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NORM OF REACTION ON HIGH CONTENT OF β-CAROTENE IN *Capsicum annuum* var. *annuum* L. GENOFOND IN THE CONNECTION WITH BREEDING ON QUALITY

O.O. Timina[1], O.Yu. Timin[2], S.K. Fedorov[3]

[1] Scientific Research Laboratory "Bioinformatics", Pridnestrovian State University, Tiraspol 3300, Pridnestrovian Moldavian Republic
e-mail: otimina@mail.ru
[2] The State Botanical Garden, Tiraspol 3300, Pridnestrovian Moldavian Republic
e-mail: otimin@mail.ru
[3] Institute of Food Technologies, Kishinev 2200, Pridnestrovian Moldavian Republic
e-mail: fyodoroff@mail.ru

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Summary

For clarification of a variation character for "high content of β-carotene in fruits" determinant in C. *annuum*. var. *annuum* in connection with the genotype, the growing conditions and the interaction between the genes, controlling this determinant, and the genes, controlling the anthocyanins color of vegetative and generative organs the authors analyzed more 100 variants, relating to cultivars *Grossum* L. (Sendt), *Longum* D.C., *Pomifera* Fingerh и*Fascicullatum* Sturt,including the varieties and the hybrids, from own selection. It was shown, that the results of the β-carotene content testing by the GOST 8756.22 and by the high performance liquid chromatography method are correlated (r = 0.99), that is relatively comparable. The intensity of fruits color during the phase of technical ripeness exactly correlates with provitamin A content, however, the fruits color and provitamin A content during the phase of biological ripeness doesn't interrelated. To obtain the new initial pepper material with a complex of practical valuable determinants, including a high content of provitamin A, ascorbic acid and anthocyanins in fruits, the authors recommend to use the tame mutant forms of pepper of natural and artificial origin with *A*,*Im* and *bc* genes.

Keywords: vegetable pepper, quality selection, β-carotene, anthocyanin, cultivated mutants.

β-Carotene, vitamins C and E are very important components of human diet. At the same time, the deficit of β-carotene and carotenoids has been established in residents of Moscow, Novosibirsk and Norilsk upon the data of blood tests performed in different groups of people in Russian Federation (1). A normal dose of β-carotene (15-50 mg/day) usually prevents serious chronic diseases of cardiovascular, respiratory, urinary, digestive systems and inhibits the development of certain cancer types (2). This fact determines a creation of agricultural crops varieties with increased contents of β-carotenoids in fruit. Vegetable pepper contains up to 30 different pigments (3), which makes interesting to clarify the interrelation of genes encoding these pigments with genes providing high content of β-carotene. In particular, there are almost no data on possible interactions of a mutant gene *bc* encoding this trait with *A* and *Im* genes controlling anthocyanin synthesis.

The purpose of this work was a genetic identification of individuals carrying the determinant "high content of β-carotene in fruit" along with assessing its reaction norm depending on growing conditions and interactions between genes controlling synthesis of anthocyanin and β-carotene in vegetable pepper.

Technique. The object of study - a population of *C. annuum* var. *annuum* L. represented by more than 100 samples – the cultivated varieties-types *Grossum* L. (Sendt), *Longum* DC, *Pomifera* Fingerh and *Fascicullatum* Sturt including the varieties and hybrids of own selection (4, 5), as well as selected forms created by crossbreeding with natural mutants for anthocyanin coloration (*A, Im*). The sample Oranzhevaya Kapiya obtained through radiation mutagenesis was kindly provided by Prof. S. Daskalov (Institute of Genetics, Bulgarian Academy of Sciences, Sofia). Designations of genes correspond to the accepted by D. Wang and P. Bosland (6). Plants were grown using a conventional technique in unheated film greenhouses, in glass greenhouses as the second crop after seedlings and in open field.

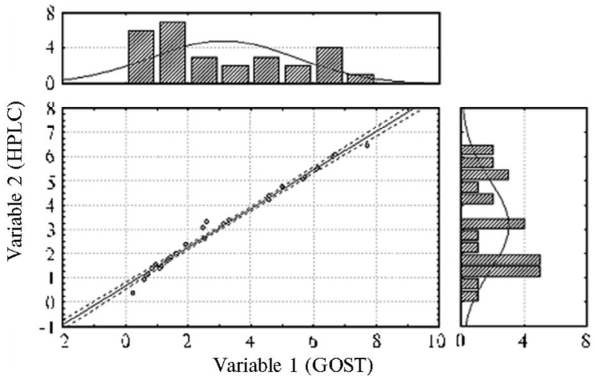
The vitamin content in fruit was determined in the phase of biological maturity according to GOST 8756.22-80 and by high-performance liquid chromatography (HPLC) on the high pressure liquid chromatograph Agilent technologies 1200 series ("Agilent technologies", USA) with diode-array detector at λ=436 nm . The mobile phase - acetonitrile: chloroform (92:8), flow rate 1,5 ml/min. Chromatographic column Zorbax XDB (4,6×150 mm), sorbent C18 (particle diameter 5 um) ("Agilent technologies", USA). Injected volume of a sample 20 ml, time of testing one sample 30 min. Standard - β-carotene free from a λ-form ("Sigma", USA). Calibration curve was designed considering 8 points (for solutions with concentrations of 4,000; 2,857; 2,222; 2,000; 1,818; 1,538; 0,800 and 0,342 mg/100 ml). Preparation of samples included treating with acetone in an alkaline medium with Na_2CO_3, Na_2SO_4 and quartz sand until complete decoloration. The extraction was performed in a separatory funnel; 15 ml hexane was added, shaken and left to separate water-acetone and hexane phases. The procedure was repeated until the complete removal of acetone traces. Hexane extract was dehydrated by passing through sodium sulfate and then injected into the column using a dispenser ("Rheodyne", USA). Vitamin C was determined using a standard technique of GOST 24 556 in the average sample of 4-5 fruits collected from branches of the 1^{st} and 2^{nd} orders. The material from lines and F1 hybrids was analyzed in 4 replications, the material from unknown genotypes - without replications. The presence of anthocyanin was determined visually by color of fruit.

The data were statistically calculated in Statistica 6,0. The three-factor analysis of variance, the coefficient of variation (Cv), the coefficient of adaptation (A) and the linear coefficient of interaction between the genotype and environment (L) were determined as described (7).

Results. Carriers of high contents of vitamin C and β-carotene in the population of C. annuum var. annuum.

Since the content of β-carotene content was determined by two methods, comparability of obtained results was assessed in a group of 28 samples (Table 1). The high degree of correlation between these methods was established (*r* = 0,99) (Fig.); though, this value varied by 20-37% in 18% samples (Fig.). Knowing that even the most precise HPLC implies an error within ± 15% at all stages of determining carotenoids (http://rudoctor.net/medicine/bz-bw/med-amjuc/pg-5.htm), the data obtained by these two methods can be considered as relatively comparable and used in this work (determination of total content of carotenoids according to GOST includes a conversion into β-carotene).

Biochemical evaluation of vitamin C and β-carotene content in populations of different cultivars-types revealed their relative uniformity in respect of these parameters. At the same time, intrapopulation variability of Cv for β-carotene content was high. An equalized level of the trait “high content of β-carotene” was the result of a targeted genetic selection, while high Cv values indicate the presence in this population of contrasting genotypes with high and low contents of provitamin A (Table 2).



Correlation between the content of β-carotene determined according to GOST 8756.22 (variable 1) **and by high-performance liquid chromatography** (HPLC) (variable 2) **in pepper *Capsicum annuum* var. *annuum* L. different genotypes.** Central chart - dispersion of values relative the regression line (95 % confidence interval): variable 2 = 0,66496 + 0,79646 × variable 1; r = 0,99384. Diagrams show the distribution of corresponding variables relative a normal distribution.

Variable 1: N = 28, x = 3,096786, σ = 2,307415, max = 7,730, min = 0,260; variable 2: N = 28, x = 3,131429, σ = 1,849163, max = 6,430, min = 0,420.

1. β-carotene content (mg/100 g wet weight) **determined by different methods in biologically mature fruits of pepper *Capsicum annuum* var. *annuum* L. genotypes most contrasting by phenotype** (non-heated film greenhouses, Tiraspol, 2008).

Genotype	Phenotype	GOST 8756.22-80	HPLC	To HPLC, %
L 49	Dark-red-purple fruit	2,55	2,62	97
L 48	Dark-red intense purple leaves, flowers, fruit	1,62	2,00	81
L-1/03	Fruit orange-red	7,73	6,43	120
$F_1$1/03 ½ L 48	Red-purple leaves, flowers, fruit	3,13	3,21	98
$F_2$1/03 ½ L 48 (1)	Orange-red fruit; purple leaves, flowers, fruit	4,57	4,37	105
$F_1$1/03 ½ L 49	Dark-red partially purple fruit	1,45	1,84	79
$F_2$1/03 ½ L 49 (1)	Orange-red-purple fruit	6,09	5,49	111
F_2Л-147 ½ L 49 (1)	Pale-yellow fruit with low expressed anthocyanin presence	Traces	0	0
Korichnevyi 1	Fruit of a chocolate color	2,44	3,09	79
F_3 Paramo	Yellow-orange fruit	Traces	0	0
Oranzhevaya Krasavitsa	Orange-red fruit	3,30	3,38	98
Kolobok	Темно-красные плоды без антоциана	0,75	1,19	63
L 125	Orange-red fruit without anthocyanin (silvery-white in the phase of commodity ripeness).	1,08	1,37	79

Note. HPLC – determined by high-performance liquid chromatography; (1) – individual selection from a combination

2. Contents of β-carotene and vitamin C (mg/100 g dry weight) **in fruit of pepper *Capsicum annuum* var. *annuum* L. cultivars-types in the phase of biologically ripeness** (non-heated film greenhouses, Tiraspol, 1998-2003)

Cultivar type	Number of investigated samples	β-Carotene		Vitamin C	
		X±*m*	*Cv*, %	*X*±*m*	*Cv*, %
Grossum	78	36,06±3,04	68,7	2427,41±178,85	25,5
Pomifera	10	28,43±5,07	56,4	3236,44±265,21	18,3
Fasciculatum	32	30,46±4,31	80,1	2700,21±219,85	18,2
Longum	6	44,15±8,68	48,2	3772,65±156,75	5,9

Note. Quantitative determination was preformed according to GOST 8756.22-80; *Cv* — coefficient of variation.

The cultivars-types *Grossum* and *Fasciculatum* insignificantly differed by the content of vitamin C. On the contrary, the types *Longum* and *Pomifera* were reliably different in this trait. (Table 2). Slight intrapopulation diversity in the content of vitamin C was found in the cultivar-type *Longum*, the medium level – in *Pomifera* and *Fasciculatum,* while in *Grossum Cv* it exceeded 20%.

The content of β-carotene varied in fruit of different genotypes as well. The most promising varieties were Cv Kolobok, Oranzhevaya Kapiya, lines L 49 and L 29. Cv Kolobok demonstrated a number of outstanding economically valuable traits: high content of β-carotene (almost equal to that in the mutant Oranzhevaya Kapiya targeted selected for this trait), resistance to Verticillium wilt, high content of vitamin C, thick and sweet pericarp. However, the content of β-carotene in its fruit greatly varied by years of study. For example, the contents of pro-vitamin A (mg/100 g dry weight) in biologically ripe fruits harvested in different years were, respectively: 2000 - 36,39; 2001 – 64,43; 2008 - only 7,24. The line 29 was obtained through synthetic selection by crossing Moldovan lines related to the type *Fasciculatum* with cv Oranzhevaya Kapiya. High content of β-carotene was also established in the line L 49 of the type *Grossum* - a natural mutant for pericarp color (anthocyanin purple color of fruit in the phases of commodity and biological ripeness).

The trait “anthocyanin coloration” can be easily identified visually depending on doses of genes *Im* and *A*. In general, the studied population of *C. annuum* var. *annuum* had average content of β-carotene varying from 28,43 to 44,15, vitamin C - from 2427,41 to 3772,65 mg/100 g dw. Therefore, in the studied population the content of vitamin C was sufficiently high, while the content of β-carotene averaged to 34 mg/100 g dw; this fact clearly indicates the predominance of samples with low content of pro-vitamin A. There was revealed a correlation between the intensity of fruit color and the content of β-carotene: fruits colored white or creamy at the phase of commodity ripeness were, as a rule, poor in β-carotene when they get biologically ripe. At the same time, color of fruits in the phase of biological ripeness wasn't correlated with β-carotene content.

Diversity of the trait "high content of β-carotene" in representatives of cultivars-types Grossum and Fasciculatum. The content of β-carotene was determined in fruits grown in the open field, film and glass greenhouses; the obtained data showed a differentiated variation in expression of this determinant in genotypes both by replications and in different growth conditions (Table 3). Low and average values of Cv depending on the genotype were observed in lines L 29 and L49, medium and high - in cv Prometey. In changed growing conditions the studied genotypes (especially L 29) showed high variation. Considering the maximum value of the mean and Cv of the analyzed trait in L 29, it can be assumed high plasticity of this genotype compared with others.

The coefficients L and A were used to assess the correlation of growing conditions with manifestation level of the trait "β-carotene content in fruits". Adaptability value of the studied samples was found as <1, therefore, the studied group didn't include genotypes capable to develop high content of β-carotene regardless of growing conditions. So, despite the relatively high content of β-carotene, this trait is insufficiently stable. At the same time L → 0 confirmed the presence in the genotypes of a linear dependence between β-carotene content and environmental conditions. In this case, the effects of selection for enhanced manifestation of a desired trait depend on stability and general adaptability of the genotype for this trait.

3. β-carotene content (mg/100 g dry weight) **in fruits of pepper *Capsicum annuum* var. *annuum* L. different genotypes determined in the phase of biological ripeness over the years of research at various growing conditions** (Tiraspol).

Genotype	Year	Variant	Content of β-carotent, $X \pm m$	Standard deviation, σ	Cv, %
Line L 29	1999	1	40,97±1,02	1,761	4,30
		2	30,33±1,12	1,943	6,40
		3	37,60±1,09	1,900	5,10
	2000	1	64,33±7,88	13,650	21,20
		2	135,43±1,80	3,150	2,30
Line L 49	1999	1	27,57±1,36	2,350	8,50
		2	46,10±0,81	1,400	3,00
		3	20,13±1,47	2,550	12,70
	2000	1	43,93±1,07	1,850	4,20
		2	43,90±1,21	2,100	4,80
Cultivar Promoter	1999	1	5,10±1,31	2,260	44,31
		2	33,07±3,68	6,380	19,29
		3	13,60±2,52	4,370	32,10
	2000	1	24,63±1,42	2,450	9,95
		2	27,03±2,90	5,030	18,61

Note. 1 – glass greenhouses, the 2[nd] crop grown after seedlings, 2 – non-heated film greenhouses, 3 – open field.

The process of carotene synthesis is controlled as a quantitative trait, and such determinants can vary within a reaction norm depending both on the genotypic environment and on growing conditions. Earlier, variability of qualitative and quantitative composition of carotenoids has been studied in detail in tomato and carrot (8). Diversity of this trait in *C. annuum* has been studied mainly in the cultivar-type *Longum* (3, 9-14). Results of this study confirm the available data about diversity of β-carotene content in fruits of the vegetable pepper. Along with it, manifestation of this trait was studied in representatives of the cultivar types *Fasciculatum* and *Grossum*, though no genotypes with stably high expression were found.

4. β-carotene content (mg/100 g dry weight) **in fruit of pepper *Capsicum annuum* var. *annuum* L. different genotypes shown considering the presence and distribution of anthocyanin coloration** (non-heated film greenhouses, Tiraspol, 2008-2009)

Sample	Genotype	Content of β-carotene	Degree of domination of the determinant in F_1 hybrid
Line 1/03	*bcbc Crtz-2Crtz-2 aa*	71,62±7,89	
Line L 49	*Crtz-1Crtz-1 Crtz-2Crtz-2 ImIm*	39,13±5,10*	
Line L 48	*Crtz-1Crtz-1 Crtz-2Crtz-2 AA*	10,03±1,21*	
Cultivar Kolobok	*Crtz-1Crtz-1 Crtz-2Crtz-2 aa*	36,02±2,49*	
$F_1$1/03 ½ L 49	*bc/Crtz-1 Crtz-2Crtz-2 Im/+*	44,01±9,20*	–0,70
$F_1$1/03 ½ L 48	*bc/Crtz-1 Crtz-2Crtz-2 A/+*	27,36±9,19*	–0,44
$F_1$1/03 ½ cv Kolobok	*bc/Crtz-1 Crtz-2/Crtz-2 aa*	21,01±6,81*	–1,84

Note. Gene *A* — anthocyanin coloration of a whole plant with fruit, Im — anthocyanin coloration of only fruits in the phase of commodity ripeness. The content of β-carotene was determined by high-performance liquid chromatography.
* The value reliably differs from that in the line 1/03 (P = 0,05).

Since the line L 49 simultaneously carries the determinants "β-carotene content" and "anthocyanin content" (as bioactive component of the pericarp), it was interesting to reveal the possible interaction of genes encoding synthesis of these pigments. This was done using instead of L 29 the line 1/03 more promising for selection; 1/03 has a common origin with L 29 and carries the same gene *bc* providing high content of β-carotene.

The obtained results indicate (Table 4) that *bc* gene at homozygous state along with the absence of anthocyanin genes provides the highest content of β-carotene in fruits. In line L 49 it was lower than in 1/03, but higher than that of L 48. In lines L 49 and L 48, heterozygous state of genes for anthocyanin coloration (*A* and *Im*) was phenotypically manifested as a partially dominant trait. Apparently, *A* and *Im* genes also provide differentiated genotypic background resulting in variable expression of the mutant gene *bc* in heterozygotes. Therefore, different F1 hybrids carrying the same dose of this gene can vary in average content of β-carotene in fruits. It was found that the interaction of *bc* and *Im* genes as well as *bc* and *aa* determines in corresponding F1 hybrids the dominance of low levels of this trait. At the same time, there was observed a trend to increased synthesis of β-carotene in heterozygotes carrying in the genotype the combination of genes *bc* and *A* whose interaction results in intermediate manifestation degree of this trait in the hybrid. It is possible that enhanced expression of *bc* gene due to its interaction with *A* gene can be used in heterosis selection and multiple combinations of crosses will provide a stable trend to improving biochemical composition of fruits and other economically valuable traits.

Thus, it has been revealed the diversity of determinant "high content of β-carotene in fruits" in *Capsicum annuum* var.

annuum L. depending on the genotype, growing conditions and the interaction of genes encoding this trait with genes controlling anthocyanin coloration of vegetative and generative organs of a plant. It has been shown that intensity of fruit color at the phase of commodity ripeness is directly correlated with β-carotene content in biologically ripe fruits, but the content of provitamin A in biologically ripe fruits doesn't depend on fruit color. To obtain the new source material of pepper having multiple economically valuable traits including high contents of provitamin A, ascorbic acid and anthocyanin in the fruit, it is recommended to use the cultivated mutant pepper forms of natural and synthetic origin carrying the genes *A, Im* and *bc*.

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