EVALUATION OF SEVERAL YIELD TRAITS FOR SOME REGENERANTS OF *MOMORDICA CHARANTIA* L. UNDER FIELD CONDITIONS

EVALUAREA UNOR TRASATURI DE CALITATE LA CATIVA REGENERANȚI DE *MOMORDICA CHARANTIA* L. ÎN CONDIȚII DE CAMP

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Abstract

Momordica charantia L. is a valuable medicinal herb used in the treatment of many diseases, especially diabetes. The conditions of in vitro cultivation and the passage of specific stages of this technology determine the occurrence at the level of regenerants of some character changes which, although they are epigenetic, are valuable for the productivity of this species. Our research has been aimed at testing in the field crop conditions (three years 2014-2016) of 5 lines of bitter cucumber regenerants (Momordica charantia L.) obtained in vitro. The obtained results allow us to assert that through the in vitro culture it is possible to obtain the variability of certain production characters in the bitter cucumber, which is kept in the descendent and allows their use in order to improve the productive features of this species in the temperate climate zone.

Introduction

Bitter cucumber (*Momordica charantia* L.), an important medicinal plant originated in the hot climate zone of the Asian continent (India, China, Malaysia), is frequently used with encouraging positive results in the treatment of various diseases, such as infections, parasitoses, cancer, and especially diabetes. Due to temperate climate conditions, bitter cucumbers are cultivated with low productivity in Romania. The application of indirect regeneration methods, via calus, leads to the production of plants that show superior productive qualities, which allows the selection of valuable genotypes for the temperate climate zone. Numerous authors have studied the regenerative capacity of various varieties of *Momordica charantia* (Sultana and Bari, 2003; Manye, et al., 2004; Agarwal and Kamal, 2004; Malic et al., 2007; Devendra et al., 2009; Mishra et al., 2012; Chao et al., 2012). The study of genetic variability in bitter cucumber tissue lines and regenerants obtained in vitro demonstrates that different methods of artificial cultivation generate morphological, biochemical, molecular changes, which are of great practical importance when they are selected and characterized (Dalamu et al., 2012; Guo et al., 2012; Ali and Tariq, 2013; Saxena et al., 2014; Shukla et al., 2015).

In this paper we present the results of the crop production in the field of 5 lines of bitter cucumber regenerants, compared to the donor plants from which they came from.

Materials and methods

We have experimentally used 5 genotypes of bitter cucumber (A) selected from a culture located in the western area of Romania. The tissue callus was obtained from aseptically cultivated cotyledons on Murashige-Scoog medium supplemented with phytohormones NAA (naphtalen acetic acid 1,5 mg/L) and BAP (benzylaminopurine 1 mg/L) (Agarwal and Kamal, 2004; Malic and colab. 2007). Undifferentiated tissue was cultured on the same basic medium supplemented with phytocormones IAA (1 mg / L indole-acetic acid) and Kin (kinetine 0.2 mg / L). Regenerated plants were acclimated to normal conditions and transferred to the field (R). Their cultivation was done under normal conditions, without chemical or organic fertilizers, providing the necessary water during the drought (Botau et al., 2010). Fruit and seed production was taken into

account. Within three rehearsals, the results on regenerative productivity were compared to those of the donor plants from which they came from. The studied characters were: Fruit weight/pl. (**FWP**), Fruit nr/pl. (**FNP**), Fruit weight (g) (**FW**), Fruit lenght (cm) (**FL**), Fruit diameter (cm) (**FD**), Seeds nr/fruit (**SNF**) and Seeds weight/fruit (g) (**SWF**).

Statistical data analysis

The data were analyzed using different statistics techniques: Jaccard similarity coefficients, UPGMA cluster analysis (Fielding, 2007), principal components. Means for the ratio between the values of regenerants and donors, were compared using least significant difference test (Ciulca, 2006). The significance of differences was expressed based on letters, being considered as significant the differences between genotypes marked with different letters. The distance matrix was used for cluster analysis based of the unweighted pair-group method with arithmetic averages (UPGMA), using the Neighbor program of the Phylip package, version 3.5c.

Research results

Considering the ratio of the values of the production characters to the regenerators compared to the donor genotypes, Table 1 shows that for the weight of the fruit on the plant only genotypes 3R and 5R showed increases of 10.1-31.8%. Regarding the superiority of regenerants to donor forms, genotype 5R was particularly highlighted by showing values of this ratio significantly superior to other genotypes. Also, genotype 3R showed a significantly higher production potential than genotypes 1R, 2R and 4R. For the number of fruit per plant, it is observed that only the genotype 5R recorded a significant increase of 30.1%, whereas in the rest of the regenerative genotypes the values of this character were near or inferior to the donor form.

Table 1

Ratio between the values of regenerants and donors of Momordica charantia concerning different yield traits under field conditions during 2014-2016

Traits	**FWP**	**FNP**	**FW**	**FL**
Genotype	$\overline{x} \pm s_{\overline{x}}$	$\overline{x} \pm s_{\overline{x}}$	$\overline{x} \pm s_{\overline{x}}$	$\overline{x} \pm s_{\overline{x}}$
1 R/A	0.683±0.009 d	0.850±0.012 b	0.913±0.028 bc	1.088±0.009 b
2 R/A	0.706±0.005 cd	0.882±0.015 b	0.757±0.015 c	1.075±0.036 b
3 R/A	1.101±0.011 b	0.973±0.014 b	1.550±0.384 a	1.092±0.017 b
4 R/A	0.849±0.004 c	0.889±0.009 b	0.979±0.005 bc	1.074±0.021 b
5 R/A	1.318±0.022 a	1.301±0.025 a	1.139±0.014 b	1.184±0.020 a
LSD 5%	0.161	0.148	0.343	0.074

Traits	**FD**	**SNF**	**SWF**
Genotype	$\overline{x} \pm s_{\overline{x}}$	$\overline{x} \pm s_{\overline{x}}$	$\overline{x} \pm s_{\overline{x}}$
1 R/A	0.898±0.057 d	1.027±0.041 b	0.886±0.076 b
2 R/A	0.941±0.043 cd	0.841±0.034 c	0.895±0.058 b
3 R/A	1.104±0.038 b	1.115±0.025 ab	1.151±0.073 a
4 R/A	1.049±0.031 bc	1.061±0.012 b	0.931±0.046 b
5 R/A	1.379±0.047 a	1.215±0.022 a	1.154±0.085 a
LSD 5%	0.145	0.112	0.096

Concerning the average weight of the fruit, the 3R genotype showed the highest increase (55%) significantly superior to the rest of the regenerants. In the case of genotype 5R the fruit mass was higher by about 14% relative to the donor form. For the other regenerants, the values of this character were lower by 2.1-24.3% compared to the donor genotypes.

For all regenerants the fruit length was superior to donor forms, with increases ranging from 7.4% for genotype 4R and 18.4% for 5R. Thus, the increase in genotype 5R was significantly higher than in the other four genotypes with near performance.

As respects of fruit diameter, 3R, 4R and 5R genotypes were superior to donor forms. The increase in genotype 5R (37.9%) was significantly higher than the increases in genotypes 3R (10.4%) and 4R (4.9%).

Most regenerants (except 2R) showed a higher seed number / fruit than donor forms. At genotype 5R, the highest increase was 21.5% and significantly superior to the rest of the regenerants, which showed increases between 2.7% (1R) and 11.5% (3R).

For the seed weights we can see that 3R and 5R genotypes recorded a significant increase of 15.1-15.4% of this character, while in the rest of the regenerative genotypes the values of this character were near or inferior to the donor form by 6.9-11.4%.

According to the matrix of similarity presented in Table 2 it is noted that the highest phenotypic diversity between regenerants and donor forms with regard to the seven characters was recorded at genotype 5R (45.35%) and 2R (34.39%) respectively, in the year 2014. For genotypes 3R and 4R, phenotypic diversity versus donor forms was considerably lower (8.51-8.73%).

Between donor forms, phenotypic diversity was between 75.92% for 2A-5A and 3.07% for 1-4A. Concerning regenerants the highest intergenotypic diversity was recorded between: 1R-5R (37,42 %); 2R-5R (24,86 %); 1R-3R (23,44 %). The lowest phenotypic diversity was recorded among regenerants: 3R-5R (3,57 %); 3R-4R (3,69 %); 4R-5R (8,50 %).

Table 2

The phenotypic similarities between Momordica charantia genotypes in 2014

Genotype	**1R**	**2R**	**3R**	**4R**	**5R**	**1A**	**2A**	**3A**	**4A**	**5A**
1R	1	0.9064	0.7656	0.8956	0.6258	0.7737	0.4601	0.8125	0.6476	0.8396
2R	0.9064	1	0.8458	0.9114	0.7514	0.8357	0.6561	0.8407	0.7829	0.7579
3R	0.7656	0.8458	1	0.9631	0.9643	0.9881	0.8509	0.9149	0.9757	0.6956
4R	0.8956	0.9114	0.9631	1	0.9150	0.9621	0.7521	0.9233	0.9127	0.7682
5R	0.6258	0.7514	0.9643	0.9150	1	0.9534	0.8409	0.8560	0.9759	0.5465
1A	0.7737	0.8357	0.9881	0.9621	0.9534	1	0.8076	0.9292	0.9693	0.7451
2A	0.4601	0.6561	0.8509	0.7521	0.8409	0.8076	1	0.5727	0.8163	0.2408
3A	0.8125	0.8407	0.9149	0.9233	0.8560	0.9292	0.5727	1	0.911	0.8775
4A	0.6476	0.7829	0.9757	0.9127	0.9759	0.9693	0.8163	0.911	1	0.6258
5A	0.8396	0.7579	0.6956	0.7682	0.5465	0.7451	0.2408	0.8775	0.6258	1

According to the dendrogram of Fig. 1 it is observed that the 10 genotypes were grouped into two main clusters, between which there is a phenotypic similarity of about 72%. The first cluster includes genotypes 1R, 2R and 5A, which have a diversity of approximately 20%. Between the genotypes of the second cluster there is a phenotypic similarity of approximately 77%.

Referring to variance analysis for the seven carcasses studied at between regenerative genotypes: 2R-4R (92,10 %); 4R-5R (91,86%); 1R-2R (86,59%). A low level of similarity and high phenotypic diversity was found between the genotypes: 1R-5R (34,31 %); 1R-3R (21,93 %); 2R-3R (33,81 %).

regenerants and donor forms in 2014 (Table 3), it is noted that high and significant variance values were recorded for fruit / plant and fruit diameters respectively. The lowest variability among genotypes was observed for the fruit length and seed weight of the fruit.

Considering the data presented in the matrix of similarity in Table 4, it is noted that on the basis of the seven characters, the highest phenotypic similarity occurs

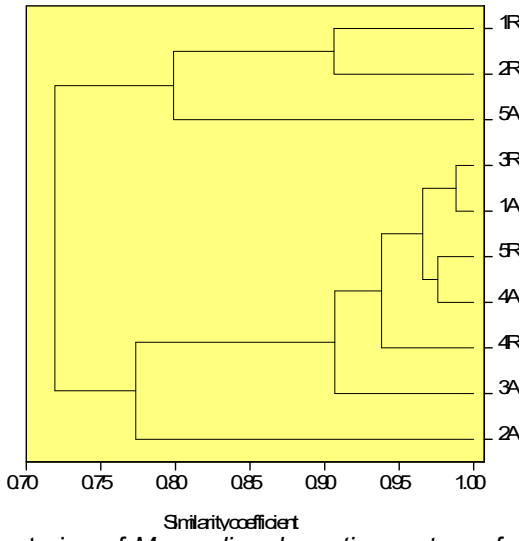

Fig. 1. UPGMA clustering of *Momordica charantia* genotypes for yield traits in 2014

Table 3

Variance analysis for the yield traits of Momordica charantia genotypes in 2014

No.	Traits	Between groups		Within groups		F Test
		SS	DF	SS	DF	
1	Fruit weight/pl.	6.45	1	2.55	8	20.18**
2	Fruit nr/pl	4.66	1	4.33	8	8.61*
3	Fruit weight	3.55	1	5.45	8	5.20*
4	Fruit lenght	0.26	1	8.73	8	0.24
5	Fruit diameter	6.54	1	2.55	8	20.51*
6	Seeds nr/fruit	3.22	1	5.82	8	4.42
7	Seeds weight/fruit	1.62	1	7.50	8	1.72

Table 4

The phenotypic similarities between Momordica charantia genotypes in 2015

Genotype	**1R**	**2R**	**3R**	**4R**	**5R**	**1A**	**2A**	**3A**	**4A**	**5A**
1R	1	0.8659	0.6807	0.8441	0.6569	0.8286	0.6618	0.7551	0.7105	0.7932
2R	0.8659	1	0.7619	0.9210	0.7995	0.7502	0.8463	0.6403	0.7615	0.5270
3R	0.6807	0.7619	1	0.8128	0.8484	0.7951	0.8903	0.6838	0.8233	0.4531
4R	0.8441	0.9210	0.8128	1	0.9186	0.8647	0.9120	0.7743	0.8975	0.5646
5R	0.6569	0.7995	0.8484	0.9186	1	0.8349	0.9709	0.7561	0.9302	0.4151
1A	0.8286	0.7502	0.7951	0.8647	0.8349	1	0.7594	0.9656	0.9518	0.8531
2A	0.6618	0.8463	0.8903	0.9120	0.9709	0.7594	1	0.6362	0.8567	0.3115
3A	0.7551	0.6403	0.6838	0.7743	0.7561	0.9656	0.6362	1	0.9273	0.8489
4A	0.7105	0.7615	0.8233	0.8975	0.9302	0.9518	0.8567	0.9273	1	0.6570
5A	0.7932	0.5270	0.4531	0.5646	0.4151	0.8531	0.3115	0.8489	0.6570	1

Compared to the donor form, the 5R genotype manifested the highest diversity (58.49%) followed by 3R with 31.62%. Genotype 4R recorded the highest similarity to the donor form (89.75%), along with the 2R genotype with 84.63%. The highest intergenotypic similarity was observed between donor forms 1A-3A (96.56%) and 1A-4A (95.18%), while between genotypes 2A and 5A there was a very high diversity of 68.85%.

Hierarchical classification of genotypes (Fig. 2) performed by cluster

average method based on phenotypic similarity groups the genotypes into two main clusters with an average diversity of about 29 %.

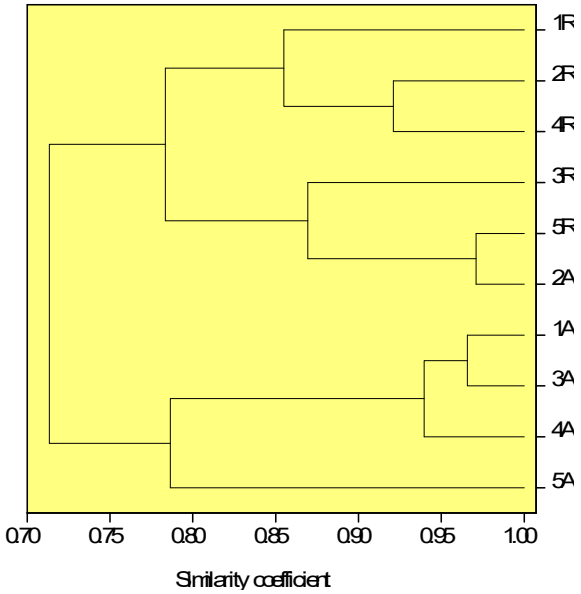

Fig. 2. UPGMA clustering of *Momordica charantia* genotypes for yield traits in 2015

The first cluster is composed of two subgroups with an average similarity of approximately 80%. One of these consists of the regenerants 1R, 2R, 4R, with an average phenotypic similarity of 86%. The second subgroup is represented by the regenerants 3R, 5R and the donor form 2A, between which there is a diversity of approximately 23%. The second cluster comprises the donor forms 1A, 3A, 4A and 5A with a similarity of approximately 78%.

Concerning variance analysis for production characters in 2015 (Table 5), it is noted that high and significant variance values were recorded especially in the fruit diameter followed by seed weight / fruit. The lowest variability among genotypes was observed for the average fruit weight and fruit production / plant.

Table 5

Variance analysis for the yield traits of Momordica charantia genotypes in 2015

No.	Traits	Between groups		Within groups		F Test
		SS	DF	SS	DF	
1	Fruit weight/pl.	1.96	1	7.04	8	2.22
2	Fruit nr/pl	2.27	1	6.75	8	2.69
3	Fruit weight	1.36	1	7.64	8	1.42
4	Fruit lenght	4.55	1	4.43	8	8.22*
5	Fruit diameter	6.92	1	2.19	8	25.29**
6	Seeds nr/fruit	3.46	1	5.58	8	4.96*
7	Seeds weight/fruit	6.12	1	2.97	8	16.50**

According to the matrix of similarity presented in Table 6, it is noted that the highest phenotypic diversity between the regenerants and donor forms in the seven-character aspect was recorded in genotype 5R (40.65%) and 2R (39.89%) respectively, in the 2015 year. For genotypes 3R and 4R, phenotypic diversity versus donor forms was considerably lower (6.89-8.35%).

Between donor forms the phenotypic diversity was between 49.19% for 2A-5A and 5.26% for 1-4 A. Concerning regenerants the highest intergenotic diversity was recorded between: 1R-5R (46,62 %); 2R-5R (25,53 %); 1R-3R (27,04 %). The lowest phenotypic diversity was recorded among regenerants: 3R-5R (4,24 %); 3R-4R (3,0 %); 4R-5R (6,72 %).

Table 6

The phenotypic similarities between Momordica charantia genotypes in 2016

Genotype	**1R**	**2R**	**3R**	**4R**	**5R**	**1A**	**2A**	**3A**	**4A**	**5A**
1R	1	0.9086	0.7296	0.8093	0.5338	0.7449	0.4555	0.7736	0.5892	0.9334
2R	0.9086	1	0.8592	0.9280	0.7447	0.8215	0.6011	0.8448	0.7572	0.7914
3R	0.7296	0.8592	1	0.9700	0.9476	0.9801	0.8690	0.9311	0.9696	0.7497
4R	0.8093	0.9280	0.9700	1	0.9328	0.9578	0.8093	0.9254	0.9165	0.7711
5R	0.5358	0.7447	0.9476	0.9328	1	0.9227	0.8399	0.8798	0.9614	0.5935
1A	0.7449	0.8215	0.9801	0.9578	0.9227	1	0.8812	0.9236	0.9474	0.7584
2A	0.4555	0.6011	0.8690	0.8093	0.8399	0.8812	1	0.7207	0.8529	0.5081
3A	0.7736	0.8448	0.9311	0.9254	0.8798	0.9236	0.7207	1	0.9384	0.8817
4A	0.5892	0.7572	0.9696	0.9165	0.9614	0.9474	0.8529	0.9384	1	0.6969
5A	0.8334	0.7914	0.7497	0.7711	0.5935	0.7584	0.5081	0.8817	0.6969	1

Based on the dendrogram of Fig. 3 it is noted that under the circumstances of 2016 year, genotypes are classified into two groups with a diversity of 27%. The first cluster is made up of genotypes 1R, 2R and 5A that exhibit a phenotypic differentiation of about 18%. In the second cluster there are two main subclusions with genotypes (3R, 1A, 4R, 5R, 4A) between which there is a phenotypic similarity of 94% and, at the same time, they present a diversity of about 10% relative to 3A and 4A.

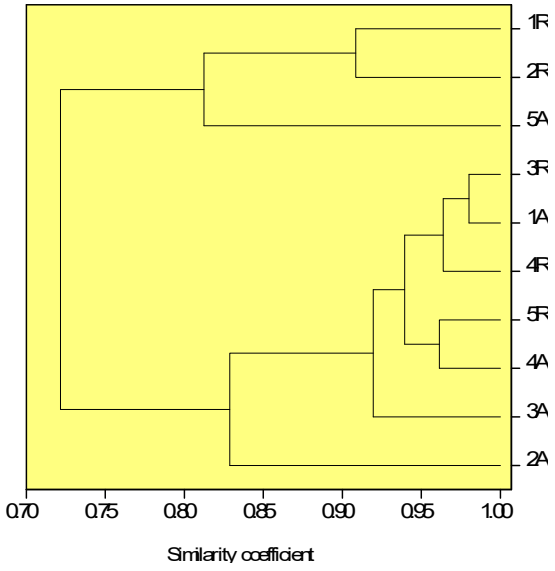

Fig. 3. UPGMA clustering of *Momordica charantia* genotypes for yield traits in 2016

Table 7

Variance analysis for the yield traits of *Momordica charantia* genotypes in 2016

No.	Traits	Between groups		Within groups		F Test
		SS	DF	SS	DF	
1	Fruit weight/pl.	6.90	1	1.42	7	33.92**
2	Fruit nr/pl	3.91	1	2.42	7	11.28*
3	Fruit weight	0.90	1	6.54	7	0.97
4	Fruit lenght	2.83	1	2.88	7	6.88*
5	Fruit diameter	3.09	1	3.38	7	6.41*
6	Seeds nr/fruit	1.17	1	4.84	7	1.69
7	Seeds weight/fruit	3.34	1	5.47	7	4.27

With regard to variance analysis for production characters this year (Table 7), it is noted that high and significant variance values were recorded especially in the case of production / plant, which generated the greatest differences between both clusters and genotypes of each cluster. The lowest variability among genotypes was observed for the average fruit weight and seed / fruit number.

Conclusions

Genotype 5R exhibited the highest overall superiority compared to donor form, recording significant increases for all seven characters, with values ranging from 15.4% for seed weight / fruit and 37.9% for fruit length, respectively an increase of 31.8% for fruit production / plant.

In the case of the 3R genotype, the highest increase in fruit weight and higher values than donor-form was demonstrated by 9.2-15.1% for most other characters.

The highest average phenotypic diversity between regenerants and seven-character donor forms was recorded in genotype 5R (48.17%) and 2R (29.89%), respectively. For genotypes 3R and 4R, phenotypic diversity versus donor forms was 9.11-15.68%.

For genotype 5R, the high diversity of donor form was associated with an increase in the value of the seven characters, whereas at 2R genotype the diversity was associated with an average decrease of the seven characters by about 13%.

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