PRIMARY EFFECTS OF GAMMA RADIATION (Cz^{137}) ON THE MORPHOLOGY OF LEAVES AT SOME SWEET CHERRY VARIETIES

Melpomena Popovska[1], Bojan Popovski[2], Viktor Gjamovski[1]

[1]University ,,Ss. Cyril and Methodius" Institute of Agriculture – Skopje, R. Macedonia
[2]University ,,Ss. Cyril and Methodius" Faculty of Agricultural Sciences and Food – Skopje, R. Macedonia

Corresponding author: m.popovska@zeminst.edu.mk

Abstract

II. The study has been conducted based on the presence of atypical leaf morphology, in relation to leaf shape, size, color, leaf nervation, leaf stem length and leaf stem thickness, as a primary effect product in the first MV1 generation after the gamma radiation with Cz^{137} on Bigareau Burlat, Pobeda Krimska and Kozerska cherry varieties**.** The selected leaf radiomorphosys were used as conservative and accurate signs for early detection of somatic mutations caused by radiation. Graft branches were exposed with dosages of 25Gy, 35Gy and 45Gy at the Institute of Radiobiology and Radio-preservation in Sofia. The grafting was performed with dormant buds onto Prunus mahaleb rootstock. The dosage augmentation caused average reduction of leaf dimensions and leaf stem lengths in all of the tested varieties. Also, with the augmentation of the radiation dosage, the percentage of chlorophyll deficiency leaves increased from 1,1% at 25Gy to 7,4% at 45Gy. An average of 47,5% of the analyzed leaves have an atypical shape, without significant differences between the varieties, and 14,5% of the leaves have an atypical leaf nervation . The highest percentage of leaves with atypical shape and leaf nervation are represented in plants treated with a dose of 45Gy.

Keywords: *Prunus avium* L., ionizing dosage, leaf shape, leaf size and color, leaf stem length.

Introduction

The primary effects of gamma radiation on fruit species are noticed as a visible phenotypic changes in the first MV1 generation following the treatment. The radiation usually results with lethal or sub-lethal damages of the cells and tissues and with atypical growth and development of plants. Appearance of the modification changes at fruit plants can be used as a diagnostic measure for somatic mutations (Nybom, 1961), (Bishop, 1967), (Равкин,1973), (Миленков, 1974,1979), (Donini,1975,1991), (Lapins, 1983). The effects are result of the direct damage of the apical meristem of the leaf buds, as well as the secondary effect of the physiological disbalance which emerges in the affected cells (Guncke and Sparrow, 1961). The authors mention that the regeneration area of the affected cells emerges in the primary brunches with an atypical position of the buds, significantly fattened nodal regions, and an atypical brunch color, presence of furcations and fasciations and atypical leaves morphology. These changes present a promising starting material for further selection and creation of new interesting genotypes. For the appearance of various morphological changes in the leaves as a result of the effect of ionizing radiation in the fruit species adduce Zwintcher (1955, 1967), (Жуков,1963), (Петров и Сермяжко, 1963), (Шепотъев, 1968), (Колесникова, 1970), (Lapins and Hough,1970), (Миленков, 1974). The authors of the available literature refer to the various changes in the leaf color, size, leaf-forms, the length of leaf stem, changes in the leaf serration and in the leaf nervature. The selected leaf radiomorphosys are conservative and accurate indications for early detection of somatic mutations. Zwintcher (1955) even declare that the propagation of leaf buds with radiomorphosys is more efficient and more economical than the propagation of all buds located in the plant's foundation. Considering the fact that there are not enough data for this scientific area in the available new literature, the aim of this

paper is to analyze the presence of leaf radiomorphosys, as a primary effect product of radioactive treatment and indicators for early detection of somatic mutations on three cherry varieties with different dosages of Cz^{137}.

Material and methods

Dormant buds from Bigareau Burlat, Pobeda Krimska and Kozerska cherry varieties were treated with radioactive Cz^{137} in doses of 25Gy, 35Gy and 45Gy. Graft branches were exposed to radiation at the Institute of Radiobiology and Radiopreservation in Sofia. Prunus mahaleb L. was used as a rootstock for grafting the buds in two following seasons, right after the treatment on 30th of August. Untreated buds from each variety were used as a control variant. Each variant was grafted onto two hundred rootstocks. An early diagnosis of the primary effects of the radiation was made in the first MV_1 generation following the treatment. Basic criteria for first choice was made according to the appearance of plants with decreased vigorousness and irregularly positioned leaf buds, presence of furcations (bi-, three- and polyfurcations), atypical leaves and expressive outspread of the plants (Popovska et al.,2011). A study has been conducted on the presence of atypical leaf morphology, in relation to leaf shape, leaf nervation, size, color, leaf stem length and leaf stem thickness, from total number of leaves at 195 selected plants, in the first year after the treatment of dormant buds. The measures were made in august, when leaves were properly differentiated. The leaf size is classified according to the leaf length in five groups: very small (< 5cm); small (5-8cm); medium size (8-12cm); large (12-16cm) and very large leaves (>16cm). Leaf stem length is classified in three groups: short (<3,5cm), medium length (3,5 – 5cm) and long leaf stem (>5cm). Control variants have dark green leaf- color. Atypical leaf color is noticed as various shades of green color, leaves with deficiency of chlorophyll and dark green leaves with yellow leaf-nerves. The research was performed at the experimental field in the Institute of Agriculture in Skopje. The soil type is silt - clay loam, suitable for cherry production, with moderate alkaline pH according to its reaction in water and neutral according to its reaction in KCl, carbonate, with a low amount of humus, with a good amount of hydrolyzing nitrogen and optimal amount of easily obtainable phosphorous and potassium. The trial was watered with a drop irrigation system.

Results and discussion

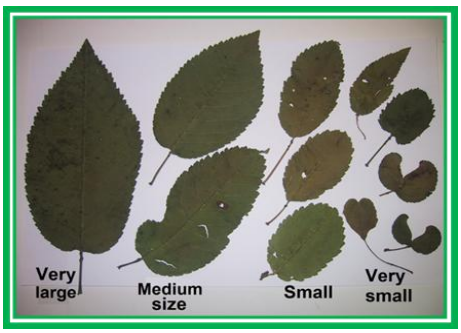
According to the basic criteria for early diagnostic of the effects of treatment with radioactive Cz137, 195 or 46,9% of the total number of plants received after the treatment, have been selected in MV1 generation (Popovska et al.,2011). The decreased vigorousness of the selected plants has the most participation of 29,3% from all present primary effects, followed by furcations (23%), then plants with atypical leaves (6,1) and then with expressive outspread (4,3%) (Popovska et al., 2011). Respective to the variety, 88 plants are selected from Bigareau Burlat, 54 plants from Pobeda Krimska and 53 are from Kozerska. Related to the dosage, 83 are selected from radiation of 25Gy, 58 from 35Gy and 54 from 45Gy (Popovska et al., 2011). The control variants are all with large and very large leaves. An average dimensions of 15,8 cm in length and 8 cm in width are present at Bigareau Burlat. This variety is by far the most versatile in terms of leaf size in comparison to the remaining control varieties (Table 1). All leaves are with a dark green hue, are smooth and have thick and mostly medium length leaf stems (Table 1-3), of about 3,6 cm. Pobeda Krimska has the most uniformed as well as the largest leaves, 18,5 cm long and 9,5 cm wide, out of all the varieties. The leaves are consistently smooth, dark green although slightly brighter than those of the remaining two varieties, while their leaf stems are of a medium thickness and long (about 5,5 cm in length). The Kozerska variety is comprised of very large leaves (16,8 cm long and 9 cm wide) with a smooth texture, dark green, and with thick and long leaf stems (5,2 cm). The data from Table 1., clearly indicate that the gamma rays more or less consistently lower the size of the leaves. This is easily confirmed by the 75,9% of large leaves with the control variants that has plummeted to 4,3% within the leaves of selected plants. The medium sized and small leaves fall 2-3 times away from the dimensions of controls, with an inconsistent percentage throughout the tested variants, but they are

still characterized with an average rise that accompanies the increased dosage with the values being at their highest at 45 Gy. The Kozerska variety is the most bountiful in small leaves while Pobeda Krimska is characterized by having very small leaves.

Table.1. Leaf size and leaf roughness of selected plants, %

Variety	Dose	leaf size, %					leaf roughness, %	
		very small	small	medium size	large	very large	smooth leaves	rough leaves
Bigareau Burlat	Control			13,3	56,7	30,0	100,0	
	25Gy	9,3	12,4	47,9	28,0	2,3	90,8	9,2
	35Gy	2,5	16,7	44,7	26,1	10,1	65,2	34,8
	45Gy	11,3	20,5	38,0	23,5	6,6	73,0	27,0
	25-45Gy	7,6	15,7	43,3	27,1	6,2	79,2	20,8
Pobeda Krimska	Control					100,0	100,0	
	25Gy	7,1	24,7	48,4	17,8	2,2	72,2	27,8
	35Gy	9,0	16,7	55,5	12,7	6,2	63,2	36,8
	45Gy	7,3	27,7	43,8	17,5	3,6	59,1	40,9
	25-45Gy	7,8	21,9	48,1	17,2	5,0	68,2	31,8
Kozerska	Control				16,7	83,3	100,0	
	25Gy	5,7	14,1	49,2	29,5	1,5	81,1	18,9
	35Gy	5,0	35,3	36,7	18,3	4,8	83,7	16,3
	45Gy	6,4	27,4	52,2	12,4	1,7	96,2	3,8
	25-45Gy	5,0	22,1	45,8	24,5	2,6	83,6	16,4
Average of controls				4,4	21,7	73,9	100,0	
Average 25 Gy		7,4	17,1	48,4	25,1	2,0	81,4	18,6
Average 35 Gy		5,5	22,9	45,6	19,0	7,0	70,7	29,3
Average 45 Gy		8,3	25,2	44,7	17,8	4,0	76,1	23,9
Average 25 - 45 Gy		7,1	21,7	46,3	20,6	4,3	76,0	24,0

Picture 1. Tipical leaf of controls (very large with tick and long stem)



Picture 2. Different leaf sizes, stem length and thickness and atypical leaf shapes

Leaves with sizes lower than 4 cm in length, 2 cm in width, and 1,5 cm in leaf stems were also noticed by (Шепотъев *(*1968) with units established by radiation of cherry seeds in a 0,5; 1,5; 2,0; 2,5, 3,0 and 3.5 kR variety of dosage with the control variety being of 12,5 cm long, 6 cm wide and leaves with 3 cm long leaf stems. Миленков (1974), had also noticed leaves two or three times the size of the control variety and attributed such changes to the larger radiation dosages. With the equal number of leaves (85) between a control and a 20Gy treated variety the Droganova Zolta exhibited an assimilate surface of 5323,55 cm^2 and 2721,35 cm^2 respectively. The samples with tiny leaves exhibited and assimilated surface of only 397,95 cm^2. According to the data in Table 1, all of the control variants leaves are of a smooth texture, while 24% of the treated material exhibits a rough leaf texture especially with the 35 Gy dosages. The leaf stem is a conservative asset to the

morphological description of the varieties and it consistently thick with the Bigareau Bulrat and Kozerska controls, and medium-thick with Pobeda Krimska control variant (Table 2, Picture 1-2).

Table 2. Thickness and length of leaf stem , %

Variety	Dose	leaf stem (thickness and length), %					
		thin	medium thickness	thick	short	medium length	long
Bigareau Burlat	Control			100,0		66,7	33,3
	25Gy	13,7	58,1	28,2	9,6	68,0	22,4
	35Gy	19,0	45,8	35,2	19,3	61,4	19,3
	45Gy	27,8	47,7	24,5	13,4	73,3	13,4
	25-45Gy	19,8	48,8	31,4	13,4	66,7	19,9
Pobeda Krimska	Control		100,0				100,0
	25Gy	45,4	37,3	17,3	10,9	59,9	29,2
	35Gy	24,6	60,3	15,1	11,3	66,4	22,3
	45Gy	51,1	23,4	25,5	29,2	26,3	44,5
	25-45Gy	35,8	45,6	18,6	13,3	59,0	27,7
Kozerska	Control			100,0			100,0
	25Gy	25,7	29,0	45,3	17,9	56,3	25,8
	35Gy	20,8	48,8	30,4	17,7	55,5	26,8
	45Gy	22,5	52,2	25,3	12,9	48,4	38,7
	25-45Gy	20,5	37,8	41,7	16,1	55,1	28,8
Average of controls			33,3	66,7		22,2	77,8
Average 25 Gy		28,3	41,4	30,3	12,8	61,4	25,8
Average 35 Gy		21,5	51,6	26,9	16,1	61,1	22,8
Average 45 Gy		33,8	41,1	25,1	18,5	49,3	32,2
Average 25 - 45 Gy		27,9	44,7	27,4	15,8	57,3	26,9

The selected plants are characterized mostly by a medium-thick leaf stem (44,7%) which closely follows the large sized leaves (Table 2, Picture 2). The small and tiny leaves exhibit thin leaf stems while the large and very large leaves are accompanied by thick leaf stems (Picture 2). The percentage of leaves with medium-thick leaf stems with Rana Bulratova closely follows the control variant. The number of leaves with long leaf stems has been decreased, and the percentage with short leaf stem has significantly risen with the highest value at 35Gy dosage (Table 2). All the leaves from the control variants of Pobeda Krimska and Kozerska are with long leaf stems whereas all the selected plants are characterized by leaves with medium-long leaf stems (55-59%). According to the data, the rise in dosage raises the short leaf stem percentage from 12,8% with 25Gy to 18,5 % with 45Gy radiation dosage. The change of leaf color is quite amusing. The gamma rays treatment can directly influence the synthesis of the biogenic elements which, in turn, directly influence the plants nourishment. The disruption of this synthesis most commonly manifests itself as a chlorophyll deficiency which reveals a certain discoloration with the leaves (Gunckel,1957). This deficiency can also occur as a side-effect from ionized radiation that inhibits the DNA, RNA and protein synthesis (Lapins,1983). The discoloration, as a modification, may disappear with following generations or be permanently adopted if the mutagen treatment directly inhibits the genetic material that controls chlorophyll synthesis in which case it is a matter of chlorophyll mutation (Петров и Сермяжко, 1963), (Lapins and Hough, 1970), (Миленков,1974). The control variants within this research refer to the dark-green leaves. There has been a myriad of hues detected with the treated material. The most characteristic ones were the light-green, chlorophyll deficiency leaves (Picture 3) and the dark-green leaves with a yellowish leaf nerves (Picture 4).

Picture 3. Chlorophyll deficiency (Bigareau Burlat 35 Gy) yellowish leaf nerves

Picture 4. Dark green leaves with a (Pobeda Krimska 45 Gy)

Table 3. Leaf color, %

Variety	Dose	leaf color, %			
		dark green	light green	chlorophyll deficiency leaves	dark green with yellowish nerves
Bigareau Burlat	Control	100,0			
	25Gy	92,2	5,3	2,5	
	35Gy	88,1	7,7	4,2	
	45Gy	93,1	0,8	6,2	
	25-45Gy	91,3	4,5	4,2	
Pobeda Krimska	Control	100,0			
	25Gy	98,5	1,5		
	35Gy	89,9	3,5	6,6	
	45Gy	72,3	2,9	16,1	8,8
	25-45Gy	90,3	2,7	5,6	1,4
Kozerska	Control	100,0			
	25Gy	96,4	2,7	0,9	
	35Gy	87,5	12,5		
	45Gy	90,3	9,7		
	25-45Gy	92,8	6,5	0,7	
Average of controls		100,0			
Average 25 Gy		95,6	3,3	1,1	
Average 35 Gy		88,5	7,9	3,6	
Average 45 Gy		85,2	4,5	7,4	2,9
Average 25 - 45 Gy		89,8	5,2	4,0	1,0

Approximately 5,2% of the tested leaves have displayed a light green hue (Table 3). This type of discoloration was most persistent with the 35Gy dosage with all the varieties especially with Kozerska (12,5%). On average, about 4% of the leaves are with deficiency of chlorophyll, with a yellowish tint which is not bound to any particular area of the leaf. The Bigareau Burlat 35Gy sample is characterized with a yellow tint between the leaf nerves while the nerves themselves are intensely green (Picture 3). These symptoms are very similar to that portraying iron deficiency in leaves. These types of leaves are also closely related to the radiation dosage in terms of them being more prominent with the rise of dosage (1,1% with 25Gy and 7,4% with 45Gy). The Pobeda Krimska variety has the most leaves with chlorophyll deficiency. Only four samples of the same variety displays an intriguing yellow hue along the lead nerve line (Picture 4) which has not yet been discovered in thus far available literature. Миленков (1974), noticed such discoloration of leaves acquired with a 50Gy radiation over mature cherry cuttings and 30Gy radiation over cherry green cuttings. The discoloration varied from light green, greying-green to dark green. The author analyzed the discoloration according to Krumel's table (1936) which distinctly clarifies the discoloration. The

discolored surface reached from 30 to 80 mm^2 and, with some samples, it even covered over 50% of the leaf surface. The yellowish discoloration was either present over the entire leaf surface or solely covered the edges of the leaf. All the symptoms resembled a nourishment microelement deficiency however, no matter how similar, these changed could not be attributed to any such changes for there were perfectly normal and green leaves along the same branch. The buds were further clipped and the same mutation was kept for the following three generations which points towards the mutation of the genetic material in charge of the organic matter synthesis. They can cover entire leaf surfaces or a selected area while the agrochemical analysis hinted at a certain Mn deficiency (Петров, Самяжко (1963), (Шепотъев, 1968), (Колесникова, 1970), (Lapins and Hough, 1970). They also mention of different hue discoloration ranging from light green to burgundy and purple. Петров и Самяжко (1963) noticed a mutagenic change in terms of a chlorophyll deficiency which went on to be transferred onto vegetative progeny. The ionizing radiation in our trial influenced on shape of the leaves from the treated material. The shape of the leaves is a conservative and accurate sign used to determine the morphological description of the varieties. The control variants are of unified slightly elongated elliptically shaped leaves with Bigareau Burlat displaying a more distinct narrowing at the tip of the leaf (Pictire 1).

Table 4. Atypical leaf shape and leaf nervation %

Variety/ Dose	leaf shape				leaf nervation			
	25 Gy	35Gy	45Gy	Average	25 Gy	35Gy	45Gy	Average
Bigareau Burlat	46,5	49,6	46,1	47,0	6,4	11,6	10,3	8,9
Pobeda Krimska	46,7	44,6	59,9	47,8	10,4	16,8	21,9	16,9
Kozerska	49,3	49,3	44,6	47,6	24,5	11,2	18,0	17,6
Average	47,5	47,8	50,2	47,5	13,7	13,2	16,7	14,5

Some of atypical forms include: oval leaves, extremely linear leaves, elliptical leaves without a distinguished tip, asymmetrical leaves, heart shaped leaves, wedge shaped, leaf stems with multiple leaves in different shapes, double tipped leaves, a double petioled leaf with an additional leaf attached to it, leaves with different sorts of cuts and different depths of cuts along the surface of the leaf. These myriads of anomalies make it difficult for each atypical shape to be accurately described. Approximately 47,5% of the analyzed leaves deviate in shape from the control varieties with no major differences between the varieties and dosages (Table 4). The control variants display a typical nervation with a central nerve symmetrically branched out into secondary fishbone nerves along the entire surface of the leaf, and more intensive tertiary nerves towards the edge of the leaf. A leaf's nervation is a pretty distinct mark of the fruit variety. About 14,5% of the leaves from the selected plants display atypical leaf nervation which does not correspond to that of the control variants. The atypical nervation was greatly prevalent under a 45Gy dosage and, in terms of variety, within Kozerska at 25Gy (24,5%) (Table 4.). Most commonly, an atypical nervation closely follows an atypical leaf shape. Some type of nervation are quite difficult to describe, for instance, there has been a case where the secondary nerves branch out upwards and parallel to the central nerve, a case where the central nerve splits in two at the very tip of the leaf, a case of absence of the central nerve, parallel nervation turned upward or down, all sorts of intense branching of the secondary nerves throughout the entire leaf surface or towards the edge of the leaf, parallel secondary nerves on the opposite side of the leaf, extreme nerve branching on the opposite side of the leaf, secondary nerves with an acute angle at the lower part of the leaf while the top part nervation is typical and follows the elongated and asymmetrical leaves etc.

Conclusions

Gamma radiation with doses of 25 Gy, 35 Gy and 45 Gy of Cz137, causes primary effect in relation of changes in the morphological properties of the leaves in the examined cherry varieties Bigareau Burlat, Pobeda Krimska and Kozerska, in the first MV1 generation after the radiation. The dosage augmentation causes average reduction of leaf dimensions and leaf stem lengths, and increasing of

the percentage of leaves with chlorophyll deficiency, in all of the tested varieties. The Kozerska is the most bountiful in small leaves while Pobeda Krimska is characterized by having very small leaves. With the augmentation of the radiation dosage, the presence of leaves with short leafy steam increased from 12,8% at 25Gy, up to 18,5% at 45Gy, and the presence of leaves with chlorophyll deficiency from 1,1% at 25Gy to 7,4 % of 45 Gy. The effect of ionizing radiation is reflected on leaves shape and on the leaf nervation. An average of 47,5% of the analyzed leaves have an atypical shape, without significant differences between the varieties, and 14,5% of the leaves have an atypical leaf nervation . The highest percentage of leaves with atypical shape and leaf-nervation are represented in plants treated with a dose of 45Gy.

References

1. Bishop C.J. (1967). Radiation induced mutations in vegetative propagated fruit trees. Proceedings of the XVII International Horticultural Congress, Vol 1:83-95.
2. Donini B. (1975). Improvement of vegetative propagated plants through induced mutations. Proceedings of Research Coordination Meeting, Tokai, Vienna. IAEA, Vol.1:35-51.
3. Donini *et.al*. (1991). Mutation breeding programmes for the genetic improvement of vegetative propagated plants in Italy. Proceedings of an International Symposium on the Contribution of Plant Mutation Breeding to Crop Improvement. Vienna. IAEA., (1): 237-254.
4. Gunchel, J.E. Sparrow A.H. (1961). Ionizing radiations:biochemical, physiological and morphological aspects of their effects on plants. Encyclopedia of Plant Physiology, 16, 555-611.
5. Жуков О.С. (1967). Изменения в характере роста и цветения костичковых култур под десйтвием рентгеновских лучей. Труды ЦГЛ им.Мичурина, N°9.
6. Колесникова А.Ф. (1970). Индуцированный мутации вишни. Селъскохозяйственная биология., No 4. Москва.
7. Lapins K.O., Hough L.F. (1970). Effects of gamma rays on apple and peach leaf buds at different stages of development.II. Injury to apical and axillary meristems and regeneration of shoot apices. Radiation Botany, 10: 59-68.
8. Lapins K.O. (1983). Mutation breeding. Methods in fruit breeding. Edited by James N. Moore and Jules Janick. Purdue University Press West Lafayette. Indiana.
9. Миленков М.М. (1974). Индуцированный мутагенез у черешни. Диссертация на соискание ученой степени кандидата биологических наук. Академия наук СССР, Сибирское отделение. Обединенный ученый совет по биологическим наукам. Новосибирск.
10. Миленков М., Георгиев С. (1979). Някои биологични особености на мутантни форми от черешовите сортове Хеделфингенска и Дроганова жълта. Постиженuа на научноисследователския Институт по овощтарство Кюстендил през периода 1969-1979. Издателство Хр. Данов, Пловдив, 61-67.
11. Nybom, N. (1961).Theuse of induced mutations for the improvement of vegetative propagated plants. Symposium on mutation and plant breeding. Washington, pp. 252-294.
12. Петров Д.Ф., Сермяжко И.А. (1963). К вопросу о значении воздействия ионизирующей радиации на черенки для получения новых сортов яблони, устойчивх в условиях Сибири. Новосибирск.
13. Popovska M., Popovski B., Gjamovski V. (2011). Criteria for early diagnostics of primary effects of the treatment with gamma rays (Cz137) at some cherry varieties. Plant Science, 48: 47-52.
14. Равкин А.С.(1973). Исполъзование ионизирующей радиации и химических мутагенов в селекции плодовых и ягодных култур. Москва.
15. Шепотъев Ф.Л. (1968). Опыты по мутагенезу у дрвесных ратений. Применение эксперименталъных мутаций в селекции растений. Киев.
16. Zwintzscher M., (1955). Die Auslösung von Mutationen als Methode der Obstzüchtung I. Die Isolierung von Mutanten in Anlehnung an primäre Veränderungen. Züchter, 25, 290 – 302.
17. Zwintzscher M., (1967). On variability of isolated the sour cherry variety Schatenmorelle for a mutation treal. Induziere Mutationen und ihre Nutzung. Berlin. N°2.