

# Research Journal of Pharmaceutical, Biological and Chemical Sciences

## ***Momordica Cochinchinensis* (Lour.) Spreng. (Cucurbitaceae) In Culture In Vitro.**

**Valeriy K. Tokhtar<sup>1\*</sup>, Zhang Doang<sup>2</sup>, Liudmila A. Tokhtar<sup>1</sup>, Oleg I. Korotkov<sup>3</sup>, and Galina I. Safronova<sup>4</sup>.**

<sup>1</sup>Belgorod State University, Pobedy St. 85, Russia, Belgorod, 308015.

<sup>2</sup>Hanoi University, Km 9 Nguyen Trai - Thanh Xuan, Hanoi, Vietnam

<sup>3</sup>Nikitskiy Botanical Garden, Nikita settlement, Republic of Crimea, Yalta, Russia 298648,

<sup>4</sup>Volgograd Regional Botanical Garden, Metallurgov settlement, 68, Volgograd, Russia 400007

### ABSTRACT

*M. cochinchinensis* (Lour.) Spreng. (Cucurbitaceae) belongs to a group of dioecious plants, forming the seeds with a very strong woody seed coat. Earlier experiments on reproduction of dioecious species *Momordica* by growing plants from seeds, collected in nature, had no results due to their low germinating capacity. To study the possibility of clonic micropropagation of *M. cochinchinensis* plants, the experiments were carried out by the introduction of this type in culture *in vitro*, and selecting the most appropriate methods of reproduction. It is found that explants are successfully sterilized in 7% solution of lysoformin during 10 minutes. The plants of *M. cochinchinensis*, set out after adaptation in the open ground, form well-developed spears that indicates about prospectivity of this plants reproduction in culture *in vitro*.

**Keywords:** *Momordica cochinchinensis*, the hardly rooted dioecious species, *in vitro* culture.

**\*Corresponding author**

## INTRODUCTION

The intensification of large-scale studies of the *Momordica L.* species, in recent years taking place all over the world, is primarily determined by the interest of international community to the search of new non-traditional food and medicinal plants. High content of useful elements in plants of the genus *Momordica* has been established long ago (Guichard & Bui, 1941). Since olden times, the local people in tropical regions of Southeast Asia gathered fresh spears, leaves and fruit of momordica, which were used as additional sources of vitamins and minerals (Upolf, 1968). Many species of *Momordica* genus are widely used in folk medicine in many tropical and subtropical countries (Anuradha & Prakash, 1989; Jaiswal et al, 1990).

At present, these non-traditional plants for growing in Europe conditions are considered as promising sources of raw materials for the food and pharmaceutical industries. Furthermore, almost all parts of plant are used: leaves, fruits, caules and seeds (Taylor, 2002). Severe hypoglycemic effect of momordica plants is due to their content of three groups of compounds known as harantin, insulin-like peptides and alkaloids. (Akhtar et al, 1981; Day et al, 1990). There is high content of fatty acids, vitamin E and carotenoids in the fruit pulp. Moreover, the substantial part of carotenoids compounds beta-carotene. (Vuong, King, 2003; Kuhnlein, 2004; Ishida et al, 2004). The content of lycopene in aril (arillus) of momordica comes to 308 mg/g, which is nearly 10 times higher than in other fruits, rich by lycopene content in fruits and vegetables (Aoki et al, 2002; Vuong et al, 2003).

In Vietnam, Thailand, Cambodia, India, Malaysia, China and the Philippines, *Momordica cochinchinensis* Spreng is especially popular, it is also known under the name Gac. This plant has rather large fruits, which until quite recently were grown only for the use in food. Just relatively recently in Vietnam it was started the production of valuable oil from the seeds of this plant, rich for  $\alpha$ -eleostearic acid.

## MATERIALS AND METHODS

*M. cochinchinensis* belongs to a group of dioecious plants, which form hard seeds with a very thick and very strong woody seed coat (Joseph, 2004). Earlier experiments on reproduction of dioecious species *Momordica* by growing plants from seeds, collected in nature, had no result due to their low germinating capacity (Watanabe, 1988).

To study the possibility of clonic micropropagation of *M. cochinchinensis* plants, the experiments were carried out by the introduction of this type in culture *in vitro*, and selecting the most appropriate methods of reproduction.

Two types of primary explants of *M. cochinchinensis* plants were used in the research: the seeds with dense seed coat (with damage of the coat in micropyle zone) and the seeds without seed coat. The sterilization of explants was performed as follows: the seeds, soaked in 96% ethanol solution, were burned in a flame of alcohol burner for not more than a minute, and then they were sterilized in lysoformin solutions of different concentrations: 10% (7 min.), 10% (10 min.), 7% (10 min.), 7% (15 min.). Six seeds were used for each variant of sterilization.

Explants were cultured on the breeding ground Murashige-Skooga (MS) without the addition of phytohormones at a temperature of 22-24° C, relative humidity not less than 70%, in the light with 16-hours photoperiod (Murashige, Scooge, 1962). The following breeding grounds were used: for the seeds with seed coat – the liquid medium without adding agar to the seed, for the seeds without seed coat – the solid medium with the addition of 6% agar. Preparation and sterilization of breeding grounds for the cultivation, was carried out according to the offered recommendations (Butenko, 1999).

## RESULTS AND DISCUSSION

According to the research results, for the explants without seed coat, as well as for the explants with damaged seed coat, the best method was the sterilization of seeds in 7% solution of lysoformin during 10 minutes (Table 1).

**Table 1: The results of sterilization of *Momordica cochinchinensis* Spreng explants.**

The way of sterilization	The result of sterilization	
	Affected by infections	Vitrification
The seeds without seed coat		
10%, 7 min.	2 explants	1 explant
10%, 10 min.	3 explants	3 explants
7%, 10 min.	1 explant	1 explant
7%, 15 min.	2 explants	2 explants
The seeds with damaged seed coat		
10%, 7 min.	2 explants	-"-
10%, 10 min.	1 explant	-"-
7%, 10 min.	0 explant	-"-
7%, 15 min.	1 explant	-"-

In explants which were sterilized in seven percent solution of lysoformin during 10 minutes, is observed only one affection by infection. In this way of sterilization, only one explant, having undergone the vitrification, was nonviable. Twelve days later, after setting on the breeding ground, there were the first changes in the structure of the cotyledons. During this period, it was observed the appearance of the growing-point. On the thirteenth day of cultivation, the cotyledons opened. In the area of emerging of the first roots and apex, some plants were marked by vitrification. On the fifteenth day of cultivation, some explants had fully open cotyledons. After sixteen days of cultivation, intensive root formation and increasing the size of the apex were observed. The first leaves appear on the twenty-first day. Appearance of the first microsperms was noticed in a week's time after the appearance of the leaves, although they were vitrified. At the same time, the root system was well developing. On the forty-seventh day of cultivation, the microsperms were already well developed. In some cases, they occupied all the available space in the cultivation area. The average length of plants spears was more than 10 cm. At this stage of development, the germinants were divided by one internode and were planted in test tubes on a nutrient medium WPM (Woody Plant medium) for woody plants. After one month of cultivation, the average length of plants-regenerators reached 12 cm, and the roots - 6 cm. At this stage, the plants were set out in the ground for adaptation to non-sterile environmental conditions.

Thus, the research, carried out by us for the first time, allows to grow plants *M. cochinchinensis* in conditions *in vitro*. It is found that explants are successfully sterilized for 10 minutes in 7% solution of lysoformin. After adaptation, the plants, set out in open ground, form well-developed spears that indicates about prospectivity of *M. cochinchinensis* plants reproduction in culture *in vitro*.

**REFERENCES**

[1] Akhtar, M.S., Athar, M.A. and Yaqub, M. 1981. *Effect of Momordica cizarantia on blood glucose level of normal and alloxan-diabetic rabbits* // Planta medica J. Med. Plant Rcs, 42: 205-212.

[2] Anuradha, V. and Jamuna Prakash. 1989. *Dietary fiber contents of selected vegetables and fruits* // Journal Of Food Science And Technology, 26 (6): 354-356.

[3] Aoki, H., Kieu, N.T., Kuze, N., Tomisaka, K., Van Chuyen N. 2002. *Carotenoid pigments in GAC fruit Momordica cochinchinensis SPRENG* // Bioscience. Biotechnology and Biochemistry, 66: 2479-2482.

[4] Butenko R.G. *Biology of higher plants cells in vitro and biotechnology based on them* // FBK-PRESS. 1999, 172p.

[5] Day, C., Cartwright, T., Provost, J., Bailey, C.J. 1990. *Hypoglycemic effect of Momordica charantia extracts* // Planta Med., 56 (5): 426-429.

[6] Gopalan, C., Rama, Sastri B.V., Balasubramanian, S. C. 1980. *Nutritive value of Indian foods* // National Institute of Nutrition, Indian Council of Medical Research, V. 2.: 204.

[7] Guichard, F., Bui, D.S.. 1941. *La matiere colorante du fruit du Momordica cochinchinnensis Spr.* // Annales de l'ecole Superieure de Medecine et de Pharmacie de l'Indochine, № 5: 41-42.

[8] Ishida, B.K., Turner, C., Chapman, M.H., McKeon T.A. 2004. *Fatty acid and carotenoid composition of gac (Momordica cochinchinensis Spreng) fruit.*// J. Agric. Food. Chem, 52 (2): 274-279.

- [9] Jaiswal, R.C., Sanjeev, Kumar, Manoj, Raghav, Singh, D.K. 1990. *Variation in quality traits of bittergourd (Momordica charantia L.) cultivars.* // *Vegetable Science*, 17 (2): 186-190.
- [10] Joseph, J. K. 2004. *Studies on ecogeography and genetic diversity of the genus Momordica L. in India.* // *Thrissur, Kerala*: 76-82.
- [11] Kuhnlein, H.V. *Karat, Pulque, and Gac: Three shining stars in the traditional food galaxy.* 2004. *Nutrition Reviews*, 62: 439-442.
- [12] Murashige, T., Scoog, F. 1962. *A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures.* // *Physiologia plantarum*, 15 (3): 473-497.
- [13] Taylor, L. 2002. *Technical Data Report for Bitter Melon (Momordica charantia).* *Herbal Secrets of the Rainforest.* 2nd edition. Sage Press, Austin: 123- 126.
- [14] Watanabe, H. K. Iseki and K., Kedo. 1988. *Studies on the cultivation of Momordica cochinchinensis. Relation of the morphologic variation on the seeds to germination and saponin content in roots.* *J. Takedo Res. Lab.*, 47: 69-76.
- [15] Uphof, J.C. 1968. *Dictionary of Economic Plants.*// Lubrecht & Cramer Ltd; 2 edition: 17-21.
- [16] Vuong, L.T., Dueker, S.R., Murphy, S.P. 2003. *Plasma beta-carotene and retinol concentrations of children increase after a 30-d supplementation with the fruit Momordica cochinchinensis (gac).*// *American Journal of Clinical Nutrition*, 75: 872-879.
- [17] Vuong, L.T., King, J.C. 2003. *A method of preserving and testing the acceptability of gac fruit oil, a good source of beta-carotene and essential fatty acids* // *Food and Nutrition Bulletin*, 24: 224-230.