MICROSATELLITE ANALYSIS OF *Y1* AND *VP5* MAIZE GENES

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Accession

Investigation of genomes plant genetic diversity resources is important goal of modern molecular genetics and biology. In our days for the genetics and breeding purposes the studing of repetitive genome fraction is actual [1], in particulaly so-called microsatellite loci (Simple sequence repeats, SSR). SSRs are widely using as molecular markers of genes of important qualitative and quantitative traits of crops, including maize [2].

Maize (*Zea mays* L.) is a widely using for production of food, feed for livestock, biofuels, *etc*. In 2009, the maize nuclear genome sequencing (line B73) was completed [3]. SSRs are considerable fraction of maize genome [4].

One of the perspective areas of maize breeding is enhancementing of the grains nutritional value quality, including increasing the carotenoids content [5]. According to the World Health Organization almost 40 % of the world population feels carotenoids and their derivatives deficiency in food [6]. That is why genes that encode of carotenoid biosynthesis enzymes need for special attention.

Products of *Y1* and *VP5* genes are important elements in the biosynthesis cycle of carotenoids and abscisic acid in grain cereals [7].

Gene *Y1*, which is located in the long arm of chromosome 6, encodes phytoene synthase (PSY), consists of 6 exons and 5 introns [8]. In *Y1* gene microsatellite p-y1SSR with *cca* repeating unit was found. The product of *Y1*

gene catalyzes the two-stage reaction in which two molecules of geranylgeranyl pyrophosphate condense for phytoene forming.

Gene *VP5*, encoding phytoene desaturase (PDS), located in the short arm of chromosome 1. The product of this gene is important for stereochemistry of fitoins to the next step in carotenoid biosynthetic pathway [9]. In *VP5* gene microsatellite umc1070 with *tc* repeat was found.

As the microsatellite fraction is the source for molecular markers, the aim of our studing was to analyze the maize genotypes diversity for microsatellite loci p-y1 and umc1070 of *Y1* and *VP5* gene, respectively.

Materials and Methods

Plant materials were 44 maize lines / varieties of Ukrainian and foreign breeding that vary in grain color according to the classification of National Plant Germplasm System and "Classifier-reference of *Zea mays* L.» [10], obtained from the National Center for Plant Genetic Resources of Ukraine and National Plant Germplasm System - Stock Center (USA) (Table 1).

Table 1. Maize lines / varieties for molecular genetic analysis of microsatellite loci *Y1* and *VP5* genes

Endosperm color			
White		Yellow	
Name of the lines / varieties *	Country of origin	Name of the lines / varieties *	Country of origin
Misceviy* (UB0108925)	Ukraine	UHK 507	Ukraine
HWSA(FG)C1	USA	Kosara 194*	Ukraine
Tx807	USA	Misceviy* (IR 14122)	Ukraine
KyWS1	USA	UHK 3	Ukraine
KyWS3	USA	ЗК 340-2	Ukraine
KyWS4	USA	UHK 35	Ukraine
KyWS6	USA	AD 40/16 MB, CB	Ukraine

KyWVS	USA	UHK 587	Ukraine
H126W	USA	H125	USA
HWSB(FG)C1	USA	B93	USA
CML 22	USA	NDB(MS)C8	USA
CML 43	USA	Florida 32	USA
TZEEI 11	USA	LH132	USA
Mo21R	USA	HP68-07	USA
7-118*	Romania	Chetnostebelnaya*	USA
D 1-08*	Albania	NDSCD	USA
LU 08*	Albania	LH123HT	USA
KO 1-08*	Albania	Mo12	USA
POOL 16 C 18*	Mexico	LP Oh43 RP1TD	CIIIA
H POOL 31 C 20*	Mexico	HP62-02	CIIIA
		Tx802	CIIIA
		HP72-11	CIIIA
		MoECB2(S1)C5	CIIIA
		H POOL 33 C 23*	Mexico

Extraction of DNA was performed by modified CTAB method from seeding mix of each line / varieties [11]. The quality and quantity of isolated DNA was tested by gel electrophoresis in 0,8 % agarose.

Polymerase chain reaction (PCR) was performed on termocycler «Tercyc» («DNA-technology», RF). The 20 mkl reaction mixture contained: 10x PCR buffer; 2,5 мМ $MgCl_2$; 0,2 мМ each dNTPs; 0,2 мкМ forward and reverse primers; 60 ng DNA; 1 un. DNA polymerase Taq. Amplification conditions were: first denaturation 96 ^oC 2 min; 30 cycles: denaturation 94 ^oC 1 min; annealing of primers 55 ^oC 30 sec; elongation 72 ^oC 1 min; final elongation 72 ^oC 2 min.

Primers design was from the database MaizeGDB [12] (Table 2).

Electrophoretic distribution of PCR fragments was performed in 3 % agarose or 10 % polyacrylamid gels with 1x TBE-buffer (89,0 мМ Tris, 89,0 мМ boric acid, 2,0 мМ EDTA) at 2,5 V/sm in apparate for vertical electrophoresis

(«Hoefer Scientific Instruments», USA). DNA fragments were visualized by silver straining [13]. The amplification products lengths were calculated by gel documentation and analysis «Image Master VDS» («AmershamPharmacia Biotech», OKB) (according to manual) and software Gel analyzer [14].

Table 2. Primers pairs for microsatellite loci of maize genes *Y1* and *VP5* analysis

Gene	Primer name	Sequence (5' –> 3')	The length of the amplification products by source
Y1	p-y1SSR F*	caagaagaggagaggccgga	200-250 b.p. [15]
	p-y1SSR R**	ttgagcagggtggagcactg	
VP5	umc1070 F*	ggtctctctatcgtccggtgagta	189 b.p. [16]
	umc1070 R**	ccggagatgggaaagaagataac	

Note: * - forward, ** - reverse

Carotenoids concentration in grain data were presented by plant biochemistry laboratory of Plant Breeding and Genetics Institute [17].

Statistical analysis was performed by Spearman's correlation coefficient and Student's t-test [18].

Results and Discussion

According to the biochemical analysis the carotenoids concentration in the maize grains ranged from 0,007 to 2,040 mg/100 g of grain (fig. 1). Statistically significant correlation between color and grain carotenoids concentration was confirmed. Spearman's correlation coefficient was K = 0,71+/-0,1. The critical value r_s by Student's test for equal t_{cr} = 1,964 for $P \leq 0,05$; t_{cr} = 2,785 for $P < 0,01$.

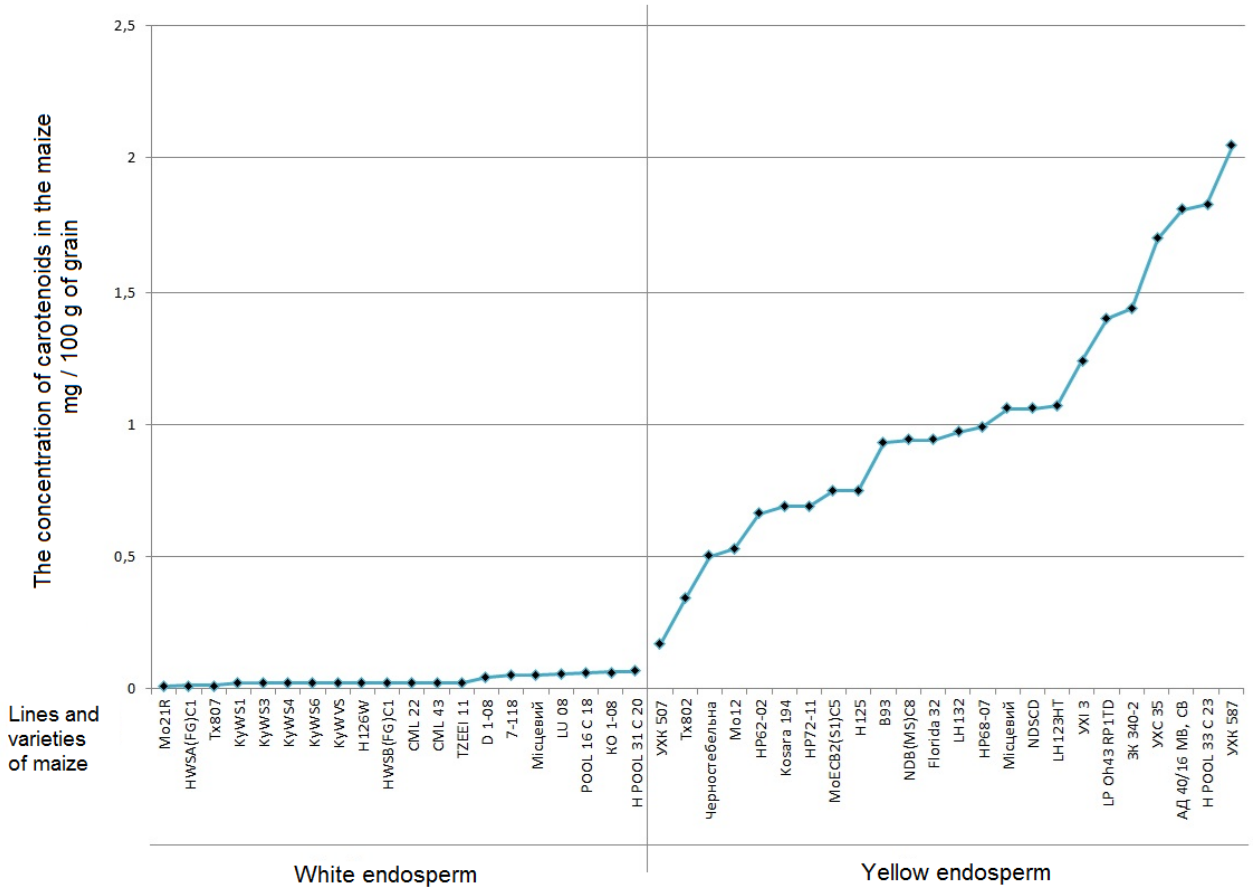

Fig. 1. The concentration of carotenoids in maize grain

Molecular-genetic PCR analysis of 44 maize samples by microsatellite region *Y1* gene using primers pair p-y1SSR was conducted. 44 maize samples distributed in 10 homozygous genotypes, amplification lengths fragments were from 197 to 224 b.p.

Analysis of relationship amplification fragments with carotenoid concentration and, consequently, grain color, demonstrated the correlation of 203 b.p. amplification fragment with yellow colour and high carotenoids concentrations, which averages 0,81 mg/100 g of grain (Table 3).

Table 3. Relationship of amplification fragments of the gene loci p-y1 with carotenoids concentration in maize grain

Genotype with fragments, b.p.	197, 197	200, 200	203, 203	206, 206	209, 209	212, 212	215, 215	218, 218	221, 221	224, 224
Carotenoids concentration in samples, mg/100 g of grain	0,02	0,02	0,05	**1,40**	0,01	0,04	0,01	0,02	0,02	0,02
		1,82	**0,34**		0,01	0,05	0,05		0,02	**0,17**
			0,50		0,02	0,06	**0,69**		**0,53**	
			0,66		0,02	0,06	**0,75**			
			0,69		0,02	**1,80**	**1,06**			
			0,75		0,02		**1,06**			
			0,93		0,06					
			0,94		**0,97**					
			0,94		**1,69**					
			0,99		**2,04**					
			1,07							
			1,24							
			1,44							

Note: Samples highlighted in bold are yellow endosperm

The Spearman's correlation coefficient of amplification fragment with length 203 b.p. with yellow endosperm was K = 0,7+/-0,1. Critical value for Student's test was t_{cr} = 2,201 for $P \leq 0,05$; t_{cr} = 3,105 for $P < 0,01$. The correlation is statistically significant.

Relationship of other amplification fragments with grain color and carotenoid concentration is not significant.

Our results of *Y1* maize gene investigation correlate with data Buckner et al. (1996) [19]. The authors researched microsatellite sequences and transposon element insertion in promoter region and established the dependence of carotenoids concentration in maize grain with allelic state *Y1* gene.

By PCR analysis of gene *VP5* maize for microsatellite locus umc1070, 44 samples were distributed in 14 genotypes, 8 homozygous and 6 heterozygous (Table 4).

Correlation umc1070 locus amplification fragments with carotenoids concentration and grain color was absent.

Table 4. Relationship of umc1070 locus amplification fragments with carotenoids concentration in maize grain

Genotype with fragments, b.p.	167, 185	167, 199	169, 181	169, 185	169, 191	169, 199	167, 167	169, 169	181, 181	185, 185	187, 187	189, 189	191, 191	199, 199
Carotenoids concentration in samples, mg/100 g of grain	**2,04**	0,01	0,02	**0,17**	0,02	**1,40**	0,02	0,02	0,91	**0,94**	0,01	0,06	0,05	0,02
		0,05			0,02		0,02	0,06	0,02	**0,97**	**0,75**	**0,34**	**0,69**	0,02
		0,53			**0,69**		0,04	**0,94**	0,02		**1,07**	**1,80**	**0,99**	
		0,66			**0,75**		0,05	**1,24**	**1,44**		**1,69**		**1,06**	
					1,06		0,06	**1,82**						
							0,5							
							0,93							

Note: Samples highlighted in bold are yellow endosperm

Resume

The 44 maize lines / varieties with different color endosperm were characterized by DNA-typing of microsatellite regions *Y1* and *VP5* gene.

According to researching of *Y1* gene microsatellite region p-y1SSR, maize samples were distributed to 10 homozygous genotypes with amplification fragments lengths 197 - 224 b.p. The correlation between amplification fragment of 203 b.p. and yellow color and high carotenoids concentrations were significantly.

By studing *VP5* gene microsatellite region umc1070, maize samples were distributed to 8 heterozygous and 6 homozygous genotypes. Reliable correlation between amplification fragments and carotenoids concentration in the maize endosperm were not found.

References

1. Inghelandt D. Population structure and genetic diversity in a commercial maizebreeding program assessed with SSR and SNP markers / D. Inghelandt, A. Melchinger, C. Lebreton, B. Stich // Theor Appl Genet. – 2010. – V. 120(7). – P. 1289–1299.

2. Jingtao Q. A genome-wide analysis of simple sequencerepeats in maize and the development ofpolymorphism markers from next-generationsequence data / Q. Jingtao, L. Jian // Qu and Liu BMC Research Notes. – 2013. – V. 6:403 . P. 1-10.

3. Schnable P. The B73 Maize Genome: Complexity, Diversity, and Dynamics / P. Schnable, D. Ware, R. Fulton et al. // Science. - 2009. – V. 326. – P. 1112-1115.

4. Vigouroux Y. An Analysis of Genetic Diversity Across the Maize Genome Using Microsatellites / Y. Vigouroux, S. Mitchell, Y. Matsuoka, et al. // Genetics. - 2005. – V.169. – P. 1617–1630.

5. Gilligan D. Biofortification, Agricultural Technology Adoption, and Nutrition Policy: Some Lessons and Emerging Challenges / D. Gilligan // CESifo Economic Studies. – 2012. – V. 58(2). – P. 405-421.

6. http://www.who.int/en/

7. Chao B. A golden era—pro-vitamin A enhancement in diverse crops / B. Chao, R. Twyman, G. Farre, et al // Plant. – 2011. – Vol. 47. – P. 205-221.

8. Gallagher CE, Matthews PD, Li F., Wurtzel ET Gene duplication in the carotenoid biosynthetic pathway preceded evolution of the grasses (Poaceae) // Plant Physiology. - 2004. - N 135. - P. 1776-1783.

9. http://www.maizegdb.org/cgi-bin/displaylocusrecord.cgi?id=12733

10. Descriptor reference book for the *Zea mays L*. species. Ykrainska akademiya agrarnih nayk. Harkiv. - 2009.

11. Ispolzovanie PCR-analisa v genetiko-selekcionnih issledovaniyah. Agrarnaya nayka. Kiev. Str. 36.

12. http://www.maizegdb.org/

13. Budowle B. DNA typing protocols: Molecular biology and forensic analysis / B. Budowle, J. Smith, T. Moretti– USA: A BioTechniques Books Publication, Eaton Publishing, 2000. - P. 130-131.

14. http://www.gelanalyzer.com/

15. Phelps. T. L. Microsatellite RepeatVariation Within the Y1Gene of Maize and Teosinte / T. L. Phelps, A. E. Hall, B. Buckner // The Journal of Heredity. – 1996. – Vol. 87 (5). - P. 396-399

16. http://www.maizegdb.org/cgi-bin/displaylocusrecord.cgi?id=12733

17. Soki fryktovie i ovoschnie. Metod opredeleniya soderganiya obschih karotinoidov. Gosstandart RF. Moskva. GOST 51443-99. – 6 str.

18. Atramentova L.O. Statistika dlya biologiv: Pidrychnik / Atramentova L.O., Ytovska O.M. – H.: «NTMT». – 2014. – 331s.

19. Brent Buckner. The yl Gene of Maize Codes for Phytoene Synthase / B. Buckner, P. San Miguel, D. Janick-Buckner, J. Bennetzent // Genetics. - 1996. - Vol. 143. - p. 479-488