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ECOLOGICAL AND GENETIC EVALUATION OF MORPHOLOGICAL AND BIOCHEMICAL CHARACTERS OF QUALITY IN *Brassica rapa* L. ACCESSIONS FROM VIR COLLECTION

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Abstract

Peculiarity of chemical composition of *Brassica rapa* L. crops (high water content and low content of fats) determine their low-calorie character. They are notable for relatively high content of carbohydrates and proteins, including all essential amino acids. Biochemical composition varies greatly among *B. rapa* members. We are the first to carry out a multifactor evaluation of valuable morphological and biochemical traits of *B. rapa* accessions from the core collection of Vavilov Institute of Plant Genetic Resources (VIR, 96 samples) in eco-geographical study (South China and Leningrad region of Russia) under field trials and under a greenhouse conditions. By means of the developed SSR (simple sequence repeats) markers which are in linked disequilibrium with QTL of morphological and biochemical traits, the molecular genetic analysis was firstly carried out in leafy, rooted, and oilseed *B. rapa* genotypes from the VIR core collection. As a result, new genetic sources for quality parameters have been found among the samples investigated. In average, the samples did not differ noticeably on plant morphology (leaf length, leaf width, petiole size, hairiness, colour) in field tests when growing in South China and in Leningrad region, whereas in a greenhouse at higher plant density the leaf size decreased, e.g. in Chinese cabbage, pak-choi, wutacai, and turnip the leaves were 5-12 cm longer and wider in the field tests. Under field trials, we revealed the significant and high significant correlations between dry matter (DM) and ascorbic acid (AA) level (r = 0.51, p < 0.05), DM and chlorophyll a (Chla) content (r = 0.59, p < 0.05), DM and carotenoids (Cd) (r = 0.55, p < 0.05), DM and carotins (Cn) (r = 0.67, p < 0.05), DM and β-carotene (β-C) (r = 0.59, p < 0.05), DM and protein (P) level (r = –0.49, p < 0.05); Chla and Chlb (r = 0.93, p < 0.001), Chla and Cd (r = 0.59, p < 0.05), Chla and Cn (r = 0.49, p < 0.05), Chla and β-C (r = 0.99, p < 0.001); Chlb and β-C (r = 0.92, p < 0.001); Cd and Cn (r = 0.49, p < 0.05), Cd and β-C (r = 0.63, p < 0.05); Cn and β-C (r = 0.49, p < 0.05). In the greenhouse, the significant and high significant r values were as follows: r = –0.59 (p < 0.05) for DM and AA, r = 0.58 (p < 0.05) for DM and Chlb, r = –0.53 (p < 0.05) for DM and Cd, r = 0.71 (p < 0.001) for DM and Cn; r = –0.59 (p < 0.05) for AA and Chlb, r = 0.83 (p < 0.001) for AA and Cd, r = 0.58 (p < 0.05) for AA and P; r = 0.74 (p < 0.001) for Chla and Chlb, r = 0.67 (p < 0.05) for Chla and Cn, r = 0.95 (p < 0.001) Chla and β-C; r = –0.48 (p < 0.05) for Chlb and Cd, r = 0.87 (p < 0.001) for Chlb and Cn, r = 0.64 (p < 0.05) for Chlb and β-C; r = 0.63 (p < 0.05) for Cn and β-C. The semi-headed Chinese cabbage Syaobaikou and Dunganskaya, pak-choi Mayskaya, and especially Ching Pang Yu Tsain with a distinctly high level of chlorophylls and carotene, are indicated as new promising genetic sources for valuable biochemical parameters under both field and greenhouse conditions. Their indices in the field trials and greenhouse tests were 5.44-7.03 and 4.20-5.40 %, respectively, for DM (that is higher as compared to mean value for the crop), 12.61-24.66 and 24.12-33.23 % for P, 32.56-46.46 and 30.00-61.47 mg/100 g for AA, 33.35-110.64 and 53.82-95.99 mg/100 g for Chla, 12.20-53.80 and 17.89-44.78 mg/100 g for Chlb, and 1.80-6.75 and 3.00-6.04 mg/100 g for β-C. It is confirmed that the SSR markers BRMS051, KS51082, BRMS043 and KS50200 may effectively screen collection accessions and breeding material for desired morphological and biochemical traits. Our original data allow to practically implement an association mapping strategy and identify genetic determinants of morphological and biochemical quality characteristics using unique *B. rapa* collection preserved in VIR.

Keywords: *Brassica rapa*, morphological and biochemical quality characteristics, molecular markers, screening of plant collection

Turnip (*Brassica rapa* L.) is the wide-spread species on the globe, which includes economically important premature ripening and productive oil, vegetable, fodder crops with valuable chemical content. Low level of fats and high content of water makes them low-calorie, and a significant amount of biologically active substances (vitamins, enzymes, etc.) positively contribute to human health, stimulates the immune system and prevents the development of cardiovascular diseases. In N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) search for sources of improved morphological signs and biochemical quality among *B. rapa* collection is being carried out. Genetic analysis of these valuable quantitative trait loci (QTL) is possible due to significant genetic variability of the crop. The biochemical composition of plants within the *B. rapa* species varies greatly [1-5]. In the past decade, the character of basic metabolite accumulation, including biologically active substances, in previously poorly studied East Asian *B. rapa* wild cabbage plants and varieties have been reported [6, 7]. The explanation for the widest variability of morphological features in *B. rapa* is suggested by K. Lin et al. [8], who found unique genes in three morphotypes of *B. rapa*, the root turnip and short-stage form, whose genomes were sequenced and compared with the genome of the headed napa cabbage.

Earlier [9-12], we mapped the loci, responsible for seven morphological and five biochemical quality traits in population lines of doubled haploid leaf, root and oil crops of *B. rapa* L. In total, 140 QTL were mapped (taking into account the control by one locus of the same characteristics under the different cultivation conditions or several characteristics simultaneously), determining the formation of these agriculturally important properties in the lines of *B. rapa* doubled haploids under field conditions and in a greenhouse. Molecular markers, genetically linked to the studied QTL, are identified, and a block genomic structure of the genetic component construction (chromosome loci and linkage groups), involved in the expression of morphological and biochemical quality attributes, is discussed. However, the achieved results not fully withhold the nature of inheritance and molecular genetic control of these traits, being of interest for studying the genetic diversity in *B. rapa*.

A specific of this paper is that a multifactorial complex assessment of morphological and biochemical quality traits that make *B. rapa* plants valuable was carried out for the first time. Analysis of the genomes of *B. rapa* various forms using molecular markers, which are in linkage disequilibrium with QTL of morphological and biochemical features, made it possible to identify promising genetic sources for quality selection in *B. rapa*. In this case, the detected molecular markers can serve as an effective tool in the mass screening of the collection samples and selection material.

The objective of the work was the morphological, biochemical and molecular genetic evaluation of the *Brassica rapa* L. collection of VIR in various ecological and geographical zones under field conditions and in the greenhouse.

Techniques. A total of 96 samples of different origin from the main VIR collection, covering botanical diversity of *B. rapa* species, were examined using taxon description by C.E. Specht and A. Diederichsen [13], with the exception of ssp. *sylvestris* (Lam.) Janchen separation as a subspecies different from ssp. *oleifera*. All samples were evaluated under the long-term field trials in the Pushkin Branch of VIR (St. Petersburg) and at the South China Experimental Station of Enza Zaden company (Guangzhou, Guangdong, China) in 2010, and also in 2013-2016 in the winter greenhouse (Pushkin Branch of VIR). The planting scheme was 70×30 cm in the field tests and 25×20 cm in the greenhouse. Morphological description and biochemical evaluation were carried out according to the previously presented methods [3, 4, 14, 15] on 20 plants of each sample.

DNA was isolated from young green leaves of plants by D.B. Dorokhov and E. Kloke protocol [16]. PCR screening was carried out as previously described [17]. Reaction mixture (25 μl) contained 10× incubation buffer (2.5 μl), 0.5 μl of each dNTP (10 mM), 1 μl of primer (10 pmol/μl), 0.5 μl of Taq DNA-polymerase (5 unit/μl) (Sibenzyme, Russia) and 20 ng of gDNA. PCR amplification was performed in a DNA thermocycler (Bio-Rad, USA) with an individual program for each type of markers in accordance to genetic marker database of vegetable crops (http://vegmarks.nivot.affrc.go.jp/). The results were visualized by DNA electrophoresis in a 1.8 % agarose gel with ethidium bromide. A documentation system (Bio-Rad, USA) was used for registration of the patterns.

The data were processed using Statistica 6.0 software (StatSoft Inc., USA). The significance level $p \le 0.05$ was used.

Results. Samples of *B. rapa* collection (Table 1) were evaluated under conditions of a greenhouse (seeding on February 1) and in field trials (seeding in Guangzhou on May 25, in Pushkin on June 25) for a number of morphological signs, which determine the productivity and consumer appeal of plants. These were leaf blade length, width, color, pubescence, the nature of the surface, as well as the petiole length and width in the best developed leaf of the middle canopy.

1. The sample list of the main collection of the *Brassica rapa* L. species from the world collection of N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR)

Subspecies	Variety	VIR accession number	Name	Origin
ssp. *pekinensis* (Lour.) Hanelt	Dungan	139	Dungan	Kazakhstan
(napa)	Xiao	53	Local	Kazakhstan
		74	Xiao Bai Kou	China
		89	Dou-shaped early ripening	China
	Chirimen	100	Hikoshima spring	Japan
	Nagasaki	238	Nagoya Market	Japan
	Shantung	58	Bi-Ze	Kirghizia
		210	Kiriba Santo	Japan
		108	Local	China
	Kasin	132	Kasin	Japan
		247	Hasinbechu	Korea
	Chosen	122	Len Sin Dzon	China
		207	Chosen	Japan
	Aichi	63	Local	China
		131	Aichi	Japan
	Nozaki	111	Nozaki early	Japan
		327	Nozaki Harumaki	Japan
	Kaga	103	Kaga	Japan
		88	Ju Sin Bao Tou Bai Zai	China
	Hotoren	127	Hotoren	Japan
	Chi Fu	48	Wong-Bok	The Netherlands
		110	Matsushima	Japan
	Kensin	222	Kensin	Japan
	Granat	164	Michihli	Canada
		71	He Tou Ven	China
	Da Zin Kou	56	Da Zin Kou	China
		128	Zushita	Japan
		198	Local	China
ssp. *chinensis* (L.) Hanelt	Piorbuy	75	Piorbuy	China
(Chinese)	Suesman	77	Suesman	China
		Vr.930	Mayskaya 8	China
	Taisai	46	Tai Na	Russia
		106	Yanzai	China
		214	Nicanme Jukijiro Taisai	Japan
	Yu Tsai (var. utilis)	195	Local	China
		203	Ching Pang Ju Tsai	China

Table 2 (continued)

var. *rosularis* (Tsen & Lee)	Ta Gu Zai	84	Hee Yu Ta Zai	China
Hanelt (rosette)		129	Ta Gu Zai	China
var. *narinosa* (Bailey) Hanelt	Chrysanthemum	154	Chrysanthemum heart	China
(broad-beaked mustard)		213	Bitamin na	Japan
var. *purpuraria* (Bailey) Bailey (purple)		391	Xing Yang	China
ssp. *nipposinica* (Bailey) Hanelt	Mibuna	115	Mibuna	Japan
(Japan)	Mizuna	159	Mizuna	Japan
		241	Shiroguki Kyona	Japan
ssp. *rapa* L. f. *Komatsuna*	Komatsuna	215	Uzuki Komatsuna	Japan
(leaf turnip Komatsuna)		242	Goseki Late	Japan
	Kurona	264	Kurona	Japan
Japan leaf vegetables	Mana	372	Bansei Mana	Japan
	Sirona	98	Osaka Market	Japan
		217	Okute Osaka Shirona	Japan
	Hiroshimana	335	Hiroshimana	Japan
Stable hybrids between sub-		96	Shantai	China
species		302	Gurin Debu	Japan
		331	White Long Petiole	Japan
		436	Benrina	Japan
ssp. *rapa* L.	Chinese	163	Local	China
(root turnip)	Ostersundomskiy	307	Ostersundomskiy	Russia
	Bortfeldskiy	385	Bortfeldskiy	Ukraine
	Karelian	738	Karelian	Russia
	Grobovskaya	821	Grobovskaya	Russia
	Milanskaya white	826	Milanskaya white	Russia
	Petrovskaya	830	Petrovskaya	Russia
	Teltower	894	Teltower	Germany
	Norfolk violet-headed	984	Norfolk	France
	Volynskiy	1050	Volynskiy	Ukraine
	Golden globe	1283	Golden globe	The Netherlands
ssp. *oleifera* (DC.) Metzger		68	Local	China
f. *annua* (spring cress)		1	Kun Min ai u-zai	China
		2	Hue Zin u-zai	China
		11	Gute	Finland
		13	Local	Argentina
		25	Zsjan Su U uan-uzai 5082	China
		63	Pahsi	India
		106	Lotni mustard	India
		108	Arlo	Sweden
		114	Local (tetraploid)	Pakistan
		163	LGL	Pakistan
		192	Mustard	Nepal
		248	Local	Spain
		251	Vat-cawte	Tanzania
		301	BHLS	Nepal
		339	Jui-cai-tai	China
		374	Local 88/47	Bhutan
ssp. *oleifera* (DC.) Metzger		166	Root mustard	Tunisia
f. *biennis* (winter cress)		337	U-zai-zsi	China
ssp. *dichotoma* (Roxb.) Hanelt		53	Local toria	India
(brown sarson)		100	Local	Nepal
		135	Ds 17	India
		161	Toria selection	Pakistan
		205	Sarson	Pakistan
ssp. *trilocularis* (Roxb.) Hanelt		131	Type 1	India
(yellow sarson)		188	Palton sarson 66	India
		299	Sangam	India
		338	Chen-du-ai-u-zai	China
ssp. *sylvestris* (Lam.) Janchen		176		Italy
(wild sylvan cress)		218	Nabo silvestre	Peru

The collection average length and width of the leaf blade under field conditions in China and cultivation in the Pushkin Branch of the VIR were not significantly different, whereas the dimensions of the leaf blade were much smaller in the greenhouse (Table 2). At the same time, in China, the size of the leaf blade was significantly larger in Chinese cabbage, broad-beaked mustard and purple cabbage (all cultures of South China origin), as well as in root turnips, being slightly more in South China rosette cabbage and cress. The dimensions of the leaf blade of the napa cabbage and turnip, as well as the length of the blade were greater during the cultivation in Leningrad region. Obviously, this is due to the very high summer temperatures in South China (35 °C and above), which limit the rapid growth of vegetative organs in some cultures. In the napa cabbage, the size of the leaf blade authentically exceeded the population average. In field tests in China, Pushkin and in the greenhouse, the length was 38.22±1.61, 41.82±1.03 and 31.87±0.71 cm, respectively, and width was 19.33±0.80, 25.02±0.78 and 16.02±0.45 cm. The leaf size of the turnip under field conditions also exceeded the average for the collection, while in the greenhouse it did not differ from the average for the species. The leaf blade of the cultivated cress was significantly less (by 30-40 %, regardless of the cultivation conditions) than the population average, and the wild cress and rosette cauliflower were the smallest. The leaf blades of napa, Chinese, broad-beaked mustard cabbages, turnip, cultivated cress under field conditions were 5-12 cm longer and wider than in the greenhouse, the leaf blades in rosette and Japanese cabbage, as well as in wild cress under the same conditions practically did not differ in size.

2. Average size of leaf in *Brassica rapa* L. grown under contrast conditions

Crop	Length, cm			Width, cm		
	field trial		P, greenhouse	field trial		P, greenhouse
	SCh	P		SCh	P	
Leaf blade						
Napa cabbage	38.22±1.61	41.82±1.03	31.87±0.71	19.33±0.80	25.02±0.78	16.02±0.45
Chinese cabbage	26.10±2.58	21.36±1.08	16.13±1.16	19.20±1.30	16.33±1.06	11.18±0.58
Rosette cabbage	14.50±0.50	12.30±2.00	13.93±1.11	11.50±0.50	10.45±1.05	10.05±1.03
Broad-beaked mustard and purple cabbage	24.70±1.86	21.50±1.98	16.73±0.42	22.30±2.61	18.60±0.38	11.17±0.42
Japanese cabbage	18.00±3.06	24.63±2.20	24.10±7.03	9.67±2.40	9.50±3.10	8.37±1.19
Turnip	24.78±3.14	19.12±1.39	19.10±0.83	19.00±1.78	12.26±0.91	15.95±0.95
Leaf turnip	27.58±2.09	33.84±2.63	21.79±1.01	18.75±1.22	23.51±1.32	12.45±0.67
Cress	19.04±1.13	16.67±0.52	12.75±0.48	13.72±0.64	12.96±0.47	10.38±0.40
Wild cress	13.50±0.50	11.50±1.00	14.03±2.01	11.50±0.50	9.50±0.50	11.85±1.86
The collection average	25.75±1.09	26.34±1.20	20.63±0.85	16.92±0.53	17.61±0.67	12.85±0.35
LSD_{05}	3.31	3.62	2.55	1.60	2.04	1.05
Petiole						
Chinese cabbage	16.00±1.79	15.41±1.74	12.35±1.35	3.33±1.39	3.39±0.23	2.06±0.09
Rosette cabbage	9.00±2.01	10.50±0.10	12.28±1.65	2.20±0.25	1.95±0.35	1.51±0.09
Broad-beaked mustard and purple cabbage	21.00±1.53	13.87±1.25	15.73±1.73	3.67±0.34	3.40±0.15	1.64±0.08
Japanese cabbage	16.30±2.17	14.43±0.66	15.76±3.06	0.97±0.03	1.10±0.15	1.03±0.09
Turnip	21.25±1.36	23.39±1.04	30.90±2.54	2.00±0.29	1.63±0.15	1.44±0.09
Leaf turnip	14.80±1.84	12.68±1.66	11.11±0.95	2.90±0.28	3.81±0.32	1.97±0.14
Cress	16.20±0.85	15.63±0.58	14.71±0.80	1.10±0.07	1.16±0.10	0.71±0.05
Wild cress	14.00±3.01	10.50±0.50	15.15±3.16	0.90±0.10	0.75±0.25	0.64±0.13
The collection average	16.53±0.64	16.07±0.65	16.33±1.37	1.98±0.14	2.04±0.13	1.29±0.07
LSD_{05}	1.93	1.95	4.11	0.43	0.41	0.22

N o t e. SCh — Southern China, Guangzhou (Guangdong Province), P — Pushkin Branch of N.I. Vavilov All-Russian Institute of Plant Genetic Resources — VIR, St. Petersburg). Field trials were carried out in China in 2010, in Pushkin in 1997-2016, greenhouse tests were conducted in 2013-2016. For Latin descriptions of cultures, see Table 1. The results were obtained by analyzing 96 samples from the main VIR collection. Average (X) and average errors (±Sx) are given.

The population average length of the petiole under all cultivation conditions was stable, the width of the petiole in Southern China and Leningrad region differed slightly, but in the greenhouse was significantly smaller (see Table 2). In broad-beaked mustard and purple cabbage, Japanese cabbage, leaf turnip and wild cress the length of the petiole in Southern China was much larger than in Leningrad region, in wild cress — slightly more, and in turnip and rosette cabbage — less than in Leningrad region. In Chinese cabbage, leaf turnip and cress the petiole was shorter in the greenhouse, than in the field; in the remaining cultures it was longer (in turnip — 2 times as long as the population average). All samples of napa cabbage had the whole sessile leaves without petioles. A long, relatively wide petiole was typical of root turnips, broad-beaked mustard and Japanese cabbage (15-30 % longer than the collection average), a short petiole was characteristic of cauliflower. In the samples, the length of the petiole under field conditions and in the greenhouse basically differed insignificantly (by 1-4 cm), except for Japanese cabbage and root turnip, in which the petiole in the greenhouse was 10-15 cm longer than in field. The widest petiole was found in specimens of Chinese and broad-beaked mustard cabbage and turnip, the thinnest ones were typical of Japanese cabbage and cress. In field tests, the petiole was significantly wider in all crops, except for Japanese cabbage and wild cress.

The leaf surface in the specimen differed depending on the botanical affiliation from smooth to folded or slightly, medium and strongly wrinkled, the nature of the pubescence varied (from absence to a strong manifestation), the color of the blade varied from light to dark green.

3. The samples of *Brassica rapa* L. distinguished by the size of the food organs when grown under contrast conditions

Sample, VIR accession number	Leaf length, cm			Leaf width, cm			Petiole length, cm		
	field trial		P, green-greenhouse	field trial		P, green-greenhouse	field trial		P, greenhouse
	SCh	P		SCh	P		SCh	P	
Napa cabbage (no petiole)									
Kiriba Santo, k-210	55.0±2.6	45.9±3.3	36.3±4.2	22.0±1.7	22.6±1.5	14.8±1.2			
Chosen, k-207	50.6±2.8	42.1±2.1	37.9±2.7	25.6±2.4	20.2±1.9	18.1±2.6			
Crop average	38.2±1.6	41.8±1.0	31.8±0.7	19.3±0.8	25.0±0.8	16.0±0.4			
Chinese cabbage									
Tai-na, k-46	24.2±2.3	22.8±2.7	16±3.1	19.0±2.6	18.2±2.2	13.8±2.8	13±1.8	22.8±3.0	16.7±2.5
Nicanme Jukijiro Taisai, k-214	26.5±2.8	23.3±2.5	12.9±2.8	21.5±1.4	18.1±1.8	10.3±1.6	25.4±2.4	19.5±2.7	17.1±2.0
Crop average	26.1±2.5	21.3±1.1	16.1±1.2	19.2±1.3	16.3±1.1	11.2±0.6	16.0±1.8	15.4±1.7	12.3±1.3
Leaf turnip									
Goseki Late, k-242	40.3±3.7	40.5±2.9	25.9±3.2	21.5±2.8	23.1±2.0	9.5±1.9	16.6±2.3	11.5±2.2	9.6±1.6
Bansei Mana, k-372	50.4±3.2	45.2±3.6	22.6±2.6	36.8±3.4	26.9±2.6	15.4±1.4	9.1±2.1	9.8±2.1	8.4±1.2
Crop average	27.6±2.1	33.8±2.6	21.8±1.0	18.7±1.2	23.5±1.3	12.4±0.7	14.8±1.8	12.7±1.7	11.1±0.9

Note. SCh — Southern China, Guangzhou (Guangdong Province), P — Pushkin Branch of N.I. Vavilov All-Russian Institute of Plant Genetic Resources — VIR, St. Petersburg). Field trials were carried out in China in 2010, in Pushkin in 1997-2016, greenhouse tests were conducted in 2013-2016. For Latin descriptions of cultures, see Table 1. The results were obtained by analyzing 96 samples from the main VIR collection. Average (X) and average errors (±Sx) are given.

As genetic sources, we selected samples in which the size of the food organs was significantly higher than the average population index (Table 3). In a number of samples, the length and width of the leaf and petioles under contrasting conditions stably exceeded the mean values for the crop, which indicated their high adaptive ability. A slight variability in these features was noted in samples of napa cabbage Kiriba Santo, Chosen, Chinese cabbage Tai-na, Nicanme Jukijiro Taisai, local Chinese turnips (k-163), which formed a commercial root yield and a high-quality salad leaf rosette. The present study confirmed, that leaf and semi-headed varieties of napa cabbage are more suitable for growing in a greenhouse, unlike the typical headed varieties, as reported earlier [6]. Adaptive properties of the collection samples of other species during comparative tests in the greenhouse and under field conditions were evaluated for the first time.

The absence of pubescence and bright color increase the consumer qualities of *B. rapa* leaf crops. In the best specimens, pubescence was absent or very poor, and the color of the leaf varied from bright light green to bright dark green.

Biochemical assay has shown (Table 4) that the dry matter content in the studied samples averaged 7.77±0.25 % under field conditions and significantly exceeded the same value in the greenhouse (5.36±0.15 %), moreover, in all crops, except for napa cabbage, the excess (by 30-140 %) was significant (see Table 4). The dry matter content authentically differ in various species and varied from 5.65 % in napa cabbage to 11.24 % in leaf turnips in field studies, and from 4.44 % in Chinese cabbage to 6.62 % in turnips in the greenhouse. Thus, the variability in the dry matter content under field conditions was significantly higher than in the greenhouse.

4. The content (X±Sx) of nutrients and biologically active substances in *Brassica rapa* L. crops under different growth conditions

Crop	Dry matter, %	Proteins, %	Ascorbic acid, mg/100 g	Chlorophyll, mg/100 g		Carotinoids, mg/100 g	β-Carotene, mg/100 g
				a	b		
Field conditions (2006-2016)							
Cabbage:							
napa	5.65±1.47	23.73±3.86	50.45±14.82	26.21±12.04	11.72±5.94	10.30±4.28	1.82±0.85
Chinese	9.99±1.91	24.85±2.73	65.21±13.36	64.51±11.96	29.03±9.87	17.60±3.62	4.12±0.67
rosette	9.41±1.98	27.80±3.24	58.03±13.69	98.87±9.06	55.46±6.79	18.74±2.05	6.34±0.63
broad-beaked mustard and purple	8.36±3.29	29.09±2.00	46.24±5.93	74.71±4.91	37.69±6.83	16.18±2.58	4.56±0.19
Japanese	9.17±0.10	23.49±3.01	46.24±1.36	86.47±6.86	40.73±5.54	21.43±3.50	5.39±0.26
Turnip:							
root, leaves	8.81±1.62	24.66±3.82	71.91±10.29	78.26±25.09	43.16±20.32	15.56±4.75	4.89±1.53
leaf	11.24±3.67	21.35±1.28	77.52±10.26	98.61±4.12	47.15±3.83	20.71±10.1	6.24±0.83
Cress	7.45±1.34	27.95±3.13	69.74±21.15	81.83±11.69	47.29±14.08	12.99±4.30	5.05±0.89
Wild cress	10.64±0.73	17.99±1.04	68.68±4.80	75.25±0.64	41.09±1.09	10.77±1.02	4.55±0.03
The collection average	7.77±0.25	24.87±0.39	58.70±1.65	59.06±3.27	30.63±2.09	14.06±0.55	3.77±0.19
LSD_{05}	0.77	1.17	4.95	9.83	6.29	1.64	0.56
Greenhouse (2014)							
Cabbage:							
napa	4.84±0.08	31.06±0.48	35.00±1.44	48.15±2.54	18.23±1.32	23.07±0.76	2.97±0.16
Chinese	4.44±0.13	24.12±0.98	30.00±4.21	73.30±4.49	36.03±2.88	27.85±3.78	4.46±0.30
rosette	4.72±0.28	23.65±0.90	19.00±3.01	63.22±0.78	33.34±0.33	20.46±0.56	4.09±0.01
broad-beaked mustard and purple	5.40±0.18	20.46±0.58	19.00±1.15	64.11±5.09	36.77±3.63	16.93±3.38	3.78±0.31
Japanese	5.44±0.12	27.23±1.51	38.00±5.20	87.60±6.67	37.41±3.47	36.39±2.42	5.45±0.38
Turnip:							
root, leaves	6.62±0.17	25.32±0.67	15.00±0.63	77.26±4.03	50.29±3.46	14.65±1.08	4.70±0.26
leaf	4.46±0.23	27.36±0.98	37.00±4.54	57.62±5.23	27.85±6.78	25.19±2.81	3.56±0.30
Cress	6.08±0.14	27.69±0.55	20.00±0.53	65.70±1.45	46.11±2.37	11.79±0.56	4.02±0.07
Wild cress	5.80±0.28	25.32±2.98	21.00±0.71	78.24±9.88	56.08±12.01	15.28±4.28	4.73±0.49
The collection average	5.36±0.10	27.50±0.40	27.03±1.16	63.24±1.71	35.15±1.72	19.77±0.94	3.88±0.10
LSD_{05}	0.29	1.2	3.47	5.12	5.16	2.81	0.31

N o t e. The protein content is indicated per dry weight, biologically active substances — per wet weight. The tests were carried out in Pushkin Branch of N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR, St. Petersburg). For Latin descriptions of cultures, see Table 1. The results were obtained by analyzing 96 samples from the main VIR collection. Average (X) and average errors (±Sx) are given

We did not find the same patterns for protein level. In field studies, its content was significantly lower than the collection average in wild cress and much higher in broad-beaked mustard and purple cabbage (see Table 4), while in the greenhouse the broad-beaked mustard cabbage, on the contrary, had the lowest index with the highest in the napa cabbage. At the collection average, the protein content increased in the greenhouse, but the range of variability (11 %) persisted regardless of the growth conditions. In Chinese cabbage, turnip and cress, the protein content in comparative tests was practically stable, in napa and Japanese cabbages, leaf turnip and cress the parameter was higher in the greenhouse, in rosette, broad-beaked mustard and purple cabbage — under field conditions. As for the last group of plants, this may be due to their relatively slow growth or to a characteristic manifestation of the analyzed sign, which is common to these botanically closely related crops.

B. rapa plants are important sources of ascorbic acid, carotenoids, chlorophylls. In our studies, the content of ascorbic acid in *B. rapa* samples varied quite widely. The minimum values corresponded to the average for the white cabbage, and the maximum values exceeded them several times. At the collection average, the indexes were 2 times higher under field conditions (58.7±1.65 vs. 27.03±1.16 mg/100 g), and this was noted for all crops, except for Japanese cabbage which had not so significant difference. In fact, the ascorbic acid content in rosette and broad-beaked mustard cabbage, root turnip and cress during the cultivation in the field was 3 times higher and more than in the greenhouse. The largest accumulation of ascorbic acid in the field was observed in Chinese cabbage, turnip and cress, in the greenhouse — in napa and Japanese cabbages and leaf turnip.

At the collection average, carotenoid accumulation was higher in the greenhouse (19.77±0.94 vs. 14.06±0.55 mg/100 g, LSD_{05} 2.81 and 1.64, respectively). This is especially important in the winter and spring periods, when food is poor in vitamins. The richest sources of carotenoids were greenhouse plants of Japanese cabbage, followed by Chinese cabbage, wild cress and leaf turnip. Under field conditions, the samples of Japanese, Chinese, rosette cabbage and leaf turnip were distinguished by this feature. In napa cabbage and wild cress, the excess of carotenoids in the greenhouse was more than 2-fold relative to that recorded in the field. In rosette, broad-beaked mustard and purple cabbage, cresses and turnips, the amount of carotenoids was relatively stable. Approximately 20 % of the carotenoid fraction are carotenes. Actually in carotene 80-90 % fraction is represented by the β-form. On the average, the content of β-carotene differed little (3.88±0.1 and 3.77±0.19 mg/100 g in the greenhouse and in the field, respectively). Carotene accumulation was stable in Chinese and Japanese cabbage, turnip, wild cress, whereas in napa cabbage it was higher in the greenhouse (2.97±0.16 vs. 1.82±0.85 mg/100 g, LSD_{05} 0.56 and 0.31, respectively), in rosette, broad-beaked mustard and purple cabbage, leaf turnip it was higher in the field, and the maximum accumulation was characteristic of Japanese, Chinese and rosette cabbage.

Chlorophyll plays a significant role in dietary nutrition: eating green leaves increases blood hemoglobin and erythrocytes. At the collection average, the content of chlorophylls *a* and *b* was insignificantly higher in the greenhouse (the sum of 63.24±1.71 and 35.15±1.72 vs. 59.06±3.27 and 30.63±2.09 mg/100 g). The practically stable content of chlorophylls (especially of chlorophyll *a*) was noted in Japanese cabbage, turnip and wild cress, its increment in the greenhouse was found both in napa and Chinese cabbages, and in the filed — in rosette and broad-beaked mustard cabbage, turnip and cress. With a deficiency of fresh salad vegetables in the winter to spring time, mainly fast growing crops (Peking and Chinese cabbage) are cultivated in greenhouses, which are high valuable due to the ability to accumulate green pigments under these conditions. As greenhouse crops, it also reasonable to recommend rosette, broad-beaked mustard, Japanese cabbage and leaf turnip, which are significantly superior to the napa cabbage in the chlorophylls content (their maximum level was noted in rosette and Japanese cabbage and leaf turnip).

The correlation analysis of biochemical characteristics showed that the amount of dry matter is significantly and unidirectionally associated with the chlorophylls, carotenes and β-carotene levels, regardless of cultivation conditions (Table 5). The interdependence between the content of dry matter and ascorbic acid, as well as carotenoids, was significant, but multidirectional (direct in field and reverse in the greenhouse). Significant invers correlations were found between the dry matter and protein content in the field. In the greenhouse, there was a significant inverse correlation between the content of ascorbic acid and chlorophyll *b*, and direct correlation for carotenoids, protein. A relationship between the amount of chlorophylls *a* and *b*, as well as their relation to the accumulation of carotenoids and carotenes (especially β-carotene) was highly reliable. An essential correlation was noted between the content of carotenes and β-carotene. Thus, samples that combine a high content of chlorophylls and carotenes can be obtained quite easily, while selection for a simultaneously high content of protein and ascorbic acid will cause difficulties.

5. Correlation coefficients between biochemical characteristics in *Brassica rapa* L. under different growth conditions

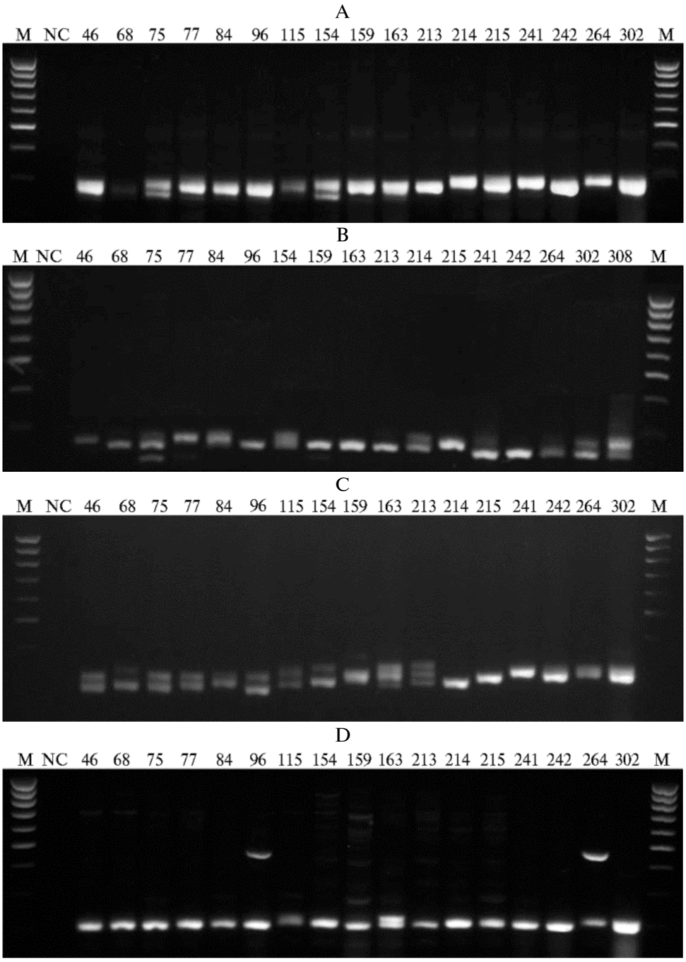
Parameter \ Parameter	DM	AA	Chl a	Chl b	Cd	Ca	β-Ca	P
			Field conditions (2006-2016)					
Dry matter (DM)		0.51*	0.59*	0.36	0.55*	0.67*	0.59*	–0.49*
Ascorbic acid (AA)			0.29	0.26	–0.02	0.33	0.30	–0.39
Chlorophyll a (Chl a)				0.93**	0.59*	0.49*	0.99**	0.16
Chlorophyll b (Chl b)					0.32	0.37	0.92**	0.28
Carotinoids (Cd)						0.49*	0.63*	0.09
Carotene (Ca)							0.49*	0.01
β-Carotene (β-Ca)								0.16
Proteins (P)								
			Greenhouse (2014)					
Dry matter (DM)		–0.59*	0.31	0.58*	–0.53*	0.71**	0.36	–0.02
Ascorbic acid (AA)			–0.11	–0.59*	0.83**	–0.42	–0.09	0.58*
Chlorophyll a (Chl a)				0.74**	0.21	0.67*	0.95**	–0.31
Chlorophyll b (Chl b)					–0.48*	0.87**	0.64*	–0.37
Carotinoids (Cd)						–0.36	0.29	0.22
Carotene (Ca)							0.63*	–0.18
β-Carotene (β-Ca)								–0.25
Proteins (P)								

Примечание. The tests were carried out in Pushkin Branch of N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR, St. Petersburg).
*, ** Significant ($p < 0.05$) and highly significant ($p < 0.001$) correlation coefficients.

Based on the data obtained, we revealed new genetic sources of the studied biochemical features with 5.44-7.03 and 4.20-5.40 % for dry matter (which exceeds crop averages), 12.61-24.66 and 24.12-33.23 % for protein, 32.56-46.46 and 30.00-61.47 mg/100 g for ascorbic acid, 33.35-110.64 and 53.82-95.99 mg/100 g for chlorophyll *a*, 12.20-53.80 and 17.89-44.78 mg/100 g for chlorophyll *b*, and 1.80-6.75 and 3.00-6.04 mg/100 g β-carotene in field conditions and a greenhouse, respectively. These are samples of semi-headed napa cabbage Xiao Bai Kou and Dungan, Chinese cabbage Mayskaya and Ching Pang Yu Tsai, which was particularly high in chlorophylls and carotene.

For molecular studies we used 8 SSR markers, suitable for subsequent screening of samples of the world collection. The markers were located on A03 (3 markers), A05 (1 marker), A06 (2 markers) and A09 (2 markers) chromosomes, encompassing linkage groups in which, according to our QTL analysis [10-12, 18] the loci are located, which control the morphological and biochemical quality features of the species. The unevenness of the markers assignment on chromosomes indirectly indicates the presence of the genome blocks of co-adapted gene suggested by us, and the existence of co-adapted blocks of genes in the genome as a whole. The presence of such blocks in the genome of *B. rapa* is confirmed by our earlier studies, which established the genetic determinants of *B. rapa* resistance to different races of *Xanthomonas campestris* pv. *campestris* causing black rot [18-20]. The effectiveness of the molecular markers selected by us should be noted, as some markers (for example, BRMS043 and BRMS034) were proved to be effective molecular genetic descriptors of the species for resistance to black rot. We used these markers to identify the collection specimens of *B. rapa* resistant to each race of *X. campestris* separately and to the pathogen as a whole [18, 21, 22].

Using 8 molecular markers to study 18 *B. rapa* samples, we detected 26 polymorphic SSR fragments from 122 to 410 bp in size. Marker BRMS051 (Fig., A) with the expected size of the 262 bp amplicon in 77 % of the samples was linked to the length and width of the leaf blade, at that in 58 % of these samples its width was 20 cm and more. In 60 % of cases, alleles which produced fragments of 262 bp and 280 bp, were associated. Fragment (allele) 280 bp was manifested in all lines with an average length of leaf blade (up to 20 cm), in all rosette cabbage samples, in all samples and varieties of Chinese cabbage and in 60 % of stable hybrids, the parent form of which was Chinese cabbage. Note that these variants of Chinese cabbage had a green (but not light or dark green) color of the leaf. Fragment (allele) 262 bp was found in 75 % of samples of turnip.



The results of PCR analysis of *Brassica rapa* L. samples using SSR markers BRMS051 (A)**, KS51082** (B)**, BRMS043** (C) **and KS50200** (D)**:** 46, 68, 75, 77, 84, 96, 115, 154, 159, 163, 213, 214, 215, 241, 242, 264, 302 and 308 are samples (VIR catalog numbers), NC — negative control, M — M16-DNA marker 100 bp (10 fragments from 100 to 1000 bp, Sibenzym, Russia). The collection of N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR, St. Petersburg).

The KS51082 (see Fig. B) was linked to the QTL of the length of the petiole. Samples k-115, k-163 and k-214 with the expected fragment 282 bp had long and very long petiole (22-40 cm). A 282 bp amplicon was revealed in all samples of Japanese cabbage and in 50 % of samples of turnip.

Using the marker BRMS043 (see Fig. B), we found the samples with the expected size of 318 bp amplicon. In total of 70 % of these samples the leaf surface was smooth and slightly wrinkled. All samples with a pair of fragments (alleles) 285 and 305 bp had smooth or slightly wrinkled leaves. Samples k-115, k-154, k-163 and k-213, combining fragments 285 and 318 bp, were outstanding on the β-carotene content of 5.1-6.2 mg/100 g. In the presence of 318 bp amplicon, 71 % of the variants had a fairly long or long leaf blade (26-44 cm) of medium width (16-23 cm), and in 75 % of samples with short leaf blades (11-23 cm) a fragment (allele) 305 bp was manifested. Allele 285 bp was found in all samples of Chinese cabbage and a hybrid of Chinese and napa cabbages, and a fragment of 318 bp was revealed in 60 % of Japanese cabbage samples. The same allele (318 bp) was found in half of the samples of leaf turnip, and in 75 % of leaf turnip we found allele corresponding to the 305 bp fragment.

The use of the molecular marker Ol12-F02 linked to the QTL for pubescence and color of the leaf blade [7] revealed four fragments, 200, 185, 175, and 140 bp. Molecular analysis of the same samples with the BRMS014 marker showed the presence of two amplicons (263 and 280 bp) and its linkage to QTL of leaf color (from light green to green) in the most part of the samples (80 %) of those in which these amplicons appeared. The same pair of amplicons was found in 70 % of Chinese cabbage samples and its hybrids.

Screening with the KS50200 marker (see Fig. D) revealed fragments 292, 280 and 260 bp. Samples that showed 260 bp amplicon in 75 % of cases had a dark green leaf blade 15-20 cm wide with a smooth, sometimes slightly wavy edge. A 280 bp amplicon was found in hybrids of Chinese and napa cabbages, and also in 75 % of leaf turnips (in the latter, the width of the blade is, at average, 20 cm, the color is dark green, the edge is from smooth to slightly wavy). In Chinese cabbage (75 % of samples with a light green and green color and a smooth edge of the leaf blade with a width of 15-21 cm), a fragment corresponding to the 292 bp allele appeared. A fragment of this size was found in 66 % of samples of Japanese turnips with a sharp-toothed cut of the leaf edge.

Screening with the BRMS034 marker linked to the color of leaf blade, as we previously reported when mapping populations of the doubled haploids lines [12], showed the presence of amplicons 122 and 144 bp (in the forms with an 144 bp amplicon the leaves were dark green), however, the studied samples did not show a reliable relationship between the detected amplicons and the sign.

In the screening with BRMS042, the amplicon of expected size (380 bp) was found for all samples. In two of the three samples of broad-beaked mustard cabbage, a 410 bp amplicon was revealed, which also appeared in one sample of Chinese cabbage (k-46). Japanese cabbage and leaf turnip produced 620-170 bp amplicons, which were not typical for the remaining samples of the studied collection.

Thus, we carried out a complex (morphological, biochemical, molecular genetic) assessment of *Brassica rapa* quality traits in environmental tests with different methods of cultivation. Note that the analysis of the *B. rapa* genomes by molecular genetic markers that are in disequilibrium linkage with corresponding QTL of the leaf color, the content of total protein, β-carotene, carotenoids, ascorbic acid, dry matter, has been carried out by anyone never before. These studies confirmed the possibility of using the SSR markers found by us to screen collection and breeding material for morphological and biochemical features. In addition, the obtained original results allow us to proceed to the practical implementation of associative mapping and identification of specific genetic determinants defining the manifestation of a number of agriculturally important and economically significant qualitative traits, using the unique material of *B. rapa* from the collection of N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR).

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