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QTL MAPPING OF ESTERASE ISOZYME FORMS IN *Brassica rapa* L. MATURE SEEDS

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Abstract

Since the 1960s, isoenzymes have been well known as one of the most common biochemical markers. Establishing the overall variability of the isoenzyme systems and identifying their genetic control retain their relevance, allowing researchers to reveal the fine mechanisms of the relationship of the organism with the environment and homeostasis and to develop effective biochemical markers for rapid assessment of genetically and selectively significant material. In this paper, the chromosome loci responsible for the activity of 13 different esterase forms of mature seeds in *Brassica rapa* L. were identified and mapped for the first time. The doubled haploid lines of two mapping populations, DH30 and DH38, were studied. All identified esterase isoforms were divided into three groups according to their electrophoretic mobility. The group of isoforms A1-A3 had a high molecular weight and low electrophoretic mobility. The group of B1-B7 isoforms exhibited an average molecular weight and an average electrophoretic mobility. The C1-C3 group consisted of isoforms having a low molecular weight and, consequently, the highest electrophoretic mobility. Each of the parental forms, as well as each of the studied lines of mapping populations, had its own unique electrophoretic spectrum of esterase isoforms. Based on the electrophoretic data obtained for both populations, a QTL analysis was carried out and chromosome loci were identified, determining the manifestation of each esterase isoform identified in the mapping lines of populations DH30 and DH38. The composite interval mapping approach, combined with a permutation test (1000 iteration) and a confidence level of $p < 0.05$, allowed us to identify and locate QTLs on chromosomes that determine the manifestation of all esterase isoforms identified by gel electrophoresis, with the exception of A1 isoform for the DH38 population and B7 isoform for the DH30 population. For these two isoforms, no QTL analysis results were obtained because of limitation in initial data on these isoforms in the corresponding mapping population. A total of 35 QTLs for esterase isoforms were mapped for DH30 mapping population and 39 QTLs for DH38 population. As a result of the QTL analysis, molecular markers genetically linked to the identified loci and the percentage of phenotypic variability determined by each of the identified QTLs were also identified. According to isoenzyme analysis, the heterozygosity of both populations in each H_l locus and total heterozygosity H_{total}, as well as $Var(H_l)$ heterozygosity dispersions for one locus and the variance of average heterozygosity within each population $Var(H_{total})$ were calculated. The identified heterozygosity was considered as the average portion of loci with two different alleles in one locus in one individual and could be defined as the observed heterozygosity characterizing the part of the genes for which the studied population is heterozygous. It was shown that in the studied populations of doubled QTL haploid lines, which determine the complex of esterases isoenzymes, are found mainly in the 2nd, 4th, 6th, and 9th linkage groups and form blocks of co-adapted genes and genomic co-adapted gene blocks, which emphasizes the importance of the contribution of

these loci in the ontogenesis and adaptability of plants *B. rapa*. In general, the carried out molecular genetic mapping and biochemical analysis of the studied biochemical traits of various manifestation of esterase isoforms in mature seeds of *B. rapa* revealed genetic determinants of the studied characters, as well as the genome distribution of mapped QTLs, which in the long term makes it possible to conduct effective molecular and genetic screening of collection accessions and breeding material of the *B. rapa* species according to these biochemical characters when performing genetic and selection investigations in this species.

Keywords: *Brassica rapa* L., biochemical analysis, esterase isoforms, mature seeds, QTL mapping, population heterozygosity

Since the 1960s, isoenzymes have been well known as one of the most common biochemical markers suitable for establishing genetic variability in both natural and artificial populations of plants [1-3] and animals [4-6]. Establishing the overall variability of the isoenzyme systems and identifying their genetic control retain their relevance, allowing researchers not only to reveal the fine mechanisms of the relationship of the organism with the environment and homeostasis during growth and development in both normal and extremal conditions [7] but also to develop effective biochemical markers for rapid assessment of genetically and selectively significant material [8].

Esterases (ES 3.1.1) combine a large number of different carboxyl ether hydrolases. Some of them have very broad substrate specificity and can hydrolyze both endogenous and exogenous ether bonds of different structures [9]. The fact that the enzymes showing esteratic activity can also hydrolyze non-ether bonds raises questions about the evolutionary adaptive physiological and ecological role of these esterases. Thus, the possibility of inhibiting α and β-esterases in plants has been described for aryl esterases in young cassava leaves and evaluated as a marker of pathogenesis after infection with bacteria [10]. Inhibition tests showed that organophosphates (insecticides) inhibit some plant esterases and activate the other ones in *Aspidosperma polyneuron* [11]. Consequently, plant esterases can serve as biochemical indicators used to detect insecticide residues and determine their toxicity in the control of environmental pollution. In general, the esterase system of organisms is characterized by the presence of a large number of isoenzymes and significant individual and population variability. In animals, most esterase enzymes are non-specific and exhibit overlapping substrate specificity [3]. In some cases, these isoenzymes are relatively specific, such as cholinesterases and carbonyl anhydrases.

The functional characteristics of plant esterases have not yet been sufficiently studied, although in plants esterases seem to represent one of the most studied groups of isoenzymes. As early as the late 1960s and early 1970s, esterases were studied in corn [1, 12, 13], barley [2], potatoes [14], cotton [15, 16], oats [17, 18], sugar beet [19], wheat [20, 21] and many other species. The most detailed genetic control of esterases is described in wheat [21-25]. In addition, the "alien"-wheat genealogy of chromosomes was defined [4, 20] and "alien"-wheat hybrids were identified [26, 27], and the variability of esterases of hexaploid genotypes was revealed [28]. All these papers were aimed at establishing not so much the physiological as the genetic component of such a biochemical system as higher plant esterases. The absence of epistatic interactions and the codominant nature of the inheritance of esterase isoforms make them a convenient tool for the rapid and effective study of the processes of biochemical adaptation to changing environmental conditions. This type of biochemical markers is convenient for solving practical problems of selection as a means of accelerating and simplifying the selection process of breeding material.

In the previous studies, a polymorphism in the isozyme profile of esterases isolated from mature seeds in samples of the varietal and line-breeding material of

hexaploid wheat (*Triticum aestivum* L.) was identified. The average heterozygosity (*H*) of the samples at 10 identified loci encoding isoforms, coding esterases, was 0.924 [29]. The most promising parental forms differing in phenotypic traits of breeding interest and the spectra of esterase isoforms – the varieties Zlata, Mera, and the homozygous lines AFI91, AFI177, ITMI7, ITMI44, ITMI83 and ITMI115 were identified and the possibility of determining the polymorphism of esterases in the hybrid generations was defined. Another study [30] shows a wide range of diversity of electrophoretic profiles of isoenzymes of mature seeds (eight isoforms with molecular masses from 37.7 to 57.6 kD) in 25 samples of radish (*Raphanus sativus* L.), which were divided into 13 electrophoretic enzyme types that differ from each other by the presence or absence of certain areas. The most common was the electrophoretic enzyme type of Gr. 1, spread among 24% of the estimated samples. The enzyme type of Gr. 5 was characterized by a maximum number of zones – 8, the enzyme types of Gr. 3 and Gr. 12 – the smallest (4 zones). The seventh and eighth zones of esterases (M_r = 39.7 kD and M_r = 37.7 kD, respectively) were monomorphic; the remaining six zones were polymorphic. The frequency of each zone for different enzyme types ranged from 6.58 to 17.11%. The obtained results allowed selecting promising source material for breeding.

However, to date, no studies have been carried out for the lines of doubled haploids on the esterase isoenzyme spectrum. The advantage of this approach is that, for example, molecular genetic maps of the mapping populations of *Brassica rapa* L. DH30 and DH38 are saturated with SSR and AFLP molecular markers with a mapping interval of 2.4-2.6 cm. The denser the markers are, the more accurate the map is. AFLP markers are currently relatively rare but still used in conjunction with other types of markers. At the same time, in the absence of other known markers of esterase isoforms, in the future for routine screening of breeding or other genetic material, it is possible to convert AFLP markers located in peaks detected by QTL into SCAR or CAPS markers. All this allows taking the issue of the reliability and reproducibility of the results when using markers obtained using certain genetic material off the table, as this question arises very often, if not always [29-31]. Different positions of loci of quantitative traits of breeding interest in the mentioned populations DH30 and DH38 have been noted in many studies, which is quite natural since the populations are different genetically [31]. However, this is even more interesting because for mass collection or selection screening the most reliable markers of loci are those in close positions in both populations.

In the present study, the isoenzyme analysis of *Brassica rapa* L. doubled haploid lines was conducted for the first time, which allowed not only identifying the localization of the loci determining the manifestation of the analyzed isoforms of esterases in the linkage groups on the chromosomes but also finding a statistical relationship between the identified QTLs (quantitative trait loci) of these isozymes and the observed heterozygosity for each locus.

The aim of the paper was the mapping of QTLs that determine the expression of different esterase isoforms of mature seeds of doubled haploid lines of *Brassica rapa* L.

Techniques. The esteratic profiles of the seeds were analyzed for 80 lines of the mapping populations of *Brassica rapa* L. (3 parent forms, 50 DH38 descending lines, 27 DH30 descending lines). The details of obtaining and using the DH30 and DH38 populations for QTL mapping of morphological and biochemical traits in *B. rapa* were described in the previous paper [31].

Mature seeds were ground thoroughly in a porcelain mortar, 100 mg of the obtained flour was placed in test tubes of the Eppendorf type, 1 ml of hexane was added, the mixture was thoroughly stirred and left for degreasing overnight in a refrigerator at 4 °C, then centrifuged 10 min at 15000 rpm, the super-

natant fluid was decanted and the sample was left under a draught to dry in air. Enzymes were extracted for 14-18 hours from fat-free and dried samples with 0.05 M Tris-HCl buffer (pH 8.3) in the presence of 2-mercaptoethanol (2 μl/ml) and glycerol (10%), at the flour to buffer ratio of 1:4 and a temperature in the range from 4 to 8 °C. The samples were centrifuged for 10 min, the supernatant liquid was collected and samples were frozen at –20 °C to prevent inactivation of the isoenzymes during storage. Before entering to the electrophoresis chamber, the samples were defrosted at room temperature. Isoenzymes were separated by native vertical electrophoresis in PAAG [32]; the concentration of separating and concentrating gels was 11 and 5%, respectively. Electrophoresis was carried out in a Mini-PROTEAN Tetra Cell chamber (Bio-Rad Laboratories, Inc., USA). Prestained Protein Ladder (Thermo Scientific, USA) was used as molecular mass markers. Protein (20 μg) was added to each pocket of the concentrating gel. The protein concentration in enzyme preparations was determined by the dye-binding method according to Bradford [33], with bovine serum albumin (Promega Corporation, USA) as a standard. Electrophoresis was performed in the cold (10-15 °C), at 10 V/cm for 2.5 h. After separation, the gel was treated with a reagent for nonspecific esterase [34]. This gel was stained in freshly prepared dye solution consisting of 100 mg α-naphthyl acetate and 120 mg β-naphthyl acetate (Sigma-Aldrich Chemie GmbH, Switzerland), dissolved in 10 ml of 70% ethanol, 500 mg of Fast Blue RR (Sigma-Aldrich Co., USA); 4 ml of propanol and 60 ml of 0.1 M phosphate buffer (pH 6.0). After the appearance of brownish-violet bands, the excess dye was removed with 10% acetic acid. The received zymograms were scanned (Epson Expression 10000XL, GE Healthcare, USA). Evaluation of each sample (R_f value of all bands in the track, calculation of molecular weight based on standards, calculation of the relative amount of each zone in the track) was carried out using Phoretix 1D Advanced software (TotalLab, Ltd., UK).

For mapping of the identified QTLs, MAPQTL 6.0 software (Kyazma B.V., Netherlands) was used [35] to detect for each esterase isoform in each population the presence and location of candidate QTL in the linkage group (at 5 cM mapping interval), to estimate LOD (logarithm of odds) values ($p = 0.05$) and the degree of phenotypic expression of the trait explained by the corresponding QTL (% Expl.). LOD significance was assessed in the permutation test (1000 iterations) [36].

The trait-marker correlation coefficients were calculated as a statistically significant association of the marker locus with the QTL conferring the trait (for $p = 0.05$ significance level) based on empiric variants for each trait-marker pair [37]. Using data on mapping of the detected QTL, the maps were graphically constructed with MapChart 2.2 software (https://www.wur.nl/en/Research-Results/Research-Institutes/plant-research/biometris/Software-Service.htm) [38]. Statistical data processing was carried out by multivariate analysis methods [35].

The degree of the observed heterozygosity H for each locus was determined, as well as its average value for several loci (total heterozygosity H_{total}). The population heterozygosity at each locus H_l and the total heterozygosity H_{total} were calculated according to the description [39, 40] by the following formulas:

$$H_l = 2n(1 - \sum_k x_k^2)/2n - 1,$$
$$H_{total} = \sum_{l=1}^{r} H_l/r,$$

where l is the sequence number of the locus, n is the population size, x_k is the estimated frequency of the k-th allele of the l-th locus, r is the total number of loci.

The variance of heterozygosity $Var(H_l)$ at one locus and the variance of the average heterozygosity within populations $Var(H_{total})$ were found [41]:

$$Var(H_l) = H_l(1 - H_l)/n,$$
$$Var(H_{total}) = \frac{1}{nr^2}\sum_l H_l(1 - H_l) + \frac{1}{nr^2}\sum_l \sum_{l \neq \hat{l}} (H_{l\hat{l}} - H_l H_{\hat{l}}).$$

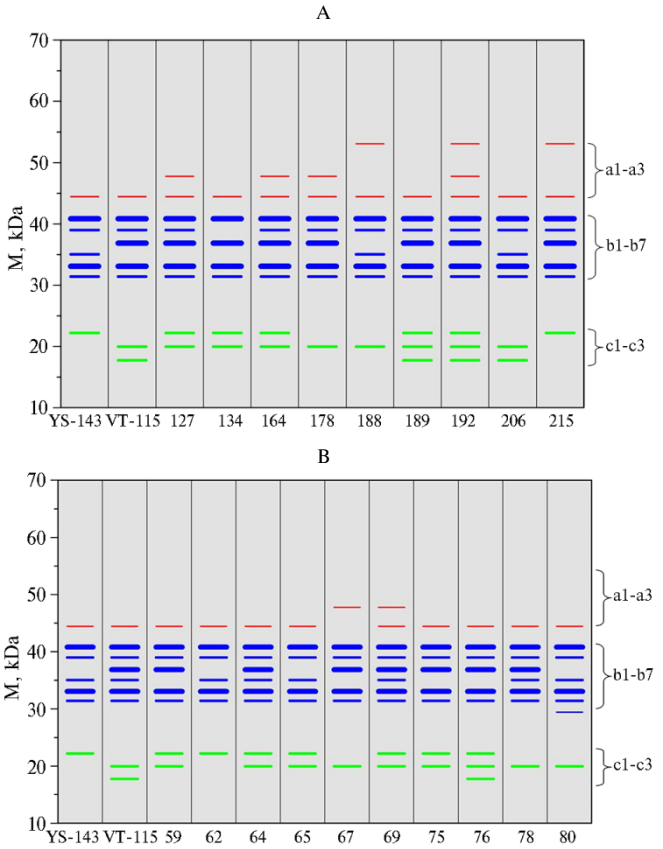

Fig. 1. The diagrams of electropherograms of esterase isoforms in the mapping populations DH30 (A) **and DH38** (B) of ***Brassica rapa* L.**: YS-143 — male parent, VT-115, PC-175 — female parents; the figures at the bottom — line numbers of mapping populations; A1-A3, B1-B7 and C1-C3 — groups of esterase isoforms of respectively large, medium and small molecular weight; M —of molecular weight marker (Prestained Protein Ladder, Thermo Scientific, USA).

Results. Electrophoretic analysis of isozymes of esterases of mature seeds for lines of doubled haploids from two mapping populations of *B. rapa* and their three parental forms revealed 13 isoforms of esteratic enzymes, which were divided into three groups by electrophoretic mobility (Fig. 1). The group of isoforms A1-A3 had large molecular weight and low electrophoretic mobility, the group B1-B7 showed medium molecular weight and mobility, the isoforms of the group C1-C3 had a small molecular weight and the highest electrophoretic mobility. Note, the intensity of the zones A1-A3 and C1-C3 varied relatively little, while B1-B7 varied significantly. In addition, in contrast to groups A1-A3 and C1-C3, including three isoforms, the group B1-B7 was represented by seven esterase isoforms.

Each of the parent forms, as well as each of the studied lines of mapping populations, had its unique electrophoretic spectrum of esterase isoforms. The group C1-C3 consisted of isoforms inherent in both the male and each of the female samples. This group of esterases exhibited a codominant type of inheritance. The group B1-B7 from the parent forms consisted of six electrophoretic zones, five of which were identical, and the area B3 absent among the male plant YS-143 was presented in both female forms, the VT-115 and PC-175, showing the dominant type of inheritance. In the case of the DH30 population, the area V4 was found in the male form YS-143 and was absent in the female form VT-115, also showing the dominant type of inheritance. In the line 97 of the mapping DH30 population, as well as the lines 80, 123, 127 and 136 of the mapping population DH38, the B7 isoform was found that was absent in all three parental forms. The appearance of this isoform in these five lines seems to be due to the transgressive nature of genetic rearrangements or changes in the genome of these lines that occurred during their creation and selection in culture in vitro, that is, due to somaclonal variability. It is also possible that this is one

of the sinapine esterases identified in *Brassicaceae* previously [42]. The same can be said for the ratio of the A1 and A2 isoforms found in a number of lines in both DH30 and DH38 populations. It should be noted that the A3 isoform is presented in all three of the parental forms and is absent in the lines 28 and 67 of the DH30 population, and the lines 1, 4, 67, 90, 124, and 134 of the DH38 population. The presence of the isoforms A1 and A2 in different lines of both populations varied. Their presence in a particular line seems to be due to the same reasons as the appearance of the isoform B7.

On the basis of electropherograms for both populations, QTL analysis was conducted and the loci on the chromosomes that determine the appearance of each of the esterase isoforms detected from lines of the mapping populations DH38 and DH30 were identified (Table 1, see the journal website http://www.agrobiology.ru).

The approach of composite interval QTL mapping used in this study in combination with the permutation test (1000 iterations) at the significance level $p < 0.05$ allowed identifying and localizing on chromosomes the loci (QTLs) that determine the appearance of all isoforms of esterases identified by means of gel-electrophoresis (see Fig. 1) except for A1 in the DH38 population and V7 in DH30. For these two isoforms, the QTL analysis results were not obtained due to the small amount of relevant baseline data for each mapping population.

The results of QTL mapping revealed molecular markers genetically linked to the identified loci, which in the future can be used for molecular genetic analysis of *B. rapa* samples, and the localization of the detected QTLs in the linkage groups was identified (Fig. 2, see at the journal website http://www.agrobiology.ru). So, in a population of DH30 for the A1 isoforms, seven loci on the chromosomes that determine its manifestation were identified. The maximum LOD values were found for the 1st (3.70) and 5th (3.76) linkage groups. For A2 in the DH30 population, three, and, in the DH38 population, six loci determining the manifestation of this isoform were found. It should be noted that the location of the QTL of the isoform A2 on the 4th and 9th chromosomes coincided in both populations, indicating the evolutionary consolidation of this trait, which apparently had adaptive significance. The maximum LOD (of 2.09) in the population DH30 was calculated for the 9th chromosome, and for DH38, the obtained LOD values ranged from 2.28 to 4.88 for different linkage groups. In contrast, the value of LOD for A3 isoforms was relatively low, 1.29 for a single QTL in the population DH30 and 1.30-1.79 for QTLs detected in DH38; however, the proportion of phenotypic variability, which determined the QTLs identified for the isoforms A3, accounted for 23.4% of the population DH30 and 14.6-19.5% in the case of DH38.

The isoform B1 is also characterized by low LOD value for both populations, i.e. 1.36-1.53 and 1.33-1.75 if the amount of phenotypic variation is 20.2-25.4 and 13.3-17.1% for DH30 and DH38, respectively. In general, three QTLs from the population DH30 and two from DH38 were mapped. QTLs of the isoform B3 were not numerous and had low LOD values. Thus, for DH30, they were 2.00 and 1.37-2.53, respectively, for DH38 — 4.00 and 1.32-1.78. However, for B3, as for A2, the location of the QTL mapped in the 6th linkage group coincided, indicating the evolutionarily adaptive nature of this isoform. The number of QTLs underlying the expression of the isoform B2 was small as well, i.e. four in the population DH30 and two for DH38. However, the LOD value (1.36-3.36 and 1.93-2.47 for DH30 and DH38, respectively) and the proportion of controlled phenotypic variability (23.9-48.0 and 19.9-24.8%, respectively) were higher than for B3. Almost the same results were obtained for the isoform B4. Two QTLs were identified for it in each of the mapping populations. The

value of LOD and the percentage of phenotypic variation ranged between 1.48-1.55 and 46.1-47.8% for DH30, 1.33-1.49 and 20.3 and 22.4% for DH38. The isoform B5, though it had a low LOD (1.32-1.52 and 1.93-2.07 for DH30 and DH38 respectively) with a small percentage of phenotypic variation (22.3-25.3 and 18.3-19.5%), however, as well as B3, had a QTL, localized in both populations at the 6th linkage group in similar positions, which confirms the conclusion about the evolutionary adaptive nature of inheritance of these isoforms of esterases of *B. rapa* seeds. The population DH30 had five QTL isoforms B6 at the 3rd, 6th, 7th, and 8th chromosomes; DH38 had three QTLs in the 8th, 9th and 10th linkage groups. LOD scores were relatively small, 1.31-1.84 and 1.31-1.48 respectively; the proportion of phenotypic variation was 23.9-29.7% and 11.9-14.9% for DH30 and DH38. It is interesting to note that for the DH30 population, the QTL identified in the 6th linkage group was located in the same place as the QTL of the isoform B5, which indicates the importance of this chromosomal locus in *B. rapa*. The B7 isoform was identified and mapped only in the DH38 population. In total, five QTLs were localized, which were located on the 1st, 2nd, 7th and 9th chromosomes. LOD values ranged from 1.53 to 2.13; the proportion of phenotypic variability due to the identified QTL was from 82.8 to 91.4%.

For C1, only one QTL was found in each of the mapping populations. The LOD values (1.39 and 1.32 for DH30 and DH38) and the percentage of phenotypic variability due to the corresponding QTL (32.9 and 18.9%) were relatively small. C2 QTLs were identified in DH30 in the 2nd, 4th and 5th linkage groups (1.46-2.20 and 31.2-43.0%), in DH38 – in the 3rd and 8th groups (1.29-2.31 and 18.2-30.7%). The C3 isoform had three mapped QTLs in the population DH30 and four QTLs – in DH38. The LOD values of the identified QTLs varied from 1.50-2.77 in DH30 and 1.51-2.51 in DH38, and the proportion of the phenotypic variability in DH38 and DH30 was 68.5-88.1 and 43.3-65.0%, respectively. QTLs for the C3 isoform mapped on the 2nd chromosome (as in the case of A2, B3, and B5) were identified in both mapping populations. This fact confirms that the specified locus of the 2nd chromosome has evolutionary significance and its structure was fixed in the process of ontogenetic and phylogenetic adaptation in the *B. rapa* species. In all probability, the stability of the typical morphological and biochemical features of the plant is associated with such adaptively significant chromosome loci and the increased adaptive ability to adverse environmental factors as well [43-45].

2. Heterozygosity and its variance in *Brassica rapa* L. mapping lines of DH38 and DH30 populations based on data of isozyme analysis of esterases

Indicator	Isozymes												
	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	C1	C2	C3
Population DH30													
H_l	0.352	0.616	0.139	0	0.139	0.475	0.491	0	0	0.073	0.475	0.391	0.391
$Var(H_l)$	0.008	0.009	0.004	0	0.004	0.009	0.009	0	0	0.002	0.009	0.009	0.009
Average heterozygosity H_{total} = 0.272; dispersion $Var(H_{ototal})$ = 0.007													
Population DH38													
H_l	0	0.213	0.213	0.078	0.149	0.424	0.476	0	0	0.149	0.465	0.465	0.368
$Var(H_l)$	0	0.003	0.003	0.001	0.002	0.005	0.005	0	0	0.002	0.005	0.005	0.005
Average heterozygosity H_{total} = 0.231; dispersion $Var(H_{ototal})$ = 0.003													

According to isozyme analysis, the heterozygosity of populations for each locus H_l and total heterozygosity H_{total}, as well as the variance of heterozygosity $Var(H_l)$ at one locus and the variance of mean heterozygosity within each of the *Var* populations (H_{total}) was calculated (Table 2).

Since the dispersion of the average heterozygosity should take into account covariations between heterozygosity at different loci (l and $\hat{l}$), which is due

to their dependence on the frequency of double heterozygotes $H_{l\hat{l}}$ at these loci, the formula proposed by Weir [41] was used. The revealed heterozygosity was considered as the average portion of loci with two different alleles in one locus in one individual and could be defined as the observed heterozygosity characterizing a part of genes on which the studied population is heterozygous. The used formulas [39-41] make it possible to calculate any polynomial in a set of variables distributed multinominally, which, in turn, allows considering the revealed heterozygosity as a measure of information polymorphism widely used in the preparation and implementation of genetic selection programs. The formation of any breeding significant properties depends on the genotype of the individual [46, 47]. At the same time, information polymorphism is nothing but a reflection of phenotypic manifestations of genetic determinants (genes, chromosome loci) distributed in the genome, determining the studied features [48].

To summarize, the QTLs determining biochemical traits, i.e. the spectra of esterase isoforms of mature seeds in mapping populations of *B. rapa* doubled haploids, were mapped by us for the first time and the molecular markers genetically linked to the QTLs of these traits were identified, which allows efficient molecular genetic screening of sample collections and breeding material. Mechanisms that control the biosynthesis and accumulation of biologically active substances such as isoenzymes that determine the biochemical characteristics of morphotypes occurs as a result of evolutionary-adaptive formation and inheritance. Modification of these mechanisms can lead to a sharp change in the number and set of isoenzymes in various organs, in particular, in seeds, which, in turn, in *B. rapa* affects the morphological and adaptive qualitative characteristics of the species. The study of genetic determinants of esterase isoforms in the future can allow controlling the process of genetic variability on these adaptively significant features by combining different genetic derivatives from different parental forms in one organism. Analysis of genetic variability is very important for understanding the genetics of quantitative traits that play a key role in breeding programs to improve economically significant properties in *B. rapa*. As a result, it would be extremely important to determine the functions of genes that affect their expression, molecular genetic mechanisms of expression and, finally, characterize the molecular variability of genomes inside and outside the species. It is interesting to note that similar data were obtained earlier for wheat [29] and radish [30], which also revealed polymorphism between samples of promising breeding material in the spectra of isoenzyme esterases forms of mature seeds. In addition, these results echo those described in the analysis of the esterase-lipase gene family in *Arabidopsis thaliana* [49] and *Olea europaea* [50]. The data obtained on these species (both in this study and earlier), in the future, are supposed to allow the identification of the genetic-evolutionary and phylogenetic relationships of the structure and functioning of genomes in mono- and dicotyledonous plants.

Note should be made of the revealed coincidence of the positions of loci that control the isoenzyme profiles of esterases and other biochemical features studied earlier, such as the content of free amino acids, phenolic compounds, carotenoids, and chlorophylls. These loci were localized in the lower part of the A03 chromosome where the gene for the transition to flowering *BrFLC5* is situated, as well as in the middle and in the lower part of the A06 linkage group [31].

Thus, molecular genetic mapping and analysis of the spectra of esterase isoforms in mature *Brassica rapa* seeds revealed genetic determinants of these traits. The distribution of mapped QTLs in the genome has been identified. It was shown that in the studied populations of doubled haploid lines, QTLs determining

a set of esterase isoenzymes are located mainly in the 2nd, 4th, 6th and 9th linkage groups and form the blocks of co-adapted genes and genomic co-adapted blocks of genes, underscoring the importance of the contribution of these loci in the ontogeny and adaptiveness of *B. rapa* plants. In general, the coincidence of localization of the detected QTLs supports the observed biochemical correlations (e.g., the location of esterase isoforms on the electrophoretogram), and the blocks of genes combined into a genome areas in the linkage groups A02, A03, A04, A06 and A09, and the QTLs identified in the linkage groups reflect the genetic correlations between such characteristics as spectra of esterase isoforms and other biochemical properties of plants.

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