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Sensitivity of watermelon variety Bojura to mutant agents ^{60}Co and EMS

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Article info:
Received: 6 April 2016
In revised form: 15 April 2016
Accepted: 25 April 2016

ABSTRACT

A study on the sensitivity of watermelon variety Bojura to mutagenic agents was carried out in 2013-2014. The goal was to establish effective doses for mutagenic treatment of dry seeds with ^{60}Co gamma rays (80, 100, 200, 250, 350 and 450 Gy) and swollen seeds with water for 24 hours were treated with ethyl methanesulfonate (EMS) at a concentration of 2%. Dominant mutations were not observed in the M_1 generation. Morphological changes in 14 of 1395 M_2 plants were observed. Phenotypic variations changes were the colour of the seed coat, chlorophyll disorders of cotyledons, leaves, petals, and alterations of the location of the fruit set in the central stem. Visible changes of the morphological characteristics of the fruit were not observed. The doses induced certain morphological changes, however, higher doses or combined gamma rays ^{60}Co and EMS treatments would induce mutations more efficiently. Subsequent experiments are required to obtain mutants with changes that affect flowers and fruits. The results are important for increasing mutation efficiency in watermelon breeding.

Key words: *Citrullus lanatus*, induced mutagenesis, phenotype

Introduction

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is one of the most important vegetable crops in Bulgaria as well as the world. Contemporary breeding programmes are aimed at obtaining F_1 hybrids with higher productivity, earliness, high fruit quality and transportability. These goals are often addressed by creating novel genetic variation. The two main techniques for doing so are intraspecific hybridization and mutagenesis. Induced mutagenesis is explored to obtain new traits and properties that are not observed or non-existent in the respective species or genus (Whitaker & Davis, 1962). This technology has proven effective for a number of important crops such as wheat, rice, maize, sunflower and others (Kozgar et al., 2014). The investigation of new hereditary variations of *Cucurbitaceae* species are focused on characteristics of the fruit, seeds, tetraploidity and male sterility (Bates & Robinson, 1995; Burger et al., 2006, Tadmor et al., 2007, Wehner, 2007). So far in Bulgaria, melon and watermelon breeding has been mainly directed at studying natural variation from spontaneous mutations (Alexandrova et al., 1994). This includes male sterility in melons, which is still used in new breeding programmes (Mihov & Lozanov, 1983). The same mutation has been found in watermelons, but subsequent studies have not been done (Lozanov, 1974). The principal problem is reduced female fertility in male-sterile watermelon lines (Murdock et al, 1990; Zhang & Wang, 1990; Guner & Wehner, 2004).

Ethyl methanesulfonate (EMS) and ^{60}Co are among the most commonly used mutagenic agents in breeding

DOI: 10.69085/jbb20161105

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programmes. The dosage and concentration of the treatments are central issues. There have been few watermelon studies and in 2000, only three mutant varieties had been developed (Maluszynski et al., 2000). Tadmor et al. (2007) reported the use of 1% EMS as the most suitable concentration for treatment of melon seeds. The use of 2% EMS is recommended to induce mutations in watermelon (Y. Tadmor, personal communication).

Specific reactions of different watermelon varieties depend on the concentration of mutagens. Thus additional studies on specific varieties are required. As a rule, high-quality varieties that are well adapted to the local conditions and have certain morphological markers are preferred in mutation breeding programmes (Gomez-Pando, 2014). Most watermelon varieties have leaves with different degrees of deep cut. Non-lobed leaves (*nl*) are inherited as a recessive character, which is a morphological marker useful for breeding. Crosses between parental components with the two types of leaf produce F_1 plants with lobed leaves. The lobed leaves are used as early stage phenotypic evidence for successful production of the F_1 hybrid plants (Wehner, 2007). The Bojura variety is maintained in our watermelon collection since it has a good fruit taste and has non-lobed leaves. Experiments with this variety provide the opportunity to start a new breeding programme aimed at increasing genetic diversity. The aim of this study was to evaluate the sensitivity of watermelon variety Bojura to mutant agents ^{60}Co and EMS.

Materials and Methods

The experiments were carried out in the Maritsa VCRI, Plovdiv, in 2013-2014.

Plant material

The Bulgarian watermelon variety Bojura (open pollinated) was used as plant material. The variety has a *monoecious* type of flowering, non-lobed leaves and a vigorous plant habit. The fruit belongs to the Crimson type with red flesh and very good taste.

Gamma rays ^{60}Co and EMS treatments

Dry seeds were treated with ^{60}Co gamma rays at the following doses: 80, 100, 200, 250, 350 and 450 Gy. After being metabolized for 24 hours, seeds were treated with 2% EMS. Non-treated seeds (10%) were used as a control. The number of treated seeds was 30-50 for each variant (M_0). M_1 seeds were obtained. M_1 plants were grown and self-pollinated to develop M_2 populations. M_1 and M_2 generations were observed for phenotypic alterations and surviving plants were also registered.

Growing conditions

Plants were grown in greenhouse conditions. Seeds were sown a in perlite substrate on 18-20 March 2013; plantlets were pricked in 0.5 L pots on 27-28 March; plants were transplanted on 20-25 April; a double-row system was used; the scheme of transplanting was 240 cm between the centres of each pair of rows, 80 cm between the two rows within a pair, 50 cm between plants in the rows. Plant density was 0.8 plants/m^2; fruits were harvested until 2-4 August.

Phenotyping

Seeds were sown in a perlite substrate after treatment. The duration of seed germination and the number of germinated seeds from each variant in the M_1 generation were recorded. Changes in plantlets in M_1 and M_2 generations were reported in the seedling stage. After planting, all plants were visually observed. Fifteen plantlets and mature plants from each M_2 family were phenotyped. The following was calculated:

$$\text{Surviving plants in } M_1(\%) = \frac{A}{B} \times 100, \quad (1)$$

$A -$ number of grown plants,

$B -$ number of sown seeds.

Coefficient of efficiency (C.E., %) in M_2 (Walther, 1969):

$$C.E.\% = \frac{S(\%) \times M(\%)}{100}, \quad (2)$$

$S -$ surviving plants in $M_1(\%)$,

$M -$ number of mutations in M_2 (%).

Results

The results show that in cotyledon stage of M_1, there were progeny differences in the rate of seed germination (Table 1). The 100 Gy dose had a stimulating effect on germination and caused seeds to germinate in just five days, three days faster than the control seeds. The control seeds and seeds treated with 80 Gy germinated eight days after sowing. Hence a dose of 80 Gy is very weak for this variety. The seeds treated with 200, 250, 350, 450 and Gy ^{60}Co and 2% EMS germinated between 8 to 14 days after sowing. The rate of germination and growth speed of these seeds was not uniform, nor was the number of surviving plants.

DOI: 10.69085/jbb20161105

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Table 1. *Survival of plants after irradiation of dry seeds from cv. Bojura with gamma rays ^{60}Co. Survival of plants after treatment of 24 hours metabolized cv. Bojura seeds with EMS.*

Mutagen	Treatment dose (Gy)/ concentration (%)	Treated seeds (number)	Survived plants (number)	Survived plants compared to control (%)	Days to germination (number of days)
	control	30	29	100.00	8
	80	30	27	93.10	8
	100	30	29	100.00	5
^{60}Co	200	30	23	79.30	8-14
	250	50	38	78.62	8-14
	350	50	32	66.20	8-14
	450	50	30	62.07	8-14
EMS	2.0	30	23	79.31	8-14

Table 2. *Observations in M_2 generation after ^{60}Co and EMS treatment.*

^{60}Co/EMS (doses/concentration)	80 Gy	100 Gy	200 Gy	250 Gy	350 Gy	450 Gy	2% EMS	Total
Planted progenies	10	20	15	15	12	10	11	93
Plants per progeny	15	15	15	15	15	15	15	105
Studied plant	150	300	225	225	180	150	165	1395
Mutant plants	0	1	2	3	3	3	2	14
C.E., %	0.00	0.32	0.68	1.01	1.07	1.20	0.93	0.77

Legend: Coefficient of efficiency (C.E., %).

The percentage of surviving plants varied widely, from 62.07 to 100%. Survival was 100% in plants treated with 100 Gy. Survival was 78-79% in seeds treated with 200 and 250 Gy ^{60}Co and 2% EMS. The lowest survival rate (62-66%) was in seeds treated with 350 and 450 Gy.

No dominant mutations were observed during the vegetation period of the M_1 generation. No visible changes in fruit traits were observed during ripening stage. Seeds (M_2) from a total 93 M_1 progeny were obtained (Table 2).

During the second year of the study, 15 plants from each M_2 generation were investigated, a total of 1395 plants. Fourteen mutant plants were identified, none of which were in the group treated with 80 Gy. The calculated coefficient of efficiency for those treated with 100-450 Gy was 0.32-1.20%.

Changes were divided into groups of seeds, cotyledons, leaves and flowers. Changes of the seed coat colour were observed (Figure 1). Five M_2 plants produced seeds with an intense red colour as compared to the control. Three plants had beige seeds of different intensities. Some recessive mutant changes were identified after seed germination. Chlorophyll disorders appeared at the cotyledon stage, as well as plantlets with pale green true leaves (Figure 2). The chlorotic abnormalities in upper leaves appeared in the next stages of plant development (Figure 3). Chlorotic changes were observed in the sepals.

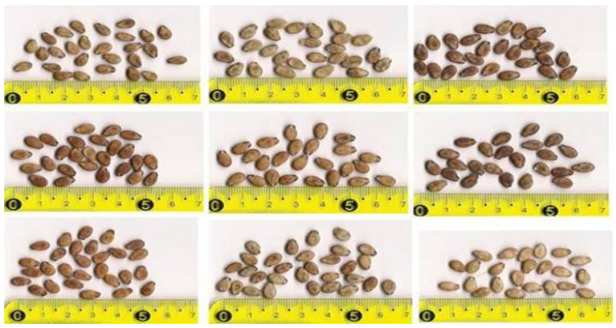

Figure 1. *Changes in seed coat colours in the M_2 generation and the control variant (upper left corner).*

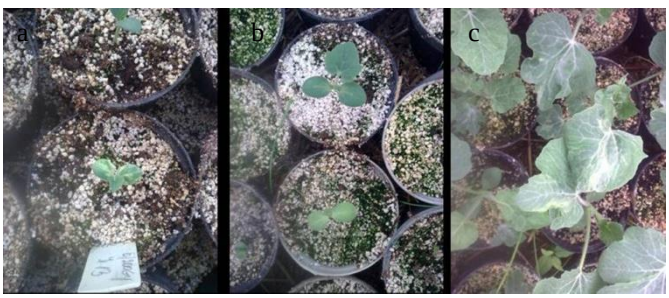

Figure 2. *Mutations in M_2 plantlets: a) chlorophyll disorders in cotyledons, b) pale green and c) chlorophyll disorders on true leaves stage.*

DOI: 10.69085/jbb20161105

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Figure 3. *Chlorophyll mutation in an M_2 plant.*

The male flowers of the treated variants produced abundant pollen during the flowering stage. Male-sterile plants were not observed. These findings on pollen viability have not yet been supported by cytological studies. The size of the flowers of the mutant plants was similar to the control. All M_2 plants had normal growth and development of male and female flowers (Figure 4). The plants had normal formation of flowers and strong vegetative growth, which did not differ from the control. The fruits of 10 plants were formed on the central stem and located between nodes 5-12.

Figure 4. *Male and female fertile flowers from M_2 generation plants.*

From the breeding point of view, fruit morphology and quality are the most important characteristics. Visible differences between M_2 fruits and the initial variety were not found (Figure 5). The colour, texture, and taste of the fruit of treated plants were typical for the Bojura variety.

Figure 5. *M_2 generation plants at fruiting stage.*

Discussion

The application of mutagenesis as a tool to obtain new hereditary variations is associated primarily with initial studies on the sensitivity of genotypes to different doses of treatment and their efficiency. The doses of the treatment showed typical reactions. There was increased vitality of M_1 at low doses and reduced vitality at higher doses. Treatment of 100 Gy caused stimulation in vegetative growth of plantlets. Higher doses successively reduced vegetative growth of plantlets and reached maximum effect at 350 and 450 Gy (Table 1). The highest doses resulted in a low number of surviving plants, close to the LD_{50}. Stimulating and depressing effects of certain doses of treatment are common phenomena in M_1 progeny (Maluszynski et al., 2009).

The FAO/IAEA Joint Division recommends three doses. In the first, plant survival is 70-90%. Two additional doses are recommended: 10-15% lower and 10-15% higher. One of these doses may be the optimal mutagenic frequency (Tomlekova et al., 2007). In our study, the optimum percentage of surviving plants (78-79%) was obtained with a dose of 200 Gy and 250 Gy ^{60}Co and a concentration of 2% EMS.

Treatment with 200 Gy and above and 2% EMS had a suppressive effect, which was observed through slower germination and weak growth of seedlings. Seed germination is a commonly used indicator for sensitivity to mutagens. According Masuda et al. (1998), irradiation with high doses has a negative effect on seed germination and seedling development.

Higher doses produced stronger alterations in M_2 plants (Table 2). The variation of the coefficient of efficiency was similar to that reported for peas (0.58-3.65%) (Mehandjiev et al., 2001).

Chlorophyll disorders in some M_2 plantlets were detected (Figure 2). Only one plant with a chlorophyll mutation on the flower petals survived to the end of the M_2 growing season (Figure 3). Tadmor et al. (2007) reported chlorophyll disorders and dwarfism in 2% of the mutant generations of melons.

Sterility in mutant offspring has been reported (Zhang et al., 2012). In our experiment, there were no sterile plants or changes in the structure of flowers. The presence of sterility in flowers is one of the markers associated with determining the effectiveness of the treatment dose (Reddy & Anadural, 1992). Colour and size changes of flowers and fertility of male flowers were obtained by treating melons with 1% EMS

DOI: 10.69085/jbb20161105

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(Tadmor et al., 2007). Variation of the location of the fruits was observed depending on the height of the plants – fruits were located on node 5-12 of the main stem or above. To a certain degree, this indicates the effectiveness of treatment doses. According to the IAEA and FAO Mutant Varieties Database, watermelon mutant varieties Luxigua 1 and Gibrid 218 were obtained after treatment with 200 and 500 Gy, respectively. The combined application of gamma rays and EMS, which have a synergistic effect, is another mean for increasing mutation efficiency (Mehandjiev et al., 2003; Kosev, 2012).

Typically, watermelon fruits form on the lateral branches of the first and subsequent branch order, and their number on the central stem is significantly lower. The location of fruits below the twelfth node on the central stem, as observed in the present study, usually correlates with early ripening and is used as a marker for precocity (Whitaker & Davis, 1962).

These results are important for further mutation programmes that seek to improve breeding efficiency. In our study, the optimum percentage of surviving plants (78-79%) was obtained with the doses of 200 and 250 Gy and concentration of 2% EMS. The data suggest treating Bojura seeds with higher doses or both gamma rays and EMS.

Subsequent experiments are required to obtain a collection of mutants with changes to flower and fruit characteristics. The early mutant generations will be further developed and screened for mutations with economic importance such as abiotic and biotic stress tolerance.

Using of TILING (Henikoff et al., 2004) or other molecular techniques for analysing the alterations in specific genes may help detect mutations in the available populations, and therefore enrich collections of watermelon varieties.

Acknowledgement

This research was carried out with the support of the FAO/IAEA TC project BUL/5/013.

References

Alexandrova M, Kostova D, Hristov N, Angelov D, Hristova H, Ivanov D. 1994. State and problems in breeding of cultures of *Cucurbitaceae*. Plant Science, 31(3-4): 127-130.

Bates DM, Robinson RW. 1995. Cucumbers, melons and watermelons. – In: Smartt, J. & Simmonds NW. (eds), Evolution of Crop Plants, 2nd ed., Longman Scientific, Essex, UK, p. 89-96.

Burger Y, Sa'ar U, Paris HS, Lewinsohn E, Katzir N, Tadmor Y, Schaffer AA. 2006. Genetic variability for valuable fruit quality traits in *Cucumis melo*. Isr. J. Plant Sci., 54(3): 233-242.

Gomez-Pando L. 2014. Development of improved varieties of native grains through radiation-induced mutagenesis. – In: Tomlekova NB, Kazgar MI & Wani MR. (eds), Mutagenesis: Exploring Novel Genes and Pathways, Wageningen Academic Publishers, Wageningen, the Netherlands, p. 105-123.

Guner N, Wehner TC. 2004. The genes of watermelon. HortScience, 39(6): 1175-1182.

Henikoff S, Till BJ, Comai L. 2004 TILLING. Traditional mutagenesis meets functional genomics. Plant Physiol., 135(2): 630-636.

Kosev V. 2012. Study of biological effect of application of gamma-rays in pea varieties, Plant Science, 49: 54-59.

Kozgar MI, Wani MR, Tomlekova NB, Khan S. 2014. Induced mutagenesis in edible crop plants and its impact on human beings. – In: Tomlekova NB, Kazgar MI & Wani MR. (eds), Mutagenesis: Exploring Novel Genes and Pathways, Wageningen Academic Publishers, Wageningen, the Netherlands, p. 167-179.

Lozanov P. 1974. Heterosis in watermelon, melon and squashes. – In: Yordanov M. (ed), The Heterosis and Their Use in Vegetable Production, Publisher house Hristo G. Danov, Plovdiv, Bulgaria, p. 254-322.

Mihov A, Lozanov P. 1983. Watermelon and melon. – Hristo G. Danov, Plovdiv, Bulgaria.

Maluszynski M, Nichterlein K, van Zanten L, Ahloowalia B. 2000. Officially released mutant varieties – the FAO/IAEA database. Mutation Breeding Review, 1(12): 1-85.

Maluszynski M, Szarejko I, Bathia C, Nichterlein K, Lagoda P. 2009. Methodologies for generating variability. – In: Ceccarelli S, Guimaraes EP & Weltzein E. (eds), Plant Breeding and Farmer Participation. Food and Agriculture Organization of the United Nations, Rome, Italy, p. 159-194.

Masuda M, Furuichi T, Takeda Y. 1998. Mutation spectrum of tomato *Lycopersicon esculentum* cv. first, induced by seed radiation with gamma-rays, and the subsequent partial chlorophyll deficiency. J. Japanese Society Hort. Sci., 67(1): 93-98.

Mehandjiev A, Kosturkova G, Mihov M. 2001. Enrichment of *Pisum sativum* gene resources through combined use of physical and chemical mutagens. Isr. J. Plant Sci., 49(4): 280-284.

Mehandjiev A, Mihov M, Noneva S. 2003. The contribution of experimental mutagenesis for genetic improvement of peas. Plant Science, 4: 325-329.

Murdock BA, Ferguson NH, Rhodes BB. 1990. Male-sterile (*ms*) from China is apparently non-allelic to glabrous-male-sterile (*gms*) watermelon. Cucurbit Genetics Coop. Rpt., 13: 46.

Reddy VR, Anadural M. 1992. Cytologycal effects of different mutagens in lentil (*Lens culinaris* Medik). Cytologia, 57(2): 213-216.

Tadmor Y, Katzir N, Meir A, Yaniv-Yaakov A, Sa'ar U, Baumkoler F, Lavee T, Lewinsohn E, Schaffer A, Burgera J. 2007. Induced mutagenesis to augment the natural genetic variability of melon (*Cucumis melo* L.). Isr. J. Plant Sci., 55(2): 159-169.

Tomlekova N, Todorova V, Daskalov S. 2007. Creating variation in pepper (*Capsicum annuum* L.) through induced mutagenesis. Plant Science, 44: 44-47.

Walther F. 1969. Effectiveness of mutagenic treatment with ionizing radiation in barley. – In: Induced Mutation in Plants. IAEA,

DOI: 10.69085/jbb20161105

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Vienna, Austria, p. 261-270.
Wehner TC. 2007. Gene list for watermelon. Cucurbit Genetics Coop. Rpt., 30: 96-120.
Whitaker T, Davis G. 1962. Cucurbits. – Interscience Publishers, Inc., New York, USA.
Zhang XP, Wang M. 1990. A genetic male-sterile (*ms*) watermelon from China. Cucurbit Genetics Coop. Rpt, 13: 45-46.
Zhang Y, Cheng Z, Ma J, Xian F, Zhang X. 2012. Characteristics of a novel male-female sterile watermelon (*Citrullus lanatus*) mutant. Sci. Hortic., 140: 107-114.

DOI: 10.69085/jbb20161105