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Cytogenetic Features of Meiosis in Hybrid F_1 Watermelon (*Citrullus lanatus* (Thunb.) Matsum et. Nakai), Depending on the Level of Ontogenesis Adaptation

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The existing interrelation between different adaptability of heterozygotes F_1 of watermelon in ontogenesis and cytogenetic parameters of meiosis is being determined. It is established that highly and low adapted hybrids of F_1 watermelon had distinctive features of passing the stages of meiosis. In particular, gradients in the frequency of chiasms across generative tiers and various sections of the anther were more likely to be observed in low adapted F_1 heterozygotes. The plants of low-adapted heterozygotes F_1 also featured by lower crossing-over accuracy, more random distribution of exchanges and their significantly higher frequency. In highly-adapted hybrids, the reaction to the influence of the investigated factors was often ambiguous. It was shown that the proportion of meiosis disturbances in plants of inadaptive F_1 hybrids did not depend on their distribution in different parts of the anther and increased under conditions of competition and reduced moisture supply of experimental plants. As a result, heterozygotes F_1 with low adaptability in ontogenesis in F_2 and later generations had a reliable increase in diversity and maximum manifestation of economic value traits. Thus, the progenies of low-adapted F_1 hybrids are expedient to apply in transgressive breeding in order to expand the availability of brand new selection-valuable genotypic variability, while the use of highly adaptive hybrids may be expedient in heterosis selection of the creation of forms resistant both to bio and abiotic stresses. *© 2020 Bull. Georg. Natl. Acad. Sci.*

Heterozygote F_1 watermelon, the parameters of meiosis, chiasm, types of disorders in meiosis

On the basis of the research carried out as well as on generalization of a large number of literature data concerning the issue of interrelation between the adaptability of F_1 heterozygotes in ontogenesis and the level of recombinant variability, and the spectrum of genotypic variability in segregating populations, A. A. Zhuchenko [1] formulated a hypothesis regarding the buffer role of high ontogenetic adaptability. According to the mentioned hypothesis, heterozygotes with an improved

potential for modifying variability provide the next generation with a smaller manifestation of genetic variability in changing environmental conditions. The posed hypothesis was confirmed on a limited number of vegetable Solanaceous plant species [2]. The manifestation of cytogenetic parameters that optimize recombination processes remains insufficiently studied. Specifically, frequency and qualitative assessment of chiasms integrally characterizing the recombination process, the formation of metabolic disturbances reflects the accuracy of the "break-up-connection" process, and atypical bivalents, indicating a decrease in interference of crossovers [3].

According to classical views, to create a valuable starting material, as a rule, highly adapted F_1 hybrids should be used [4]. However, as a result of the mechanisms of genetic adaptation, the formation of valuable genotypes in generations of low adapted heterozygotes is not ruled out. Therefore, the goal of this study was to investigate the cytogenetic peculiarities of hybrids F_1 of watermelon with the highest and lowest level of adaptation in ontogenesis.

Materials and Methods

Determining the degree of ontogenetic adaptation of F_1 watermelon hybrids. In order to assess the degree of ontogenetic adaptability, we have modified the methodology developed for *Solanaceous* plant species. It was found out that the hexagonal scheme of plant placement (3×3 cm) in Wagner vessels of 5L volume is optimal for watermelon. Seven hybrids derived from the same female form or different female forms were placed in one vessel. The repetition of the experiment was six times. As criteria for assessing the level of ontogenetic adaptability, traits of the vegetative and generative system were used.

In the phase of the first true leaves, the F_1 heterozygote plants were switched to a moisture supply mode at the level of 45% of the total moisture capacity (FW). Control plants were grown under optimal conditions. When generative organs were formed in 25% of plants, each of them was isolated from the soil and sorted according to the combination number. Then, based on the maximum and minimum mean values of the characteristics of the vegetative and generative systems, hybrid combinations with, respectively, high ([Chernogorets/Borchansky], [Ogonyok/Form 4 tsl.], [Ogonyok/№5], [Jubenica 44/Garny], [Chernogorets/L. Ms], [Chernogorets/Sugar baby]) and low ([Chernogorets/Bykovsky], [Ogonyok/№6], [Chernogorets/№5], [Ogonyok/Chernogorets], [Chernogorets/Ogonyok], [Jubenica 44/L. Ms/ Shironinsky], [Garny/Orfey]) ontogenetic adaptability were selected.

Cytological research. The cytological evaluation of intraspecific hybrids of watermelon was carried out following Brown method being modified by Zhuchenko et al. [5]. The buds were fixed in acetic alcohol for 24 hours and stored in aqueous solution of 70% ethyl alcohol at temperature 1-4°C. On the day of preparation, the material was washed with water, kept in aqueous solution of 4% of iron ammonium alum for 60 minutes, then was again washed with tap water and then with distilled water. Anthers were mashed into drops of 1% oceto-carmine and selected with cells at the meiosis stage. The specimen was contrasted with 45% acetic acid.

To study the distribution of chiasmata within the anthers, they were divided into three equal sections – apical (apex), medial (middle part), and basal (base). At the stage of diplotene – early diakinesis, the number of bivalents of various types (open, circular, and atypical) per meocyte was determined and on the basis of which the frequency of interstitial chiasm and the total frequency per one maternal cell were calculated. At the stages of "metaphase I – telophase II", the frequency of major disorders was determined.

Statistical processing of the results. The digital data were processed using the variation statistical

methods. In order to process the data expressed in percentages, the Fischer formula for rates was applied [6]. For a small number of comparisons (k =3) the Student's t-test and the Bonferroni correction were used [7].

Results and Discussion

As our studies have shown, the differences between hybrids of watermelon with different adaptability were observed both in the traits characterizing the development of vegetative organs and the number of buds (Fig. 1).

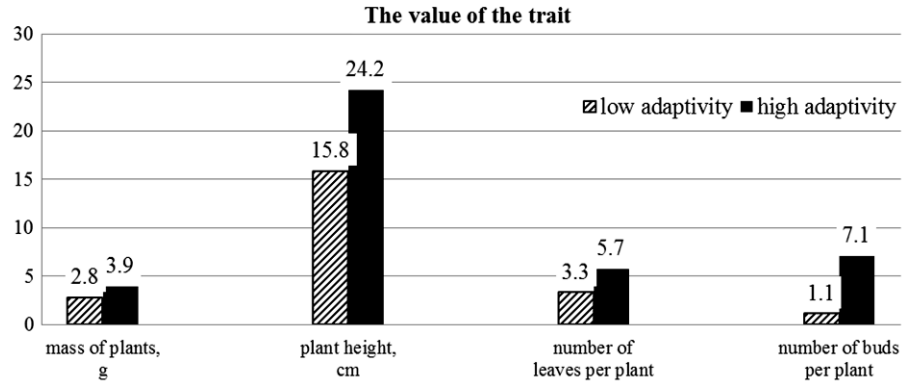

Fig. 1. Morphological features of low and highly adapted hybrids of F_1 watermelon.

Chiasm distribution within the anthers of F_1 watermelon hybrids with different ontogenetic adaptations. Taking into account the importance of the distribution of recombination events within the reproductive system or its individual elements in the processes of genetic adaptation, we investigated the distribution of chiasms in the meiocytes of transverse sections of the anthers of watermelon hybrids F_1 with different ontogenetic adaptability. The scheme used in 2006 and 2007 has been demonstrated to support the identification of hybrids with varying levels of adaptability in ontogenesis over the following years. Thus, in 2008 the highly adaptable hybrids F_1 were [Chernogorets/Borchansky] and [Ogonyok/Forma 4 tsl.], in which the frequency of interstitial chiasms did not decrease from the apical part to the base of the anther F_1. 11 bivalents were generated in the prophase. Decrease in total frequency of chiasms from apical to basal section of the anther occurred in F_1 plants of the first combination regardless of conditions. In plants of the second combination the opposite effect has been observed. Different regularities have been established for low-adapted heterozygotes. In hybrids F_1 [Chernogorets/Bykovsky] and [Ogonyok/№6] there was a decrease in the frequency of interstitial chiasms in the area of the anther base. In all cases the gradient of total frequency of chiasms (decrease in the direction from apical zone of the anther to its base) and the number of atypical bivalents was observed.

Under unfavorable conditions, the frequency of interstitial chiasms and the total frequency of chiasms in low-adaptive F_1 heterozygotes, as opposed to highly adaptive ones, tended to grow in the apical and medial areas of the anthers. The frequency gradient of chiasms (its maximum value within the free end) was also observed in the studies of 2009. Besides, despite the level of ontogenetic adaptability, clear differences in the apex and other sections of the structure were observed under unfavorable conditions, especially in the frequency of interstitial chiasms. By the number of atypical bivalents, this effect was found only in low adaptability hybrids F_1 [Chernogorets/№5]. In

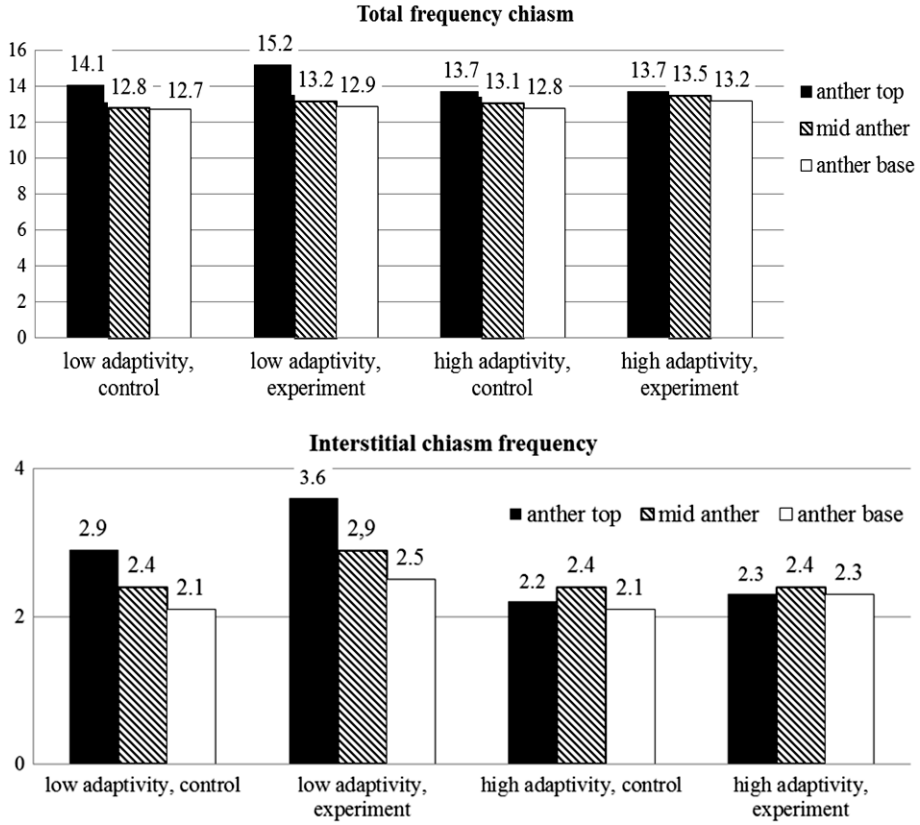

Fig. 2. Total frequency of chiasms (upper chart) and frequency of interstitial chiasms (lower chart) in meiocytes of lumbar sections of the anther in hybrids F_1 of watermelon with different ontogenetic adaptability (generalized data for 2007–2010).

terms of total frequency of chiasms reliable differences were in the variant of research regardless of hybrid combination.

In 2010, the adaptive category of F_1 hybrids ([Ogonyok/№5], [Jubenica 44/Garny]) did not have atypical bivalents. A decrease in the frequency of interstitial chiasms was observed only in the experiment with the first hybrid. However, the gradient of total chiasms frequency was observed in both hybrids of the first generation in optimal and unfavorable conditions. Whereas, in low-adapted hybrids F_1 ([Ogonyok/ Chernogorets], [Jubenica 44/L. Ms/Shyroninsky]) the manifestation of such a gradient was observed regardless of cultivation conditions. In general, the same effect, except for the control version, in plants of hybrid F_1 [Ogonyok/Chernogorets] has been established for interstitial chiasms. Within the research regarding the hybrid, the frequency of atypical bivalents authentically decreased from the free end of the anthers to its base.

The revealed regularities for the watermelon, in general cohere well with the data obtained earlier for the eggplant, a species with a different type of pollination [2], and are probably explained by the connection of the frequency of interstitial chiasms and atypical bivalents with the formation of non-traditional recombinants [8], which may be an additional mechanism of genetic adaptation. Thus, unlike low adaptive heterozygotes, the gradient of total frequency of chiasms, frequencies of interstitial chiasms and atypical bivalents was often absent, and atypical bivalents were not generated at all in some cases (Fig. 2).

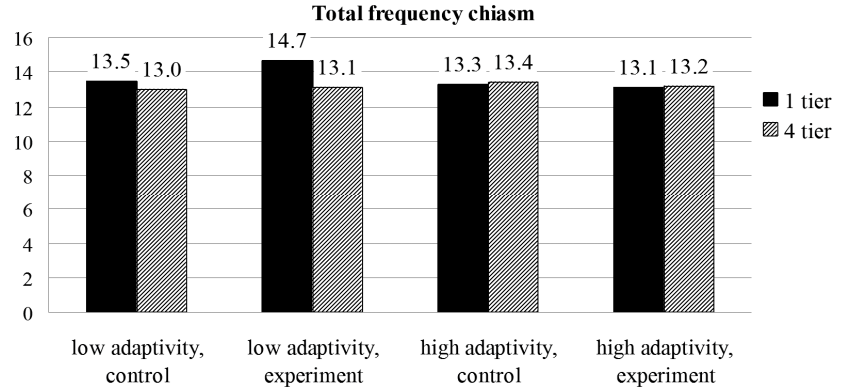

Fig. 3. Distribution of chiasms by generative levels in F_1 hybrids of watermelon with different ontogenetic adaptability (average data for 2007–2010).

Distribution of chiasms in the reproductive organ levels in hybrids F_1 of watermelon with different ontogenetic adaptability. Dependence of frequency of chiasms on the tier of vertical base of generative organs (male buds) was clearly traced in plants of low-adapted hybrids [Chernogorets /Ogonyok], [Garny/Orfey], especially in adverse conditions. So, for the first combination of crossing in the control the maximum total frequency of chiasms was within the limits of buds of the second tier, in unfavorable conditions a clear decrease in this index with the increase in tier was traced. A similar effect was also found for the second combination of a low adapted hybrid.

For the highly-adapted combination [Chernogorets/L. Ms], the differences in control sample were reliable between the first and third to fifth buds, but in the study the maximum values of chiasm frequency (interstitial and summative) were observed within the second and third tier buds. The similar regularities were observed in 2009 for other highly-adapted F_1 hybrids ([Garny/Orfey], [Chernogorets/Sugar baby]). However, there are no natural changes in the studied parameters depending on the tier of F_1 progeny with different ontogenetic adaptability. In the low-adapted hybrid [Chernogorets/№5] the frequency reduction of chiasms was observed regardless of conditions. Differences in frequency of total and interstitial chiasms in tiers of low-adapted F_1 hybrids [Ogonyok/Chernogorets], [Jubenica 44/L. Ms)/Shironinsky] remained in 2010. Similar regularities in highly-adapted heterozygotes were observed only in the progenies of F_1 [Jubenica 44/Garny] in the control variant and in the progeny of F_1 [Ogonyok/№5] in the experiment variant.

Thus, the decrease in the cytological parameters of meiosis characterizing the recombination process within the generative tiers of watermelon hybrid F_1 does not depend on the degree of adaptation. However, low-adaptive F_1 hybrids have such a gradient, especially in the total frequency of chiasms, in the majority of variants in unfavorable conditions (Fig. 3).

Meiosis disorder in F_1 watermelon hybrids, depending on the degree of ontogenetic adaptability. Another parameter that characterizes the course of meiosis is the frequency of violations. The reason for their formation is, on the one hand, anomalies in the formation of spindle of division, and on the other, chromosomal (chromatid) mutations, as well as (for so-called metabolic disorders) – changes in the normal flow of the process of "break-up-connection" during the crossover. Low adapted watermelon F_1 hybrids [Chernogorets/Ogonyok], [Garny/Orfey], with some exceptions, is characterized by an increase in the frequency of violations (chromosome emission in the metaphase I and II, the formation of bridges

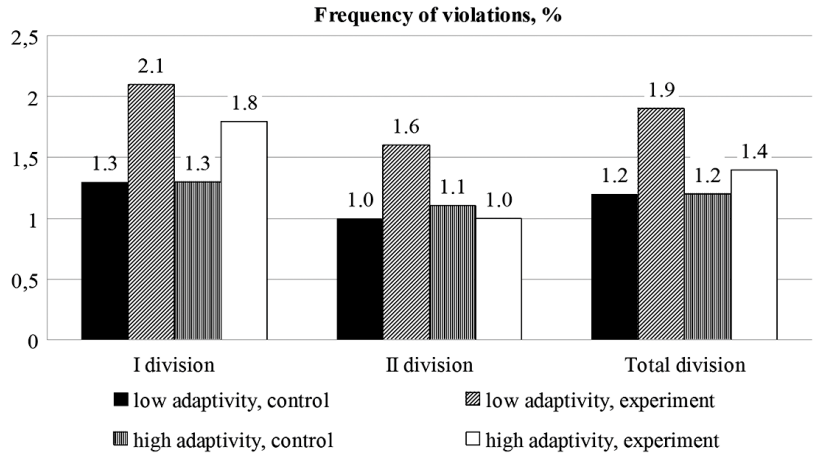

Fig. 4. Frequency and dynamics of meiosis disorders in F_1 hybrids of watermelon with different ontogenetic adaptability (average for 2007–2010).

in the anaphase I and II) in conditions of competition and low water availability. In general, for low-adapted hybrids, it was natural to increase the average frequency of violations for I and II divisions in adverse conditions by almost 2–3 times. Whereas, in highly-adapted F_1 heterozygotes, the percentage of anomalies at the stages of the second division of meiosis, as a rule, decreased. Generally, the average values of the percentage of violations for the I division increased in the framework of the competitive conditions and reduced water availability regardless of the adaptation level of the F_1 hybrids. At the II division stage, as well as for the total frequency of violations, the mentioned effect was observed only in the low-adapted F_1 heterozygotes (Fig. 4).

Basically, while analyzing the generalized values of the cytological parameters characterizing recombination, it can be assumed that the low-adapted watermelon F_1 hybrids, being under conditions of reduced water availability and competition, as well as when compared with the highly-adapted F_1 hybrids, the number of bridges, fragments, atypical bivalents and chiasm frequency is being increased (Table).

It should also be noted that a decrease in the accuracy of the "break-down" process, as well as in the interference of crossovers in low-adapted F_1 hybrids, obviously, may be a common phenomenon, and the increase in the number of exchanges may depend both on the combination of crossing and on the peculiarities of genetic adaptation of a particular species.

Table. The frequency of metabolic disturbances and conjugation of chromosomes in F_1 hybrids of watermelon with different ontogenetic adaptation, the average for 2007-2010

Level of adaptation F_1	Variant	Frequency of exchanges disorders			The frequency of chiasms per meiocyte	The number of atypical bivalents per meiocyte
		studied cells	bridges, %	fragments, %		
High	control	1875	0.96	1.01	13.04	0.03
	experiment	2210	1.04 *	1.22	13.04	0.06
Low	control	2182	1.15 #	0.87	14.94 #	0.05 #
	experiment	3274	1.65 *#	1.06	16.24 *#	0.15 *#

As a result of the established mechanisms, differences in the diversity of quantitative traits in future generations are not excluded, as confirmed by our studies. Thus, in the progeny of low-adapted F_1 hybrids, the average value of productivity components was significantly higher in comparison with the progeny of highly-adapted F_1 hybrids in control (for the number and weight of fruits per plant) or study (for the weight and diameter of the fruit).

ბოტანიკა

საზამთროს (*Citrullus lanatus* (Thunb.) Matsum et. Nakai) F_1 ჰიბრიდების მეიოზის ციტოგენეტიკური თავისებურებები ონტოგენეზში შეგუებულობის ხარისხთან დაკავშირებით

ო. მოგილნა*, ო. სამოვოლი*, ს. კონდრატენკო*, ო. სერგიენკო*, ვ. სუჩკოვა**, დ. ბედოშვილი§

*უკრაინის სოფლის მეურნეობის მეცნიერებათა ეროვნული აკადემია, მებოსტნეობისა და მებაღეობის ინსტიტუტი, ხარკოვის რაიონი, უკრაინა
**უკრაინის სოფლის მეურნეობის მეცნიერებათა აკადემია, მემცენარეობის დეპარტამენტი, კიევი, უკრაინა
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(წარმოდგენილია აკადემიის წევრის გ. ნახუცრიშვილის მიერ)

გამოვლენილია საზამთროს F_1-ჰეტეროზიგოტების ონთოგენეზში სხვადასხვა შეგუებულობასა და მეიოზის სხვადასხვა პარამეტრს შორის არსებული ურთიერთკავშირი. დადგენილია, რომ საზამთროს მაღალი და დაბალი შეგუებულობის მქონე F_1-ჰეტეროზიგოტები განსხვავდებიან ერთმანეთისგან მეიოზის თავისებურებებით. კერძოდ, დაბალი შეგუებულობის მქონე F_1-ჰეტეროზიგოტებში დიდი ალბათობით ვლინდება ხიაზმების სიხშირის გრადიენტები სამტვრე პარკის გენერაციული იარუსებისა და სხვადასხვა სექციის მიხედვით. დაბალი შეგუებულობის მქონე მცენარეები აგრეთვე ხასიათდებოდა კროსინგოვერის ნაკლები სიზუსტით, ქრომოსომების მონაკვეთების უფრო რანდომული გაცვლითა და მნიშვნელოვნად უფრო მაღალი სიხშირით. მაღალი შეგუებულობის მქონე ჰიბრიდებში რეაქცია გარემო ფაქტორებზე ხშირად არაერთგვაროვანია. გამოვლინდა, რომ ინადაპტაციური F_1-ჰიბრიდების მცენარეებში მეიოზის დარღვევები იზრდება მაღალი კონკურენციისა და ტენით არასაკმარისი უზრუნველყოფის პირობებში, მაგრამ ამ დარღვევათა სიხშირე არ არის დამოკიდებული სამტვრე პარკების

ნაწილების დანაყოფებზე. დაკვირვებამ აჩვენა, რომ დაბალი შეგუებულობის მქონე F_1-ჰეტეროზიგოტებიდან მიღებულ F_2-ისა და უფრო გვიანი თაობების ონთოგენეზში მაქსიმალურად იზრდება მრავალფეროვნება და ვლინდება სამეურნეო მნიშვნელობის ნიშნები. ამგვარად, დაბალი შეგუებულობის მქონე F_1-ჰეტეროზიგოტებისგან მიღებული თაობების გამოყენება მიზანშეწონილია ტრანსგრესიულ სელექციაში, რომელიც მიზნად ისახავს სელექციონერისათვის ხელმისაწვდომი ახალი, მაღალი სელექციური ღირებულების მქონე გენოტიპური ცვალებადობის შექმნას. ხოლო მაღალი შეგუებულობის მქონე ჰიბრიდების გამოყენება უფრო მიზანშეწონილია ჰეტეროზისულ სელექციაში ან ბიო და არაბიოსტრესების მიმართ გამძლე ფორმების გამოსაყვანად.

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