uzunluğu ve yaprak alanı değerleri kontrol ve 15 Gy Co^{60} uygulamasından elde edilmiştir. Radyasyon uygulaması göz uyanmasını bir miktar geciktirmiş fakat olgunluk zamanında gecikmeye neden olmamıştır. Diğer taraftan Co^{60} uygulaması salkım ve tane büyüklüğünde azalmaya neden olmuştur. Ayrıca yüksek dozdaki Co^{60} uygulaması stoma sayısının azaltmıştır. Sonuç olarak radyasyon uygulaması farklı karakterlerde bir miktar farklılığa neden olmasına karşın asma ıslahı açısından pozitif varyasyonlar elde edilememiştir.

Anahtar kelimeler: Asma, mutasyon, gama ışınımı

Giriş

Grape (*Vitis vinifera* L.) is one of the most important commercial fruits in the world. It is heterozygous and vegetatively propagated. Plant breeding requires genetic variation of useful traits for crop improvement. It has a long juvenility period and takes a long time to flower so new grape cultivars can be obtained in a long time for world grape production by traditional breeding methods such as clonal selection, crossbreeding and natural mutation. However artificial mutation techniques have become a proven way of changing the one or more agronomic characteristics of a popular variety using. Physical (ionized radiation such as x and gamma rays) and chemical mutagens (ethyl DES, colchicine) can be used to induce mutations and generate genetic variations from which desired mutants may be selected. Natural and artificial mutation effect appears in long time period but these studies decreased day by day (Donini and Sonnino, 1998; Predieri, 2001). 2252 mutant varieties were recorded in past seventy years in cereals, fruits and other crops (Maluszynski et al., 2000). Although number of mutant varieties, obtained by natural or artificial mutation methods, are overly but only 48 mutant cultivars of them were determined in fruits and viticulture (Ahloowalia et al., 2004). Radiation application has been used mostly for providing direct mutation effects and rate of gamma radiation utilization was 64% (Ahloowalia et al., 2004). Some modifications in agronomic characteristics that berry size, inhibition of berry falling, disease resistance, shortens the mature period have been researched in the mutation induction studies by using gamma irradiation. The objective of the study was to examine the optimum dose among gamma radiation doses investigated in Pembe Cekirdeksiz grape cultivar. Moreover, it was investigated that make less physical injury and positive effects comparing the control plants was advantages of grafting of irradiated buds on to mature vines to shorten the breeding period.

Materials and Methods

In this study, cuttings with one bud were collected in first experimental year and used for irradiation in Pembe Cekirdeksiz grape cultivar. Cuttings were irradiated by 0, 15, 25, 35 and 45 Gy gamma radiation doses. Irradiation of the cuttings was carried out at Cukurova University, Medical Science Faculty by means of blood ray apparatus. Irradiated buds on cuttings were grafted on to 20 old Vitis vinifera vines by chip bud grafting method.

Morphological, phenological, cytological observation and pomological analyses were carried out in the obtained candidates. In the first experimental year, only the rate of graft setting (%) was observed. Morphological observations (shoot length (cm) and leaf area (cm^2) and phenological observations (bud burst times (day), full blooming, veraison and ripening dates (day. month) were recorded in the second experimental year. Pomological analyses were also performed in obtained all ripened clusters in the second experimental year. Berries were sampled from mid-sections (1/3) of each cluster. Cluster and berry weights (g) were determined with a precise balance (±0.01 g, Radwag WTC200, Poland). Cluster length (cm) and widths (cm) were measured with a ruler. Berry lengths and widths (mm) were measured with a digital caliper (Mitutoyo Absolute 500-196-20, Japan). Berry volumes were measured with a graduated cylinder. Then, water soluble dry matter content (WSDC) was determined with a hand refractometer (Atago 2311, Japan) and pH was measured with a pH meter (HI 2211-02, USA). The titrable grape juice acidity (%, g/100 ml grape juice) was measured with 0.1 N sodium hydroxide titration method. Leaf samples were taken from 1/3 section of summer shoots with identical vigor at veraison period. Leaf area was measured with a planimeter (KOIZUMI-KP-90N, Japan). Shoot length was measured with a tape-meter. Cytological observations include stomata density in the 1/3 part of mature leaves that were determined all continued vigour plants after grafting. Nail polish was used in stomata density in leaves (Akal, 2001).

The experiment was distributed by randomized block experiment design as three replicated, ten buds per replicated. ANOVA analysis was used for all parameters by using JMP program version 10.0.0. Averages of applications were compared by LSD (Low Significant Differences) test at 5% level.

Results

In this study, effects of gamma irradiation doses on different characters were investigated. The data about grafting success were presented in Table 1. The grafting success was found to be 70% in control application, 53.3% in the application of 15 Gy Co^{60}, 33.3% in the application of 25 Gy Co^{60}. However, bud burst was not observed in application of 35 Gy Co^{60} and 45 Gy Co^{60}. As seen in Table 1, different doses of gamma irradiation did not markedly effect to leaf area. While the highest value in leaf area was detected in control application (184.6 cm^2), the lowest value

was determined in treated with 25 Gy application (162.7 cm^2). The lowest shoot length value was obtained from 25 Gy Co^{60} (Control: 125 cm, 25 Gy Co^{60} application: 113.8 cm).

Table 1. Effects of different Co^{60} doses on grafting success (%), leaf area (cm^2) and shoot length (cm) in Pembe Çekirdeksiz grape cultivar

Co^{60} Doses	Grafting success	Leaf area	Shoot length
Control (0 Gy)	70.0 a	184.6	125.0 a
15 Gy	53.3 ab	176.8	117.9 ab
25 Gy	33.3 b	162.7	113.8 b
35 Gy	0	-	-
45 Gy	0	-	-
LSD 5%	27.5	N.S.	10.1

N.S.: Not significant

Effects of gamma applications on bud burst time were not found significant statistically whereas four days delayed was occurred by 15 and 25 Gy Co^{60} applications as compared with control (Table 2). Gamma ray doses did not markedly effect to blooming date. While full blooming time was recorded as 71 day in control application and 25 Gy Co^{60} application, one day before was observed in 15 Gy Co^{60} application. Although veraison in all gamma applications was determined as 117 day after grafting time, 1-2 days retarded veraison was occured in control application (118 day). While the earliest ripening time was determined in 25 Gy Co^{60} application (128 day after grafting time), nearest dates to this time were observed in other applications (Table 2).

Table 2. Effects of different Co^{60} applications on bud burst, full blooming, veraison and ripening time (day)

Co^{60} Doses	Bud burst time	Full blooming time	Veraison time	Ripening time
Control (0 Gy)	35	71	118	130
15 Gy	39	70	117	131
25 Gy	39	71	117	128
35 Gy	-	-	-	-
45 Gy	-	-	-	-
LSD 5%	N.S.	N.S.	N.S.	N.S.

N.S.: Not significant

Effect of gamma irradiation on cluster weight, width and length in Pembe Cekirdeksiz was shown in Table 3. Although differences between applications was not found significant, the highest cluster weight value was obtained from control application (122.3 g). Decreases in these values was determined by increasing of application doses. However difference between applications was not noticeable. While the highest cluster width value was seen in control application (9.25 cm), 25 Gy Co^{60} application resulted in the formation of the lowest cluster (8.14 cm). As seen as cluster width, gamma applications were not caused markedly differences in cluster length but highest cluster length value was determined in control application, shortening amount of was pointed out by increases of gamma ray dose. Increases in gamma ray doses was caused of small amount of reduction in berry weight. Differences between control and application doses were appeared as important in berry weight. Lowest berry weight value was obtained from 25 Gy Co^{60}application (1.05 g). Berry weight in control application was 1.42 g. Effect of applications according to berry volume was conformity with berry weigth. While lowest berry volume value was obtained from control application (1.30 ml), lowest value was determined from application of 25 Gy Co^{60} (0.93 ml) (Table 4). When berry width results were examined, a small amount of reduction was determined with regard to increases of gamma doses. While berry width of control application was 11.80 mm, smaller berries were obtained by increasing of gamma doses as comparing with control application.

Table 3. Effect of Co^{60} application on cluster weight (g), cluster width (cm) and cluster length (cm) in Pembe Çekirdeksiz grape cultivar.

Co^{60} Doses	Cluster weight	Cluster width	Cluster length
Control (0 Gy)	122.3	9.25	21.9
15 Gy	116.4	9.00	19.7
25 Gy	98.6	8.14	19.4
35 Gy	-	-	-
45 Gy	-	-	-
LSD 5%	N.S	N.S.	N.S.

N.S.: Not significant

The smallest berries were attained by 25 Gy application (9.48 mm). Berry length results were in harmony with berry width and berry length values varied between 10.6-13.2 mm. The longest berries were obtained from control application, the shortest berries (10.6 mm) were determined in 25 Gy application (10.6 mm) (Table 4).

Table 4. Effects of Co^{60} on berry weight (g), berry volume (ml), berry width (mm) and berry length (mm) in Pembe Çekirdeksiz grape cultivar.

Co^{60} Doses	Berry Weight	Berry Volume	Berry Width	Berry Length
Control (0 Gy)	1.42 a	1.30 a	11.80 a	13.2 a
15 Gy	1.14 b	1.05 ab	10.23 ab	11.3 ab
25 Gy	1.05 b	0.93 b	9.48 b	10.6 b
35 Gy	-	-	-	-
45 Gy	-	-	-	-
LSD 5%	0.23	0.30	2.23	2.38

Effects of applications on total soluble solids, acidity and pH were not found significant statistically. The values of total soluble solids, acidity and pH varied in between 21.1-22.0%, 0.260-0.340% and 3.73-3.86, respectively. (Table 5).

Table 5. Effects of Co^{60} application on total soluble solids (TSS)(%), acidity (%) and pH in Pembe Çekirdeksiz

Co^{60} Doses	TSS	Acidity	pH
Control (0 Gy)	21.1	0.340	3.73
15 Gy	22.0	0.260	3.86
25 Gy	21.3	0.340	3.74
35 Gy	-	-	-
45 Gy	-	-	-
LSD 5%	N.S.	N.S.	N.S.

As the results of stomata density determination, decreases of stomata amount in 1 mm^2 was seen as regard to increases of gamma irradiation doses. Application that has the highest stomata density was determined in control application with the value of 170 each mm^{-2}. 15 and 25 Gy Co^{60} applications were seen in the same statistically group (142 each mm^{-2} and 122 each mm^{-2}, respectively) (Table 6).

Discussion

According to the results, a reduction of graft setting rate may be described as physiological that gamma irradiation hydrolyze cell water and formed free radicals can be caused cell division inhibition because of disrupt cell wall and cytoplasm (Kaya, 2002; Blanco, 2005).

Table 6. Effect of Co^{60} application on stomata density (each mm^{-2}) in Pembe Cekirdeksiz

Co^{60} Doses	Stomata Density
Control (0 Gy)	170 a
15 Gy	142 b
25 Gy	122 b
35 Gy	-
45 Gy	-
LSD 5%	22

It was reported that the efficient mutation dose and susceptibility to gamma irradiation can be changed from cultivars to cultivar, also can be varied in the different buds of same cutting (Coban et al., 2002; Meksem and Kahl, 2010). 3.8 krad gamma irradiation doses in ten different buds of the same cutting in Mevlana, Pembe Gemre and Misket grape cultivars was applied and susceptibility of every bud has been showed differently. Ponnuswami et al. (1991) pointed out that ED50 according to survival rate varied between 20-25 Gy in Muscat grape cultivar. Marasalı et al. (2003) reported that ED50 was found to be 31.50 Gy in Kalecik Karası cv., 27.73 Gy in Sultani Cekirdeksiz cv., 21.47 Gy in Uslu cv. In addition to these findings, Dardeniz and Tayyar (2005) determined that ED50 was found to be as 28.13 Gy in 420 A and 15.10 Gy in 5 BB. The results of this study are conformity with those of Ponnuswami et al. (1991), Marasalı et al. (2003) and Dardeniz and Tayyar (2005).

Different doses of gamma irradiation did not markedly effect to leaf area. Such relation was also reported by Einset and Pratt (1975), Dardeniz and Tayyar (2005) and Pannuswami et al. (1991) who examined the negative impact of gamma irradiation on vegetative growth. In additionaly, Pannuswami et al. (1991) also obtained that leaf area decreases according to increase gamma irradiation doses.

Conclusion

Upon the results of this study, it can be concluded that increases in gamma ray doses reduced graft setting rate in Pembe Cekirdeksiz. The closely results to control application were obtained from 15 and 25 Gy Co^{60} applications with regard to survival rate. 35 and 45 Gy Co^{60} doses were found as lethal in the cultivar. Considering the general effect of increases irradiation doses on other morphological properties, shoot length and leaf areas were affected a little from increases gamma doses. Moreover, Co^{60} treatment resulted decreasing in cluster size and berry size. In addition stomata numbers decreased with the progressive Co^{60} doses. As a consequence although same bit variations in different characteristics were determined by radiation treatments, positive variations for grape breeding were not observed at this stage.

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