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Article

Study a Collection of Watermelons for a Mutation Affecting Male Sterility

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Abstract: Systems favoring cross-pollination such as male sterility and female flowering type are of great importance in the development of new hybrid cultivars and their seed production. The advantages of male sterility are expressed in production of cheaper and competitive seeds. The presence of this characteristic in watermelon is not common, and in some cases, it is accompanied by negative manifestations. The aim of this study was to search for a genetic source of male sterility in a collection of watermelon. A collection of 150 watermelon genotypes was tested at the Maritsa Vegetable Crops Research Institute, Bulgaria over the past nine years. The results show that two mutations were found. The first mutation was found in a plant of the Asar variety, which formed completely degenerated structures in place of male and female flowers and could not reproduce. The other, a mutation affecting male flowers, female flowers and leaf shape was observed in one genotype. Male flowers produce a small amount of pollen. Female flowers are formed but they are sterile and abort at an early stage. The genotype can be propagated by pollination of the normal plants, which in the next generation derivate into mutant 25% and normal 75%. The gene source is phenotyped according to the main characteristics of the fruits and the vegetation period. The mutation found cannot be directly used in breeding program, but it is of interest for studying this important trait. The success of detecting sterility flowers depends on the volume of watermelon plants, which for the conditions of the experiment amounted to a minimum of 4492 plants at a probability of $P_3 - 0.95$.

Keywords: *Citrullus lanatus*; natural; mutant; probability; seed; production

1. Introduction

The study of traits of interest in plant collections is essential when starting any breeding program. Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is a species that belongs to the *Cucurbitaceae* family, which is characterized by wide polymorphism in a number of plant and fruit characteristics [1]. The presence of genetic systems that favor cross-pollination in cucurbits (monoecious, andromonoecious, gynodioecious type of flowering) contribute to the application of the heterosis method of selection. The heterosis method is well known and applied to other cucurbit crops such as cucumbers (*Cucumis sativus* L.), melons (*C. melo* L.) and zucchini (*Cucurbita pepo* var *gyromontia*), where the positive effect is expressed in early maturity, productivity and growth [2–4]. In addition to the well-expressed heterosis effect, it is important to facilitate seed production and obtain cheap seeds. Seed production in these crops is based on the use of different flowering types, with female flowering type in cucumber, male sterility in melon and monoecious flowering type in courgettes being used for the maternal components of F_1 hybrids. Pollination between the two parental components and the maintenance of the parental lines is most often done by bees. The process of easy pollination and obtaining a relatively large number of seeds from the fruits contribute to obtaining cheap hybrid seeds from these crops. There have been several reports of male sterile forms being found in watermelon. The first report of a male sterile form obtained by gamma radiation treatment of the cultivar 'Sugar Baby' was in 1962, which was characterized as glabrous male sterile (gms) due to the associated absence of hairs on the leaves of the plant [5]. Along with male sterility, female fertility is reduced, limiting the use of this gene source. In China, another male sterility

resulting from a spontaneous plant mutation was found in Nongmei 100 variety in 1983 [6]. A third male sterility was obtained in Russia from a natural mutation of the Kamyzyatsky variety in 1984. It manifests itself by forming only female flowers, and the males are underdeveloped and do not open. Found male sterility does not affect female fertility, making him a suitable donor [7]. A fourth male sterility was found in the progeny of a line originating from a cross between the commercial hybrid variety 'Fiesta' (Syngenta) and a high-quality breeding line, HL229 (Hungnong Seed Co.) in Korea in 1998. It differs from the sterility mentioned above [8]. All known male sterilities in watermelon are monogenic recessive, but their use in seed production is not widely practiced.

Male sterility has also been found in melon, which is used in the seed production of hybrid varieties. In Bulgaria, this has been a practice for more than 45 years, when a natural mutant was discovered in the Vidinski koravaci variety [9]. In melon, four more types of male sterility have been established, which differ in their manifestation and whose genes are not allelic [10]. The five male sterilities are obtained through natural mutations and do not affect female fertility.

Male sterility has also been found in cucumbers, but the female type of flowering is most successfully used. The expression of the female flowering type is controlled by the action and interaction of three genes, but can also be modified by the use of growth regulators [11]. Often, two female lines are used in seed production of some cultivars, which are maintained and hybridized by treatment with GA-3 or silver nitrate to produce male flowers. A female type of flowering, which is controlled by two genes, has also been identified in melon, but its use in the development of new F1 hybrid varieties and in seed production is so far insignificant [12]. The apetalous gynoeious mutant was found in the watermelon variety 'Mi Guo' in China in 2006 [13]. Inheritance of the trait is monogenic recessive and female flowers have normal fertility, making it suitable for efficient seed production [14]. Maintenance of the mutant is achieved by crossing with the wild-type monoecious or with an andromonoecious pollinator, necessitating finding an approach to self-pollinate the female line, which is a disadvantage.

In considering the issues raised, it is important to note that an efficient system for easy and cheap seed production of hybrid watermelon varieties has not yet been discovered. Systems favoring cross-pollination such as male sterility and female type of flowering are applicable to other cucurbit cultures, but in watermelon there are a number of obstacles to be introduced effectively. The search for new gene sources of these characteristics is an important condition when starting a new breeding program. Plant collections can be used to discover new donors of male sterility, which is the goal of my research.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

The experiments were carried out at the Maritsa VCRI, Plovdiv, Bulgaria during 2015-2024. A collection of 150 watermelon genotypes (PI's, cultivars, landraces, breeding lines, and introduced materials) was studied in order to find out mutations affected male flowers or male sterility.

Plants were grown in greenhouse conditions. Seeds were sown in a perlite substrate on 18-20 March; 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 centers 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/m²; fruits were harvested until 2-4 August.

2.2. Phenotype Description

Phenotyping was performed on progeny of the mutant variety Concurrent.

The following characters were measured: days to 50 % flowering, days to ripening (from pollination to mature fruit), vegetation period (from emergence of plants to harvest fruits), fruit length (cm), fruit diameter (cm), rind thickness (cm), fruit weight (kg), and total soluble solids TSS (°Bx) (measured by using a digital refractometer KERN ORA 32 BA/BB).

Characterization according UPOV [15]

Fruit: shape in longitudinal section (1 Flattened, 2 Round, 3 Broad elliptical, 4 Elliptical, 5 Pyriform, 6 Oblong);

Fruit: depression at base (1 absent or very shallow, 2 shallow, 3 medium, 4 deep, 5 very deep);

Fruit: depression at apex (1 absent or very shallow, 2 shallow, 3 medium, 4 deep, 5 very deep);

Fruit: ground color of skin (1 light green, 2 medium green, 3 dark green, 4 white, 5 yellow, 6 brown);

Fruit: margin of stripes (1 diffuse, 2 medium, 3 contrasting);

Fruit: width of stripes (1 very narrow, 2 narrow, 3 medium, 4 wide, 5 very wide); Fruit: main color of stripes (1 yellow, 2 very light green, 3 light green, 4 medium green, 5 dark green, 6 very dark green);

Fruit: main color of flesh (1 white, 2 yellow, 3 orange, 4 pink, 5 pink red, 6 red, 7 dark red);

Fruit: waxy layer (1 absent or very weak, 2 medium, 3 very strong).

The organoleptic characteristics were determined by tasting the fruits by expert assessors.

Taste (1 foul, 2 unremarkable pleasant taste, 3 pleasant taste, 4 superb taste);

Sweetness (1 absent, 2 slightly expressed, 3 sweet, 4 very sweet);

Juiciness (1 weak, 2 medium, 3 strong);

Texture (1 fibrous (stringy), 2 soft, 3 grainy melting, 4 fine grain crunchy).

2.3. Probability of Occurrence of Mutations in a Collection of Watermelons and Determination of the Minimum Number of Plants to Obtain at Least One Mutant Plant

Probability of mutations occurring:

$$p = m/n;$$

p – determining the probability of mutation

m – number of mutated plants

n – total number of plants

Determination of the minimum number of plants to obtain at least one mutant plant [16]

$$n_{\min} = \log(1 - P_3) / \log(1 - p)$$

$P_3 = 0.95$ – probability

$P_3 = 0.99$ – probability

$P_3 = 0.999$ – probability

2.4. Experimental Design and Statistical Analysis

Plots were arranged in a randomized complete block design, with two replications, ten plants per plot, and a total of twenty plants per genotype. The trait expression was estimated using all plants per genotype. For analyzing the data Descriptive statistics and Chi-square test were used. Results were processed by statistical program Genes [17].

3. Results

3.1. Screening a Watermelon Collection for Natural Mutants

The results of the last nine years show that it is possible to obtain natural mutants by screening a collection of watermelons. The frequency of mutations obtained is not high, two genotypes show mutational variability out of a total of 150 genotypes examined. The resulting mutations affect morphological changes in flower buds and changes in leaf shape. The first mutant was registered in 2015. in the Asar variety originating from Kazakhstan. Of the 20 plants tested, one was distinguished by a mutation that was expressed in a change in the structure of flower buds (Figure 1a). A granular structure surrounded by numerous sepal forms on the flower stem (Figure 1b). Pollination of the mutant flowers was not possible and the grain structure died after seven days (Figure 1c). Throughout the growing season, the plant continued to grow and form mutant flowers, and at the end of the growing season, it failed to form normal flowers and reproduce (Figure 1d). Normal watermelon flowers are characterized by the formation of five sepals, five petals, five stamens in male flowers, pistil in female flowers or stamens and pistil in hermaphrodite flowers (Figure 1e,f). The

Asar variety is characterized by an andromonoecious type of flowering, which allowed plants to self-pollinate, but no mutation was found in subsequent generations.

A second mutation was found in the Concurrent variety originating in Russia in 2019. Several plants were found to have changes in flower and leaf characteristics (Figure 1g). Most plants of the cultivar form normal male and female flowers (Figure 1h). Mutant plants can be distinguished in the seedling stage by the formation of strongly dissected leaf lobes, being densely spaced and generally having a smaller habit than normal plants. Later in the growing season, modified male flowers appear that form stamens. The stamens are initially tightly enveloped by the sepals, but they open as the pollen matures (Figure 1i). The amount of pollen is scarce and attempts to pollinate other flowers have not been successful. Mutant plants produce female flowers, but they are not fertile. The pistil of the carpel is not developed and cannot be pollinated, whereupon it turns yellow and dies (Figure 1j). No progeny was obtained from the mutant plants. Self-pollination of normally developed plants allowed subsequent offspring to be produced.



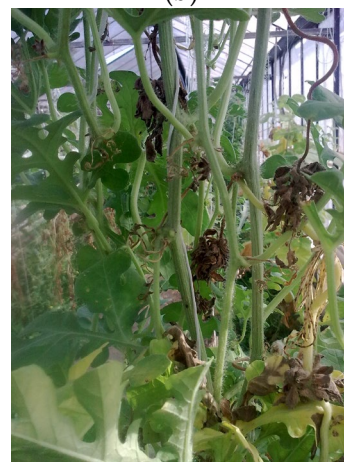
(a)



(b)



(c)



(d)



(e)



(f)

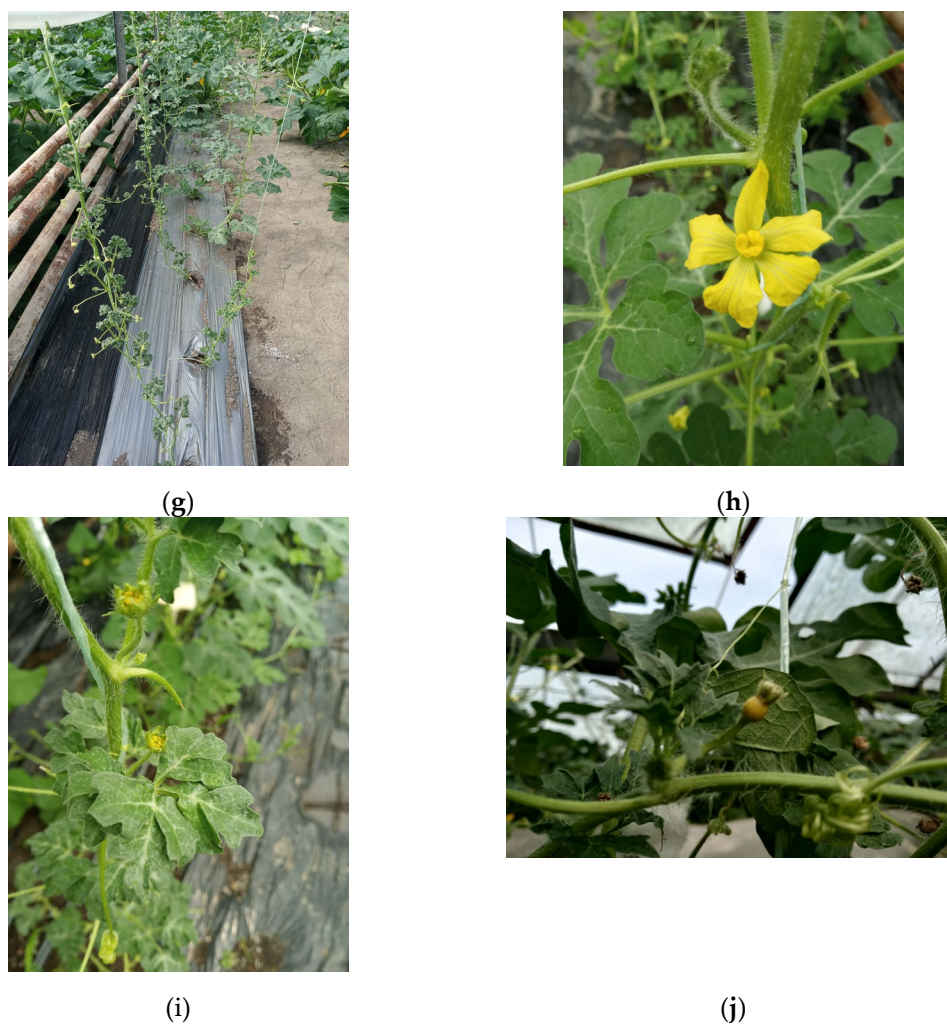


Figure 1. Mutant plants obtained: (a) cv. Asar mutated flower; (b) Structural change of the flower; (c) Dead flower; (d) The plant with dead flowers; (e) Fertile male flower; (f) Fertile female flower; (g) cv. Concurrent mutant plants; (h) cv. Concurrent fertile plant with normal leaves; (i) Mutated male flowers; (j) Mutated female flower.

3.2. Inheritance of Mutation

The resulting progeny of the self-pollinated normal plants of the Competitor variety showed that they separated into fertile and sterile. The sterile ones could not reproduce. Self-pollination of the next generation of fertile plants again resulted in the emergence of fertile and sterile. The analysis of the self-pollinated generations showed a segregation in the ratio of 3:1 fertile to sterile plants (Table 1). Fertile plants do not differ phenotypically from the original, and sterile plants also do not differ from the previous ones. Chi-square shows a good match of the experimentally obtained with the expected results, which is 3:1 fertile to sterile plants. It is obvious that the established mutation is inherited monogenically recessively. Interestingly, this segregation results from self-pollination of fertile plants. These plants are assumed to be in the heterozygous state to cause such segregation. Established segregation was seen in several offspring in 2022 and 2023, with the results having a high degree of probability P (%).

Table 1. Segregation of self-mated progeny of cv. Concurrent from fertile plants.

| Self-pollinated progeny | Total | Male fertile | Male sterile | Ratio Obs:Exp | Chi square p | Probability P(%) |
|-------------------------|-------|--------------|--------------|---------------|--------------|------------------|
| | | | | | | |

| 2022 | | | | | | | |
|------------------|----|----|----|-----|--------|-------|--|
| Concurrent 4-1 | 18 | 14 | 4 | 3:1 | 0.0741 | 78.55 | |
| Concurrent 4-4 | 18 | 12 | 6 | 3:1 | 0.6667 | 41.42 | |
| Concurrent 4-6 | 17 | 13 | 4 | 3:1 | 0.0196 | 88.86 | |
| 2023 | | | | | | | |
| Concurrent 4-1-3 | 10 | 7 | 3 | 3:1 | 0.1333 | 71.50 | |
| Concurrent 4-4-6 | 12 | 8 | 4 | 3:1 | 0.4444 | 50.50 | |
| Concurrent 4-6-8 | 14 | 10 | 4 | 3:1 | 0.0952 | 75.76 | |
| Total | | | | | | | |
| Concurrent | 89 | 64 | 25 | 3:1 | 0.4532 | 50.08 | |

3.3. Phenotyping of Some Important Traits of cv. Concurrent

The reported metric indicators of the individual offspring show that the Concurrent variety belongs to the group of medium-ripe varieties with a vegetation period of 90-110 days (Table 2). The fruits of fertile plants are of medium size (16.9 cm by 16.7 cm) and weight (2.7 kg). The rind thickness has average parameters (0.8 cm). The TSS content is within good fruit quality, averaging 8.4%. The results of the two years on the reported characteristics differ. In 2022 the indicators days to mass flowering, days to fruit ripening and vegetation period are shorter. Fruit sizes were larger but dry matter content was lower compared to 2023. In general, the fruits are of good quality, characterized by a round shape, red flesh color, pleasant taste, great sweetness, strong juicy, the texture is grainy melting (Figure 2). These qualities of the variety make it particularly suitable for breeding, which facilitates the development of a hybrid based on male sterility.

Table 2. Important fruit characteristics of cv. Concurrent.

| Genotype | Days to mass flowering | Days to ripening | Vegetation period (days) | Fruit weight (kg) | Fruit length (cm) | Fruit diameter (cm) | Rind thickness (cm) | TSS (%) |
|--------------------------|------------------------|------------------|--------------------------|-------------------|-------------------|---------------------|---------------------|---------|
| 2022 | | | | | | | | |
| Concurrent 4-1 | 56 | 35 | 91 | 3.414 | 18 | 19 | 0.9 | 8.2 |
| Concurrent 4-4 | 55 | 42 | 98 | 4.316 | 22 | 20 | 0.6 | 7.0 |
| Concurrent 4-6 | 56 | 33 | 89 | 4.156 | 19.5 | 20 | 0.9 | 8.2 |
| 2023 | | | | | | | | |
| Concurrent 4-1-3 | 61 | 50 | 111 | 1.754 | 14 | 15 | 1 | 9.0 |
| Concurrent 4-4-6 | 59 | 44 | 103 | 1.140 | 14 | 12 | 1 | 9.8 |
| Concurrent 4-6-8 | 60 | 55 | 115 | 1.446 | 14 | 14 | 0.6 | 8.0 |
| Mean | 57.8 | 43.2 | 101.2 | 2.7 | 16.9 | 16.7 | 0.8 | 8.4 |
| Standard Error± | 1.0 | 3.5 | 4.3 | 0.6 | 1.4 | 1.4 | 0.1 | 0.4 |
| Confidence Level (95.0%) | 2.6 | 8.9 | 11.0 | 1.5 | 3.6 | 3.6 | 0.2 | 1.0 |

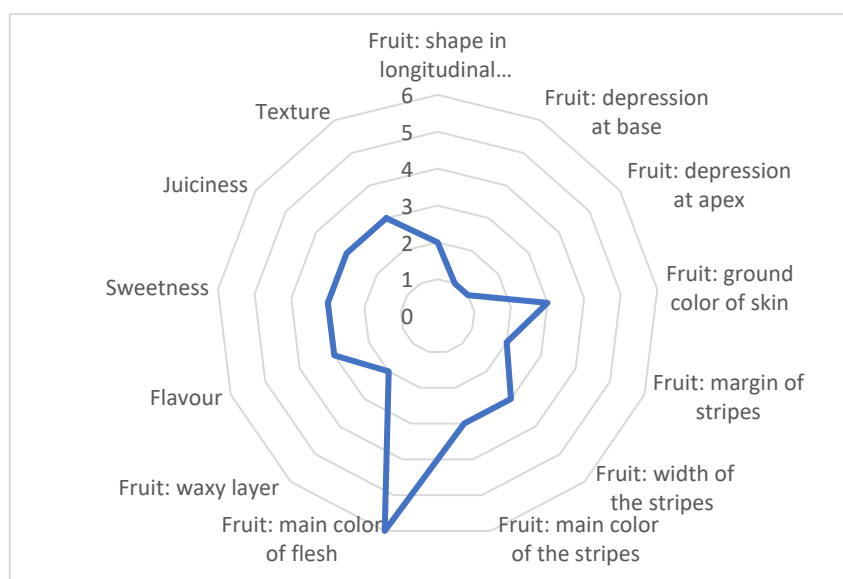


Figure 2. Organoleptic characteristics of fruits, cv. Concurrent.

3.4. Probability of Mutation

Although mutations rarely occur, with what probability and with what number of examined plants they are found is a question that contributes to clarifying the breeding strategy in general. In the present experiment, 150 progenies of different genotypes of watermelons with a total number of about 3000 plants were tested. In the last nine years I have been able to discover two mutations, of which the second mutational form can be reproduced by pollination of fertile plants. The breeding program was aimed at detecting mutations affecting male sterility and the screening was directed specifically at such plants. Calculations made to determine the probability of a mutation occurring show that it amounts to 0.067% (Table 3). The probability is not high, but it is still enough to select those with the desired changes by careful examination of the plants.

The data obtained allow to determine the minimum number of plants to select at least one mutant plant. According to the results, one mutant plant can be obtained by examining a minimum of 4492 plants at a probability of $P_3 - 0.95$; over 6905 plants at $P_3 - 0.99$ and over 10358 plants at $P_3 = 0.999$.

Table 3. Probability of occurrence of a mutation in a collection of watermelons and determination of the minimum number of plants (n_{\min}) to obtain at least one mutant plant.

| Probability of mutation occurring | Confidence probability | n_{\min} |
|-----------------------------------|------------------------|------------|
| 0.00067 | $P_3 - 0.95$ | 4492 |
| 0.067% | $P_3 - 0.99$ | 6905 |
| | $P_3 = 0.999$ | 10358 |

4. Discussion

4.1. Screening a Watermelon Collection for Natural Mutants

Screening of watermelon collections is essential for selecting forms of interest. This is also the first step towards developing a breeding program. Identification and finding of traits of interest is achieved by screening collections or by applying mutational breeding. Male sterility is one of the characteristics important for obtaining new F1 hybrid watermelon varieties. In the available collection of watermelons, I was able to obtain two mutations that originated from two varieties. The first mutation from the variety Asar was characterized by too strong a change in the parts of the

flower bud, and it was not possible to obtain seed progeny with which to continue the breeding of hybrid varieties based on male sterility (Figure 1a-d). The second mutation obtained in the Concurrent variety also showed significant changes in male and female flowers (Figure 1g-j). However, the strong changes in female colors did not allow offspring to be reproduced.

Other studies have also reported finding male sterility in watermelon, which is often accompanied by reduced female flower fertility. In some of the cases, sterility was obtained by artificially inducing mutation, in others by screening collections. In both cases, reduced fertility or low seed yield has been reported, making it difficult to use these gene sources in breeding programs [18]. In rare cases, sterility is not accompanied by negative side effects [7]. However, there is no data on the introduction of male sterility in watermelon in hybrid cultivar breeding schemes.

Five types of male sterility are known in melon, which do not affect female fertility [19]. Moreover, for the conditions of Bulgaria, the use of ms-4 is important for breeding and has been used intensively since its discovery more than 45 years ago [20]. The five male sterilities are not allelic and can be used in seed production [21].

Despite female sterility, the resulting mutant form can be maintained by self-pollination of fertile plants. The resulting mutation is reported for the first time. Usually, maintenance of the sterile line (msms) is done by hybridizing with a fertile line of the Msms genotype, where half of the plants in the next generation are sterile. Hybrid seed production is carried out by removing the fertile plants from the female line and crossing is carried out from the paternal form by insect pollinators, most often with bees.

4.2. Inheritance of Mutation

Inheritance of male sterility has been shown to be monogenic recessive in almost all studies. In some cases, it is accompanied by the appearance of another mutant trait such as glabrous foliage [22], juvenile albino [23], dwarfism [24], which are used as genetic marker. The presence of a distinct morphological marker by which male sterile plants can be identified at an early stage of their development helps in efficient seed production. All male sterile genes reduce female fertility and often have low seed yield as well.

In my study, the inheritance has the characteristics of a monogenic recessive, which is accompanied by changes in leaf shape and female sterility. It is possible to observe a pleiotropic effect affecting several traits such as leaves and flowers. On the other hand, the new mutant form was maintained by self-pollination of the fertile plants in a ratio of 3:1, indicating that the genotype of the fertile plant was in the heterozygous state (Msms). The data are not categorical and need to be investigated in more detail in future experiments. It is necessary to determine the reaction of the offspring when crossing other genotypes with normal formation of male and female flowers and to make a hybridological analysis. It is also important to establish the degree of sterility of the mutated male flowers, as well as other accompanying traits. The research has more fundamental significance for the study of sterility and, to a lesser extent, practical significance. Their use can be aimed at obtaining hybrids with a small number of seeds or without seeds by limited pollination with sterile or not fully fertile pollen. Seedless fruits of diploid watermelon were obtained by pollination with non-viable pollen treated with soft R radiation [25].

4.3. Phenotyping of Some Important Traits of cv. CONCURRENT

The quality of the cultivars is very important in determining the parental components for hybridization. The mutational breeding also selects the best quality and most promising genotypes with which the experiment will be carried out. In our collection, I had the opportunity to come across a variety with relatively good characteristics, except for sterility. The Concurrent variety belongs to the group of mid-early watermelons, forms medium-sized fruits with good organoleptic qualities, red flesh and monoecious flowering type. In any case, the combination of male sterility with good qualities of watermelon fruits greatly facilitates the selection process. In some studies, poor fruit quality has been reported in association with male sterility [23]. Considering the polygenic

inheritance of productivity elements, early ripening and fruit quality are not easy to combine in a single genotype along with male sterility.

4.4. Probability of Mutatio

The occurrence of a mutation, and especially one with a positive effect, is extremely important in plant improvement. They occur rarely in nature, but the potential for qualitative progress is huge. Spontaneous mutations are often observed when growing a significantly large number of plants. Induced mutations increase the frequency of their occurrence, but in a number of cases also cause other unwanted effects. However, there are no guarantees that a desired quality or characteristic of interest will be obtained. The efficacy of positive mutations is unclear, as they remain more of a random variable and success is not guaranteed. In my research, I tried to define some parameters with which to place certain limits on the probability. The probability of a mutation affecting plant flowers in the tested collection of watermelons is 0.067%. The minimum number of plants to be surveyed is 4492 plants at a probability of $P_3 - 0.95$. I was unable to find any other studies that used probability to detect random events in watermelons or other cucurbit crops. In my previous experiments using gamma radiation and EMS on the Bojura watermelon variety, I observed some mutational changes in the plants and seeds, but did not find the male sterility I was interested in. In the individual treatments, the efficiency coefficient varied from 0 to 1.2%, and the number of M_2 plants studied was 1395 [26]. In the context of the obtained results of the present study, it is evident that it was necessary to examine a significantly larger volume of plant material.

5. Conclusions

By studying a collection, it is possible to detect a spontaneously occurring mutational change affecting watermelon flowers. The new mutation is characterized by female sterility, male flowers produce a small amount of pollen, changes in leaf lobes and a smaller plant habit than normal. Inheritance is monogenic recessive, and the maintenance of the Concurrent variety is carried out by self-pollination of normal plants. The fruits of normal plants are characterized by good organoleptic qualities. Success depends on the volume of watermelon plants and genotypes studied, which for the conditions of the experiment amounts to a minimum of 4492 plants at a probability of $P_3 - 0.95$.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org.

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Informed Consent Statement: "Not applicable"

Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in section "MDPI Research Data Policies" at <https://www.mdpi.com/ethics>.

Conflicts of Interest: "The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results".

Appendix A

The appendix is an optional section that can contain details and data supplemental to the main text—for example, explanations of experimental details that would disrupt the flow of the main text but nonetheless remain crucial to understanding and reproducing the research shown; figures of replicates for experiments of which representative data is shown in the main text can be added here if brief, or as Supplementary data. Mathematical proofs of results not central to the paper can be added as an appendix.

Appendix B

All appendix sections must be cited in the main text. In the appendices, Figures, Tables, etc. should be labeled starting with “A” –e.g., Figure A1, Figure A2, etc.

References

- Whitaker T.; Davis G. In: *Cucurbits*. Interscience Publishers, Inc., New York. 1962.
- Ene, C. O.; Ogbonna, P. E.; Agbo, C. U.; Chukwudi, U. P. Heterosis and combining ability in cucumber (*Cucumis sativus* L.). *Inform. Process. Agric.* **2019**, *6*, 150–157. doi: 10.1016/j.inpa.2018.07.008
- Napolitano, M.; Terzaroli, N.; Kashyap, S.; Russi, L.; Jones-Evans, E.; Albertini, E. Exploring Heterosis in Melon (*Cucumis melo* L.). *Plants* **2020**, *9*, 282. <https://doi.org/10.3390/plants9020282>
- Darrudi, R.; Nazeri, V.; Soltani, F.; Shokrpour, M.; Ercolano, M.R. Evaluation of combining ability in *Cucurbita pepo* L. and *Cucurbita moschata* Duchesne accessions for fruit and seed quantitative traits. *J. Appl. Res. Med. Aromat. Plants*, **2018**, *9*, 70–77.
- Watts, V.M. A marked male-sterile mutant in watermelon. *Proc. Amer. Soc. Hort. Sci.* **1962**, *81*, 498-505.
- Zhang, X.P.; Wang, M. A genetic male-sterile (ms) watermelon from China. *Cucurbit Genetics Coop. Rpt.* **1990**, *13*, 45-46.
- Dyutin, K.E.; Sokolov, S.D. Spontaneous mutant of watermelon with male sterility. *Cytology and Genetics*, **1990**, *24*, 56-57.
- Yang, D.; Paek, K.; Hwang, J.; Park, H. Characterization of a New Male Sterile Mutant in Watermelon. *Cucurbit Genetics Cooperative Report* **2001**, *24*, 52-58.
- Lozanov, P. Breeding of male-sterile parental components for facilitating the production of hybrid seeds from melon. Proceedings of the First Scientific Conference on Genetics and Breeding, Razgrad, Bulgaria, 1983.
- Lecouviour, M.; Pitrat, M.; Risser, G. A Fifth Gene for Male Sterility in *Cucumis melo*. *Cucurbit Genetics Cooperative Report*, 1990, *13*, 34-35.
- Weng, Y; Wehner, T. C. Cucumber Gene Catalog 2017. *Cucurbit Genetics Cooperative Report* **2017**, 39 – 40.
- Pitrat, M. Melon. In: Handbook of plant breeding. Vol. 1, Vegetables I: *Asteraceae*, *Brassicaceae*, *Chenopodiaceae*, and *Cucurbitaceae*. Ed. by Prohens J and Nuez F. Springer, 2008; p. 283-316.
- LI, Y.; YANG, H.; YANG, J.; YANG, D. A Apetalous Gynoecious Mutant in Watermelon. *Cucurbit Genetics Cooperative Report*, **2009**, 31-32, 15-16.
- Xiantao, J.; Depei, L. Discovery of Watermelon Gynoecious Gene gy. *Acta Horticulturae Sinica*, **2007**, *34* (1), 141– 142.
- UPOV Code: CTRLS_LAN, (*Citrullus lanatus* (Thunb.) Matsum. et Nakai), International union for the protection of new varieties of plants, Geneva, TG/142/5 Rev. Watermelon, 2013-03-20 + 2019-10-29
- Lidanski, T. Statistical methods in biology and agriculture. Zemizdat, Sofia, Bulgaria, 1988; p. 375.
- Cruz, C. D. GENES - a software package for analysis in experimental statistics and quantitative genetics. *Acta Sci Agron*, **2013**, *35* (3), 271–276.
- Wehner, T.C. Overview of the genes of watermelon, Cucurbitaceae 2008, Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae, (Pitrat M, ed), INRA, Avignon (France), May 21-24th, 2008
- Dogimont, C. Gene List for Melon. *Cucurbit Genetics Cooperative Report* **2011**, 33-34, 104-133.
- Lozanov, P. Heterosis in watermelon, melon and squashes. In: *The heterosis and their use in vegetable production*. Publisher house Hristo G. Danov – Plovdiv, Bulgaria, 1974, p. 254–322.
- Lecouviour, M.; Pitrat, M.; Risser, G. A fifth gene for male sterility in *Cucumis melo*. *Cucurbit Genet. Coop. Rep.* **1990**, *13*, 34–35.
- Ray, D.T.; Sherman, J.D. Desynaptic chromosome behavior of the gms mutant in watermelon. *J Hered*, **1988**, *79*, 397-399.
- Zhang, X.P.; Rhodes, B.B.; Baird, W.V.; Skorupska, H.T.; Bridges, W.C. Phenotype, Inheritance, and Regulation of Expression of a New Virescent Mutant in Watermelon: Juvenile Albino. *J. AMER. SOC. HORT. SCI.* **1996**, *121* (4), 609–615.
- Huang, H.; Zhang, X.; Wei, Z.; Li, Q.; Li X. Inheritance of male-sterility and dwarfism in watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai]. *Scientia Horticulturae*, **1998**, *74*, 175-181.
- Sugyama, K.; Morishita M. Production of seedless watermelon using soft-X-irradiated pollen. *Scientia Horticulturae*, **2000**, *84*, (3–4), 255-264.
- Velkov, N.; Tomlekova, N.; Sarsu F. 2016. Sensitivity of watermelon variety Bojura to mutant agents ⁶⁰Co and EMS. *J. BioSci. Biotech.* **2016**, *5* (1), 105-110.

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