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The evaluation of somatic variability in the callus of bitter melon (*Momordica charantia L.*) using molecular methods

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Abstract The cultivation methods for callus for a long period under the influence of phytohormones allow the selection of cell lines with somatic variation, which can be useful for the production of valuable metabolites. In the present research we studied based on molecular methods the somaclonal variability that is appearing after a long period of callus cultivation from bitter melon on MS medium added with different hormonal balances. Using the RAPD markers we established that some tissue lines present modifications at DNA level of 3 years callus cultivation with hormonal balances. In these conditions it is possible that the binding sites of the used primers can be affected.

Key words

Momordica charantia, callus, somatic variability

Momordica charantia L. from the family Cucurbitaceae, named bitter gourd or bitter melon, grows in tropical and subtropical regions from Africa, Asia, America, being used as food and natural medicine (Abascal and Yarnell, 2005). This specie contains a complex of beneficial compounds like: vitamins, minerals and antioxidants that can be used for treating a wide range of illnesses. His fruits are rich in the vitamins A, C, E, B1, B2, B3, B9 (Bakare and colab. 2010) and also potassium, calcium, zinc, phosphorus, iron. The medicinal value of this plant is given by its high antioxidant properties, conferred by phenols, flavanoids, isoflavones, terpenes, anthroquinones and glucosinolates (Snee and colab. 2011).

The main constituents of bitter melon that are responsible for the antidiabetic effects are: triterpenes (charantin), proteids, steroids, alkaloids, lipid and phenolic compounds (Saeed and colab. 2010; Budrat and colab. 2008).

It is known that the prolonged cultivation techniques of undifferentiated plant tissue on medium with hormonal balances lead to somatic variability due to the mutagenic effect of growth regulators (Badea, 2001).

The aim of the present research was to assess the somaclonal variability that is appearing after a long period of subcultivation of the callus from bitter melon on MS medium added with different hormonal balances using molecular methods.

Material and Methods

Bitter melon callus was cultivated on MS medium (Murashige and Skoog, 1962) supplemented with 11 variations of hormonal balances, representing combinations of auxins, cytokinins and gibberellins in different concentrations (Table 1). Callus subculture was performed every 3 months for 3 years.

Plant samples submitted to DNA extraction using the CTAB method (Doyle and Doyle 1987) in number of 13 were represented by 11 tissues lines (calluses) and two parent plants as a control, from which it was obtained and propagated the callus. Amplification of DNA samples was performed using 4 RAPD primers, with the following sequences:

- OPAA-18 5′TGGTCCAGCC3′
- OPW-13 5′CACAGCGACA3′
- OPA-02 5′TGCCGAGCTG3′
- OPX-01 5′CTGGGCACGA3′

Table 1

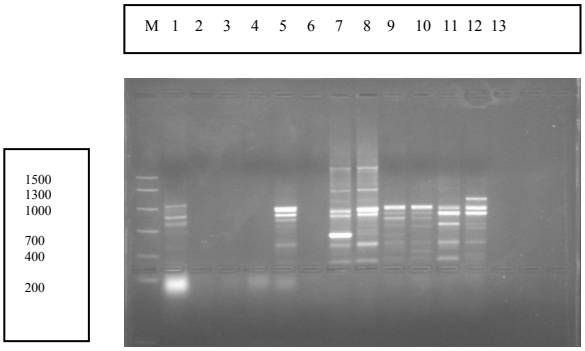
Hormonal balances

Hormonal balance	Growth regulators mg/l					
	ANA	BAP	2,4-D	KIN	AIA	GA3
M1				0,05		0,1
M2	2			0,01		
M3		1	1,5			
M4	1		1			
M5	1,5	1				
M6	1		1			
M7		5		0,5		
M8				0,01	1	
M9		0,5				
M10		2			1	
M11	0,5	0,5				

For polymerase chain reaction a Thermal Cycler Corbett was used and the reactions were submitted to the following PCR program: preliminary DNA denaturation for 5 min at 94°C, followed by 45 cycles consisting of denaturation (1 min 94°C), primer annealing (1 min, 36°C) and extension (2 min, 72°C). A final extension for 3 min at 72°C was included. The resulting amplicons were analyzed by 1.5% agarose gel electrophoresis.

Results and Discussions

Analyzing the electrophoretic it was pointed out that there are differences between DNA bands of different model tissue lines (callus) and between them and the control. The results are shown in the figures below.



Legend

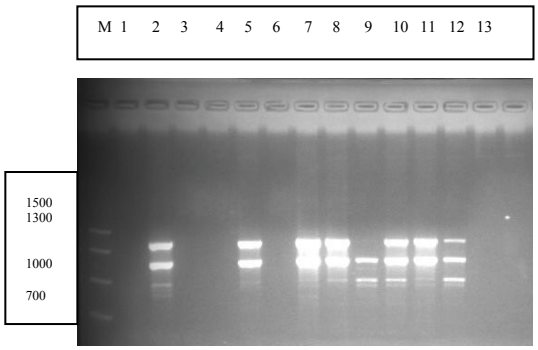
M – Marker
1. – Control
2. – Control
3. – Line 0,05 Kin + 0,1 GA3
4. – Line 2 ANA + 0,01 Kin
5. – Line 1 BAP + 1,5 2,4-D
6. – Line 1 ANA + 1 2,4-D
7. – Line 1.5 ANA + 1 BAP
8. – Line 1 ANA + 1,5 2,4-D
9. – Line 5 BAP + 0.5 Kin
10. – Line 0.,01 Kin + 1 AIA
11. – Line 0.5 BAP
12. – Line 2 BAP + 1 AIA
13. – Line 0,5 ANA + 0,5 BAP

Fig 1. Agarose gel electrophoresis of tissue lines DNA amplified with the primer OPAA-18

The results of amplification with the primer OPAA 18 (Figure 1) showed that from 13 samples the ones numbered with 2, 3, 4, 6 and 13 were not amplified. Some tissue lines (callus) multiplied *in vitro* for 3 years showed that the banding pattern is different from the control (sample 1). Thus, the 7th sample has an addition band that is stronger than the control with the corresponding DNA fragment length of 500 bp (base pairs). The samples numbered with 9, 10, 11 have a different pattern than the control strip, showing the

corresponding DNA band of the length less than 1000 bp. The absence of some bands in the tissue lines indicates that the DNA lost his binding sites for the RAPD marker.

It is obvious that the amplification with the primer OPAA 18 revealed a number of changes appeared on DNA samples extracted from callus.

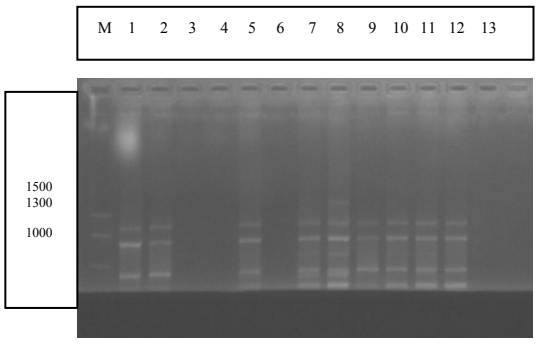


Legend

M – Marker
1. – Control
2. – Control
3. – Line 0,05 Kin + 0,1 GA3
4. – Line 2 ANA + 0,01 Kin
5. – Line 1 BAP + 1,5 2,4-D
6. – Line 1 ANA + 1 2,4-D
7. – Line 1.5 ANA + 1 BAP
8. – Line 1 ANA + 1,5 2,4-D
9. – Line 5 BAP + 0.5 Kin
10. – Line 0.,01 Kin + 1 AIA
11. – Line 0.5 BAP
12. – Line 2 BAP + 1 AIA
13. – Line 0,5 ANA + 0,5 BAP

Fig 2. Agarose gel electrophoresis of tissue lines DNA amplified with the primer OPW-13

In figure 2 it was observed that in the presence of the primer OPW 13 the sample number one representing the control and the tissue lines 3, 4, 6 and 13 didn`t amplified. Tissue line number 9 doesn`t have the band correspondent to the DNA fragment with the length of 1300 pb. The remaining tissue lines have the same pattern as the control, which indicates the absence of somatic variability.

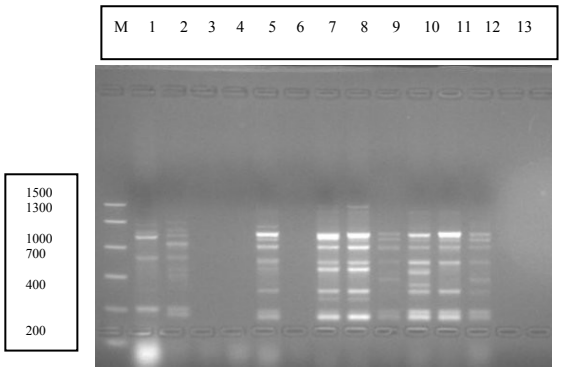


Legend

M – Marker
1. – Control
2. – Control
3. – Line 0,05 Kin + 0,1 GA3
4. – Line 2 ANA + 0,01 Kin
5. – Line 1 BAP + 1,5 2,4-D
6. – Line 1 ANA + 1 2,4-D
7. – Line 1.5 ANA + 1 BAP
8. – Line 1 ANA + 1,5 2,4-D
9. – Line 5 BAP + 0.5 Kin
10. – Line 0.,01 Kin + 1 AIA
11. – Line 0.5 BAP
12. – Line 2 BAP + 1 AIA
13. – Line 0,5 ANA + 0,5 BAP

Fig 3. Agarose gel electrophoresis of tissue lines DNA amplified with the primer OPA-02

The tissue lines numbered 3, 4, 6 and 13 shown in figure 3 have not amplified with the primer OPA-02. The other samples, exception sample number 9, have the same bands as the control. The tissue line number 9 is the only one that is presenting modification consisting in the absence of DNA fragment length of 700 bp.



Legend

M – Marker
1. – Control
2. – Control
3. – Line 0,05 Kin + 0,1 GA3
4. – Line 2 ANA + 0,01 Kin
5. – Line 1 BAP + 1,5 2,4-D
6. – Line 1 ANA + 1 2,4-D
7. – Line 1,5 ANA + 1 BAP
8. – Line 1 ANA + 1,5 2,4-D
9. – Line 5 BAP + 0.5 Kin
10. – Line 0.,01 Kin + 1 AIA
11. – Line 0.5 BAP
12. – Line 2 BAP + 1 AIA
13. – Line 0,5 ANA + 0,5 BAP

Fig 4. Agarose gel electrophoresis of tissue lines DNA amplified with the primer OPX-01

The strip of figure 4 showed the lack of amplification of DNA for the tissue lines number 3, 4, 6 and 13 when it was used the primer OPX-01. Tissue lines present the model pattern different from the control (sample 1), having an extra band corresponding to 1100 bp in length.

Thus, the tissue lines number 3, 4, 6 and 13 did not amplified with any of the primers used. We explain this by the absence of DNA binding sites of the primers used during this experiment.

Conclusions

Based on the experimental results obtained we can conclude:

1. The results of amplification with RAPD markers showed that the callus subcultivation for a prolonged time on a medium supplemented with hormonal balances: 1 BAP + 1,5 2,4-D; 1,5 ANA + 1 BAP; 1 ANA + 1,5 2,4-D; 5 BAP + 0.5 Kin; 0,01 Kin + 1 AIA; 0.5 BAP; 2 BAP + 1 AIA causes the appearance of some changes in the DNA molecules.
2. The tissue lines 3, 4, 6 and 13 lack the DNA binding sites for the RAPD primers, which entitles us to believe that hormonal balances (0,05 Kin + 0,1 GA3; 2 ANA + 0,01 Kin; 1 ANA + 1 2,4-D; 0,5 ANA + 0,5 BAP) produce variations that can affect the binding sites of the primers used in the amplification.

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