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Original scientific paper

DETERMINATION OF OPTIMAL MUTAGEN DOSAGE AND ITS EFFECTS ON MORPHO-AGRONOMIC TRAITS IN PUTATIVE MUTANTS OF 'AMASYA' APPLE

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Mutation breeding is one of the efficient ways to create new genotypes. The starting material of this study was the 'Amasya', a Turkish apple cultivar. This study aimed to identify optimal mutagen dosage that is the most important prerequisite to create a proper mutant population, and evaluate the genetic variability for morpho-agronomic traits (e.g. tree height and fruit number) in the putative apple mutants of 'Amasya' irradiated at the optimal mutagen dosage. In the first year of the study, to determine the optimal mutagen dosage, dormant 'Amasya' scion woods were irradiated at 0, 10, 20, 30, 40, 50 and 60 gray dosages using ^{60}Co source. The optimal mutagen dosage was determined as 29.01 gray for 'Amasya' concerning the 50% shoot length reduction in comparison to the non-irradiated control plants. In the following year, more or less 2000 dormant 'Amasya' scion woods were irradiated at the optimal mutagen dosage to generate a mutant population of 'Amasya' apple cultivar. Mutation frequency based on the visual observations of plant abnormalities was observed. Then three years, genetic uniformity

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was analyzed through the growth of the mutant population, and mutation frequency (%) determined. These results indicated that there was considerable genetic variability in response to irradiation for evaluated traits. Most of the plants in the putative mutant population, nearly 80%, had a significantly shorter tree height and trunk cross-sectional area than standard 'Amasya'. The mutants/genotypes identified in this study could be used improvement of new promising apple cultivars with good agronomical traits.

Keywords: fruit breeding, gamma irradiation, *Malus domestica*, *Malus slyvestris,* mutation breeding

INTRODUCTION

Apple is recognized and sought by consumers by individual cultivars, unlike many fruit crops (LUBY and BEDFORD, 2015). For this reason, cultivars have always been an essential role in the marketing of apple. Each year new apple cultivars are bred, or discovered, and released into the apple industry. Most of them originate as bud sports, which are mostly formed by a spontaneous single limb mutation. In fact, good strains of the familiar cultivars are more preferred than entirely new cultivars since they can be adopted with lower cost and risk to the industry (ANONYMOUS, 2015; ATAY *et al*., 2018). Even some strains have been more successful in some markets than their parental cultivars.

Gamma radiation has been widely used in fruit breeding to improve some traits such as fruit colour and pest/disease resistance (LAPINS, 1983; SPIEGEL-ROY, 1990; PREDIERI and ZIMMERMAN, 2001; SCHMIDT and VAN DE WEG, 2005). According to the FAO/IAEA (2018), 385 cultivars were obtained by mutation induction in vegetatively propagated crops, and approximately 25% of them were registered in fruit/nuts category. It is known that identification of the optimal mutagen dosage is the first and most important prerequisite to create a proper mutant population (KODYM *et al*., 2012). The use of optimal mutagen dosage is one of the essential elements in a mutation breeding programme (UKAI and NAKAGAWA, 2012).

'Amasya' apple cultivar has a great popularity thanks to its excellent flavour in Turkey (ATAY *et al*., 2016). However, the absence of regular bearing, good fruit skin colouring, functional tree architecture delays economic returns, limiting producer interest in its cultivation. 'Amasya' apple is predicted to have more economic consequences if superior mutants (e.g. weaker and regular bearing strains with enhanced fruit colour) is to be obtained. In this context, we have conducted a mutation breeding programme in Turkey to overcome the major problems of 'Amasya' apple. With this background, this study aimed to (1) determine optimal mutagen dosage, and (2) examine the genetic variability for morpho-agronomic traits in putative apple mutants of 'Amasya' irradiated at the optimal mutagen dosage.

MATERIALS AND METHODS

Experimental site and plant materials

The starting material of this study, which was conducted in Turkey, was the 'Amasya' apple cultivar, registered in Turkey by TAGEM Black Sea Agricultural Research Institute. The experimental site and plant materials, used here, have been described earlier (ATAY *et al*., 2018).

In summary, in February 2011, dormant 'Amasya' scion woods were irradiated at TAEA-SNTRC in Ankara at 0, 10, 20, 30, 40, 50 and 60 gray (Gy) dosages using ^{60}Co source. The water content in the woods was determined as 31.5%. Immediately after irradiation, Malling 9 (M.9) rootstocks were bench grafted with the irradiated scion woods. For each irradiation dosage, sixty trees were propagated. Soon afterwards, trees moved to a nursery at 0.8 m × 0.3 m planting distance. Fertilisers were provided through fertigation in the nursery. Weed control and other nursery practices were managed according to the usual local practices. The trees were dug in December 2011.

In 2012, nearly 2000 dormant scion woods of 'Amasya' apple was irradiated at the optimal mutagen dosage, which was determined in 2011, to generate the mutant population. At this time, the water content in the woods was determined as 27.4%. The tree propagation and nursery procedures up to the planting were the same as performed in 2011. The trees were kept two years (2012 and 2013) in the nursery without pruning. The 857 healthy mutant trees were obtained from the nursery as two-year-old, and they were planted to the orchard in January 2014. Non-irradiated five 'Amasya' trees, which were propagated with the same method as for other trees, were also planted as the control in the orchard. The tree spacing was 4.0 × 1.0 m in the orchard. All trees were supported and were maintained without pruning from planting until December 2015. Variation in morphological traits was investigated over the years 2013 to 2015. Approximately thirty mutant trees died during this period.

Data collection

In 2011, the number of surviving plants was recorded, and the percentage of surviving plants was calculated. The length of scion shoots, hereafter referred to as shoot length, to determine optimal mutagen dosage was measured. Both the percentage of surviving plants and shoot length were determined approximately 60 days after grafting in 2011.

In 2012, Soil-Plant Analyses Development (SPAD) values were assessed using a portable chlorophyll meter (SPAD-502Plus, Konica Minolta, Japan) in the first week of July. The thirty leaves which showed light green, green and dark green colour were sampled from each tree for SPAD measurements. In the same period, the percentage of surviving plants (as described above) and mutation frequency of the population was determined. Mutation frequency was based on the visual observations of plant abnormalities such as chlorophyll mutation and unusual apple leaf shapes (Figure 1) of the population (KUNTER *et al*., 2012).

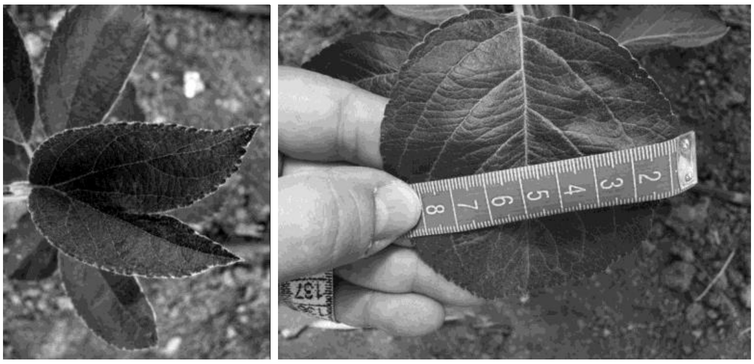

Figure 1. Unusual apple leaf shape samples in response to irradiation.

In 2014 and 2015, fruit number, tree height and trunk diameter data for each tree were collected. Tree height measurements were started one-year prior (i.e. in 2013) than others. The fruit were counted at harvest time. Tree height and trunk diameter were measured in December. The trunk cross-sectional area (TCSA) was calculated by the following formula: TCSA (cm^2) = π (trunk diameter/2)^2.

Data analysis

The data for the percentage of surviving plants and SPAD were subjected to one-way analysis of variance (ANOVA). Reduction of the 50 percent in shoot length compared with non-irradiated control plants was calculated through linear regression analysis to determine the optimal mutagen dosage. Tree height and TCSA were depicted by box plots and clustered. The discriminated clusters were subjected to one-way ANOVA. The means were separated by Least Significant Difference (LSD) multiple comparison test in ANOVA.

RESULTS AND DISCUSSION

After 60 days of growth, the impact of gamma irradiation dosages on the percentage of surviving plants is shown in Figure 2. The irradiation dosages had a significant effect on it. The percentage of surviving plants was the highest (71.19±4.74%) in non-irradiated control, while the lowest in 60 Gy (13.11±5.22%). As previously mentioned in fruit trees (ZAGAJA and PRZYBYLA, 1973; ANONYMOUS, 1976), irradiation has a significant effect on the percentage of surviving of plants. Thus, it affects the physiological and metabolic adaptation, protein composition, enzymes related activity, hormonal system and gas exchange of plants (LAGODA, 2012). It is also known that irradiation affects the free radical content of plants, particularly at higher dosages (ANONYMOUS, 1976).

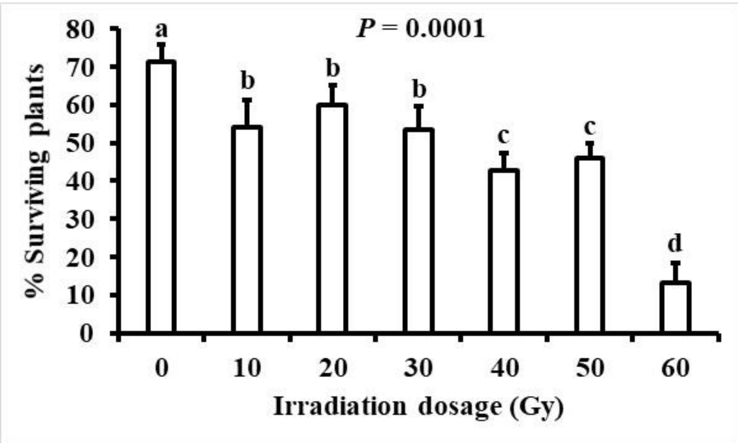

Figure 2. Effect of different irradiation dosages on the percentage of surviving plants of apple cv. ‘Amasya’. Different letters at the top of each bar indicate significant differences at $P < 0.05$. The values are means ± standard deviation (SD).

We investigated the effects of different dosages of gamma rays on the shoot length of 'Amasya' (Figure 3). Thus, length is often used as a criterion of plant reaction to a mutagen depending on the species (KODYM *et al*., 2012). A gradual decline in shoot length was apparent when increasing irradiation dosages from 10 to 60 Gy. Especially after 30 Gy, shoot length decreased considerably. The shoot length in non-irradiated control plants was 29.89±16.44 cm. Effects of mutagens are well known to dependent on dosages applied (FAO/IAEA, 2018). Reduction of 50% in shoot length was determined as 29.01 Gy that meant it appears to be optimal mutagen dosages for irradiation of 'Amasya' apple. Reduction in shoot length could be used to determine the optimal mutagen dosages of mutagens (VAN HARTEN, 1998). It became commonly accepted that dosages inducing 50% reduction in the growth of plants can result in the highest mutations rates (KODYM *et al*., 2012). In this study, the dosage determined for 'Amasya' apple is generally in agreement with the results obtained in temperature fruit trees irradiated in the dormant period (ZAGAJA and PRZYBYLA, 1973; LACEY and CAMPBELL, 1979; LAPINS, 1983; LIU and SHIRONG, 2002; CAMPEANU *et al*., 2010).

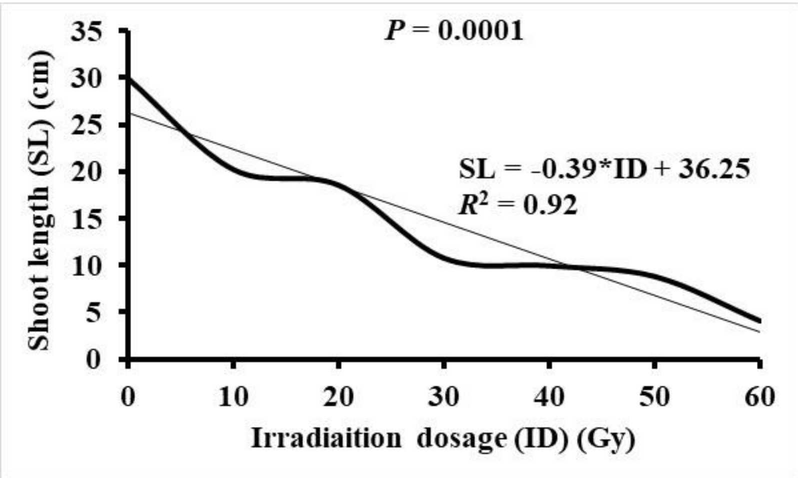

Figure 3. Effect of different irradiation dosages on shoot length of apple cv. 'Amasya'.

There was a significant influence of gamma irradiation on the SPAD values of leaves (Figure 4). SPAD value was the highest (51.97±3.61) in dark green leaves and the lowest (23.67±3.16) in light green leaves. SPAD value is correlated positively with the nitrogen content of plants (ATAY and KOYUNCU, 2013). Thus, nitrogen is an essential element for plants, and essential compounds (e.g. amino acids, enzymes, and chlorophyll) include nitrogen (JOHNSON, 2008).

The Mutation frequency of the population submitted to optimal mutagen dosage (29.01 Gy) in 2012 was determined as 2.43% (data not shown), which was similar to the values obtained previously from fruit trees (LACEY and CAMPBELL, 1979; PREDIERI and ZIMMERMAN, 2001; KUNTER *et al*., 2012). The percentage of surviving plants in this population was 55.34% (data not shown). These findings agree with the data that collected in 2011 in this study.

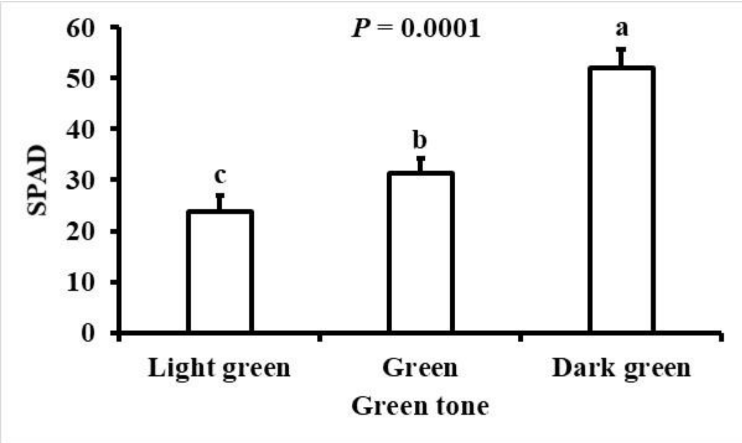

Figure 4. SPAD values of the leaves which showed light green, green and dark green colour. Different letters at the top of each bar indicate significant differences at $P < 0.05$. The values are means ± SD.

Tree height was measured to determine variation in morphological traits of the population over three years from 2013 to 2015 (Figure 5). There were marked differences in tree height among the trees. Tree height was relatively higher in 'Amasya' (190 cm, 250 cm and 280 cm in 2013, 2014 and 2015, respectively) in compared to the median values of the population, which was 122 cm, 160 cm and 210 cm in 2013, 2014 and 2015, respectively. Similarly, variability in TCSA among trees showed a visible variation (Figure 6). The median TCSA value of the population was 1.68 cm^2 and 4.18 cm^2 in 2014 and 2015, respectively, whereas TCSA was higher in 'Amasya' in both years (3.56 cm^2 and 5.89 cm^2 in 2014 and 2015, respectively) in comparison to others. Mutagens make plants to grow slowly, leading to a reduction in plant height (MALEK *et al*., 2014; GANAPATHI *et al*., 2015; ASARE *et al*., 2017). In this study, both tree height and TCSA showed marked variations among the mutants. Much of the trees (approximately 80%), had lower values for tree height and TCSA than standard 'Amasya'. Our results showing reduced growth effects of mutagens agree with LAPINS (1983) and CAMPEANU *et al*. (2010) who showed the reduction in tree vigour in apple trees after irradiation. There might be numerous factors (such as protein synthesis, leaf gas and water exchange) affected by irradiation, which may lead the reduction in tree height (DATTA *et al*., 2011; LAGODA, 2012; ALI *et al*., 2016). Also, cell division and cell elongation stages of plants are affected negatively by irradiation (ASARE *et al*., 2017).

We also created a hierarchical cluster assess similarity or dissimilarity in the putative 'Amasya' mutants for fruit number, tree height and TCSA. The hierarchical cluster resulted in twenty main clusters (Table 1). The differences between clusters were significant for all traits examined. 'Amasya' with nine genotypes formed the cluster 5. Cluster 10 with twenty genotypes had quite high value for tree height and TCSA in 2015. Similarly, clusters 9, 5, 12 and 15 had relatively high values for tree height and TCSA in 2014. Cluster 20 generally had the lowest mean values for all studied traits. Fruit number per tree was the highest in cluster 15 (47.88 ± 10.17).

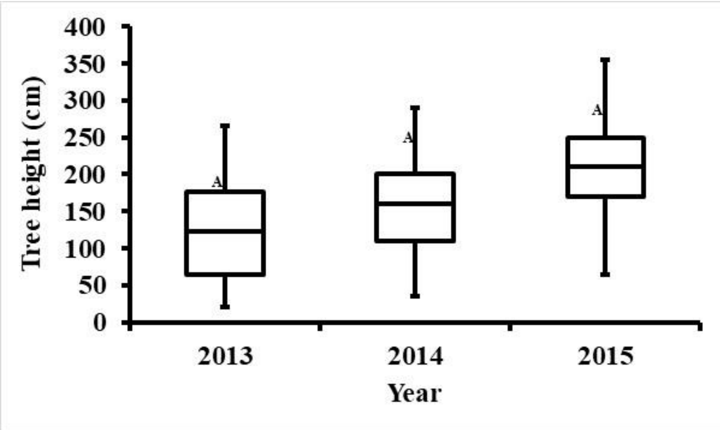

Figure 5. Box plots depicting the variation of 826 genotypes (825 mutants of ‘Amasya’ apple with the original ‘Amasya’ represented by ‘A’ in the figure) for tree height. The horizontal middle line in boxes denotes the median value. The whiskers below and above the boxes denote the minimum and maximum value, respectively.

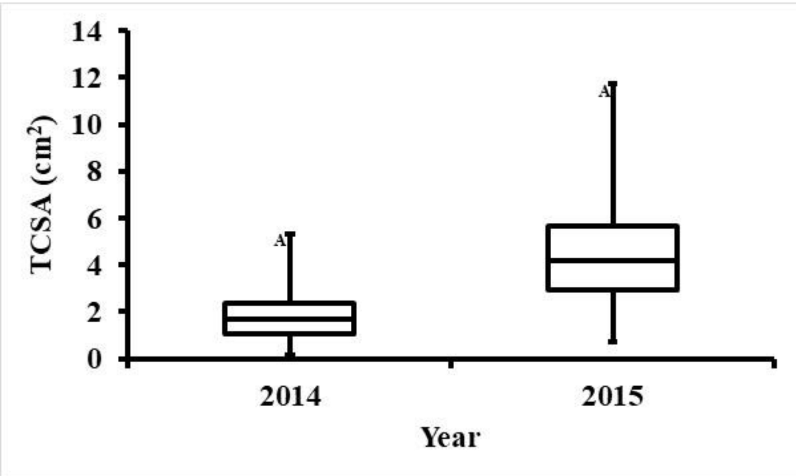

Figure 6. Box plots depicting the variation of 826 genotypes (825 mutants of ‘Amasya’ apple with the original ‘Amasya’ represented by ‘A’ in the figure) for trunk cross-sectional area (TCSA). The horizontal middle line in boxes denotes the median value. The whiskers below and above the boxes denote the minimum and maximum value, respectively.

Table 1. Clustering of 826 genotypes (825 mutants of 'Amasya' apple with the original 'Amasya') for fruit number, tree height and trunk cross-sectional area (TCSA) in 2014 and 2015.

Cluster	n	Fruit number per tree-2014	Fruit number per tree-2015	Tree height (cm)-2014	Tree height (cm)-2015	TCSA (cm^2)-2014	TCSA (cm^2)-2015
1	29	3.37 ± 0.56 c	2.48 ± 4.48 g	206.37 ±21.54 d	254.82± 21.35 de	2.31 ±0.34 e	5.61± 1.40e
2	36	1.61 ± 0.59 e	1.11 ± 1.54 h	204.02 ±19.92 d	254.89 ±23.25 de	2.26 ±0.41 e	5.47 ±1.06 ef
3	13	2.38 ± 0.50 d	0.38 ± 0.76 hı	151.15 ±17.09 g	199.23 ±16.56 hı	1.52±0.37 gh	4.00 ±0.58 h
4	19	5.36 ± 1.06 b	0.94 ± 2.27 hı	182.36±23.76 ef	224.21 ± 28.73 g	2.30 ±0.65 e	5.41 ±1.23 ef
5	10	6.30 ± 1.33 a	1.30 ± 3.12 ghı	241.00±14.68 ab	288.00 ± 11.35 b	3.14± 0.54 c	6.84 ±1.39 c
6	91	0.11 ± 0.31 jk	0.51 ± 1.10 hı	174.56 ±16.95 f	235.54 ±16.57 f	1.69 ±0.26 fg	4.35 ±0.59 h
7	35	0.00 ± 0.00 k	5.14 ± 2.80 f	180.42 ±19.18 f	229.71 ± 14.59 fg	2.34 ±0.48 e	4.92 ±0.76 g
8	55	0.00± 0.00 k	0.85 ± 1.28 h	188.54 ±19.06 e	248.18 ± 19.32 e	2.30 ±0.31 e	6.19 ±0.59 d
9	15	2.53 ± 1.06 d	12.06 ± 4.35 d	245.00 ±20.61 a	285.33 ± 27.67 b	3.76 ±0.79 b	7.23±0.74 bc
10	20	1.10 ± 1.07 f	6.65 ± 3.31 e	248.50 ±19.20 a	307.25 ± 21.67 a	3.78 ±0.50 b	8.83 ±1.49 a
11	50	0.38 ± 0.60 hı	2.72 ± 2.99 g	222.00 ±21.11 c	276.20 ± 28.50 bc	2.88 ±0.52 c	7.42 ±0.99 b
12	15	0.93 ± 0.88 fg	25.13 ± 3.87 c	240.33 ±16.19 ab	276.67 ± 19.88 bc	3.64 ±0.56 b	7.21±0.94 bc
13	13	0.61 ± 0.76 gh	27.69 ± 4.44 b	220.76 ±22.34 c	254.61 ± 34.30 de	2.47±0.46 de	3.96 ±0.95 h
14	24	0.25± 0.44 ıj	12.87 ± 3.54 d	228.95 ±18.05 bc	268.33 ± 28.03 c	2.62 ±0.30 d	5.07 ±0.88 fg
15	9	2.22 ± 1.92 d	47.88 ± 10.17 a	240.00 ±17.85 ab	267.78 ± 27.73 cd	4.12 ±0.71 a	6.79 ±1.42 c
16	131	0.01 ± 0.08 k	0.14 ± 0.59 ı	125.22 ±19.21 ı	185.00 ± 17.37 j	1.26 ±0.29 ı	3.38 ±0.50 ı
17	49	0.04 ± 0.19 jk	0.40 ± 1.05 hı	131.63 ±18.94 h	199.28 ± 14.75 h	1.39 ±0.36 h	4.96 ±0.57 g
18	20	0.05 ± 0.22 jk	7.50 ± 3.15 e	154.00 ±12.73 g	185.50 ± 15.71 ıj	1.86 ±0.72 f	2.97 ±0.63 j
19	120	0.00 ± 0.00 k	0.01 ± 0.12 ı	100.95 ±18.39 j	149.70 ± 16.63 k	0.89 ±0.25 j	2.50 ±0.68 k
20	72	0.00 ± 0.00 k	0.00 ± 0.00 ı	64.72 ±13.83 k	136.18 ± 22.19 l	0.55 ±0.18 k	1.743 ±0.48 l
P		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Within each trait, different letters indicate significant differences at $P < 0.05$. n: Number of genotypes per cluster. The values are means±SD. Original 'Amasya' is in Cluster 5.

CONCLUSION

In this study, the optimal mutagen dosage for 'Amasya' apple cultivar was determined to use in mutation breeding programme of apple. The use of this dosage enabled the obtainment of a mutated population with low physiological damage and high mutation frequency. It was generated a proper mutant population irradiated at the determined optimal mutagen dosage. This study is the first attempt to identified individuals with a target mutant phenotype. There is also considerable genetic variability among irradiated 'Amasya' apples using gamma rays for evaluated traits over the years 2013 to 2015. After that, the next important step is to screen and select mutants with desired traits. Further investigation is also needed to re-evaluate and confirm the putative mutants under well designed experimental conditions. Our ongoing mutation breeding programme in Turkey can make a vital contribution to the fruit industry with the improvement of some traits of 'Amasya' apple such as well-balanced vigour and regular bearing.

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ODREĐIVANJE OPTIMALNOG MUTAGENOG DOZIRANJA I NJEGOVIH EFEKATA NA MORPHO-AGRONOMSKE OSOBINE KOD POTENCIJALNIH MUTANATA "AMASIA" JABUKE

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Izvod

Oplemenjivanje korišćenjem mutacija je jedan od efikasnih načina za stvaranje novih genotipova. Početni materijal ovog istraživanja bila je „Amasia", turska sorta jabuke. Ovaj rad je imao za cilj da identifikuje optimalnu dozu mutagena koja je najvažniji preduslov za stvaranje odgovarajuće mutantne populacije, i da proceni genetsku varijabilnost za morfo-agronomske osobine (npr. visina stabla i broj plodova) u pretpostavljenim mutantima jabuke "Amasia" zračenih optimalnom dozom mutagena. U prvoj godini, da bi se odredila optimalna doza mutagena, dormantne Amasia reznice, su ozračene sa 0, 10, 20, 30, 40, 50 i 60 Gy doza koristeći 60Co izvor. Optimalna doza mutagena određena za sortu "Amasia" iznosila je 29.01 Gy, što se tiče smanjenja dužine izdanka od 50% u poređenju sa ne-zračenim kontrolnim biljkama. U sledećoj godini, oko 2000 dormantnih reznica sorte Amasia je ozračeno optimalnom dozom mutagena da bi se generisala mutantna populacija 'Amasia' sorte jabuke. Uočena je učestalost mutacije na osnovu vizuelnih opažanja abnormalnosti biljaka. Zatim su tri godine analizirane genetičke uniformnosti kroz rast mutantne populacije, a utvrđena je i učestalost mutacija (%).Ovi rezultati pokazuju da postoji značajna genetska varijabilnost u odgovoru na ozračivanje za posmatrane osobine. Većina biljaka u potencijalnoj mutantnoj populaciji, skoro 80%, imala je značajno kraću visinu stabla i površinu poprečnog preseka stabla od standardnog sorte "Amasia". Identifikovani mutanti / genotipovi u ovoj studiji mogu se koristiti za poboljšanje novih perspektivnih sorti jabuka sa dobrim agronomskim osobinama.

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