

## **Inhibition of plant tumors growth with antitumor preparation amitozyn and its analog amitozynoberamid**

L.N. Yurkevich, Yu.A. Potopalskaya, Ye. M. Negrebetskaya

*The Institute of Molecular Biology and Genetics of NAS of Ukraine*

Representatives of different classes of animal organisms (nematodes, insects), primitive fungi, as well as bacteria and viruses effect plants in various ways, disrupting and changing the development of cells, tissues and organs, thus leading to tumor formation. Among the factors that cause genetic formation of tumors on plants we distinguish mutations of plant genes or the presence of oncogene sequences of agro bacteria in plant genome in the process of bacterial gene transfer in evolution [1].

Plant tumors, accompanied by tissue proliferation, are frequently occurred in nature. Among them of special interest are tumors, produced by phyto pathogenic bacterium *Agrobacterium tumefaciens*, which has Ti-plasmid, responsible for “coronated gal” or bacterial cancer. Plasmid initiates the process, but further it can proceed in its absence [1-2].

Plant tumors of this line have the following general properties peculiar to malignant neoplasms of animals and humans: autonomous non-regulated growth, transplantability and aggressiveness. These tumors are able to grow in culture medium in the absence of growth phytohormones, necessary for growing normal issues. Therefore, they are used in experiments, aimed at defining certain fundamental biological concepts that provide the understanding of tumorous processes in general, as well as for studying antitumor action of some preparations [2-4].

We have performed studies on plant tumors, induced with *Agrobacterium tumefaciens*, with the aim of defining antitumor action of preparations amitozyn and amitozynoberamid offered for clinical test [6].

### **Materials and methods**

The study was performed on the tumor model of *Kalanchoe diademata* and tomato line “Ukrainian salt-tolerant”, induced with high-virulent strain *Agrobacterium tumefaciens* 8628. For experiments we took samples of *Kalanchoe diademata* and tomato plants of the same age, height and thickness of stem. *Agrobacterium* was infected into a leaf blade or by decapitation method. For this purpose *Kalanchoe* plants with 5-7 leaves were decapitated with scalpel. After 15-30 min., 48 hours’ suspension of agrobacteria was applied onto the section surface at  $1 \times 10^8$  cells dose.

The plants of “Ukrainian salt-tolerant” tomato line were inoculated with agrobacteria at fruiting and the beginning of ripening stage. The plants were kept at 25-28 C throughout the experimental period.

The changes in “coronated gal” tumors weight and the determination of antitumor activities of amitozyn and amitozynoberamid were studied on the tumors of *Kalanchoe* plant. For control the tumors were treated with water. The size of tumors was measured every 5-6 days. Their volume was calculated by the formula:

$$p = \frac{a \cdot b \cdot c}{4}$$

where a- width, b-length, c- height of a tumor.

Since the relative weight of tumor issue approximates unit, a tumor weight quantitatively equals its volume.

In order to define the inhibitory action of antitumor preparations on plant tumors, their solutions at 0,1 mkg/ml were applied as applications at 1-2 ml, depending on a tumor size, onto the tumors, beginning with day 15 after agrobacteria inoculation.

**Results.** According to the literature, first symptoms of tumors on plants at induced infection became evident on day 10-30 as small swellings and humps of white or light-green color on a stem or leaf. [2]. Similar phenomenon was observed in our experiments. According to

our data, the kinetic curve of tumor growth on a Kalanchoe stem is of S-like shape. (Fig.1). Kinetic measurements show that the rate of tumor development of Kalanchoe, induced with agrobacterium, first increases, and after 30 days it comes to a halt, probably due to some limiting factors. This evidences that tumors in plants develop according to the same kinetic laws as tumors in animals and humans [2,5,7].

After 3-time treatment of tumors in tomatoes with amitozyn we first observed the necrosis of tumor surface cells. By day 20 after the treatment dark spots became apparent on tomato tumors (necrosis), and by day 60 the tumor necrotized completely. After the tumor necrosis the tomatoes grew normally and bore fruit.

The necrosis of surface cells of tumors in Kalanchoe became apparent after 4-time treatment, and by day 70 a necrotic spot appeared.

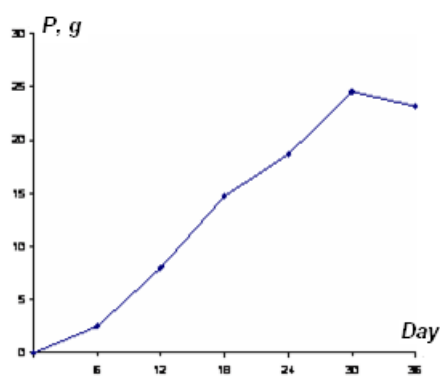
We observed no complete necrosis on secondary tumors of the plants that were not treated with the preparation, but their growth rate slowed down and a slight necrosis of surface cells appeared.

Studying the inhibitory action of amitozyn we found that tumor regression was observed starting from day 6 after the treatment with preparation. Tumor mean weight decreased from  $22,0 \pm 6,35g$  to  $13,11 \pm 4,91g$  during the period of amitozyn action (29 days), and after the same period of amitozynoberamid action on tumors in Kalanchoe, it reduced from  $32,05 \pm 6,12g$  to  $22,4 \pm 4,91g$ . It is seen from Fig. 2 that the kinetic curve representing changes in tumors weight treated with amitozyn is similar to that of amitozynoberamid. In control (water) we first observed a slight increase in tumor weight, which got stabilized after 3-time treatment. (Figure 2).

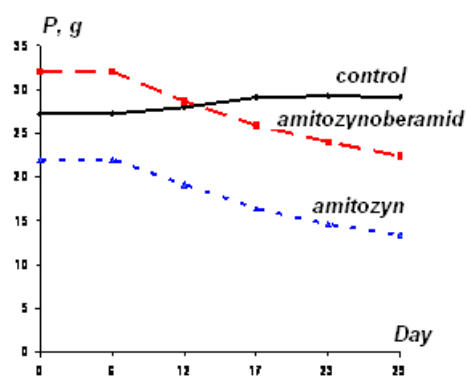
**Conclusion.** In experimental study on antitumor action of amitozyn and amitozynoberamid on Kalanchoe plant model we determined that both preparations at concentration 0,1mg/ml (during 29 days) inhibit a tumor growth, induced with *Agrobacterium tumefaciens*, with no influence upon a plant growth.

Amitozyn at concentration 0,1mg/ml on tomato tumors was found to result in a complete necrosis of “coronated gal” tumors during 60 days.

The data obtained support the advisability of pursuing research on the use of these preparations in industrial scale.



**Fig.1** Kinetic curve of the weight growth of tumors induced with *Agrobacterium tumefaciens* 8628 on *Kalanchoe daigremontiana* plants



**Fig.2.** Kinetic curves of the changes in tumors weight infected with *A. tumefaciens* 8628 under the effect of antitumor preparations

## References

1. A.S. Piruzyan, V.M. Andrianov. *Agrobacteria plasmids and genetic engineering of plants*. Moscow. “Nauka” Publ. -1985. –p.251
2. A.I. Potopalsky, Z. Yu. Tkachuk. *Plant tumors*. Kyiv, “Vishcha shkola”.-1985, p.184.

3. N.N. Zoz, A.M. Serebryany, P.V. Kotenkov. Inhibition of plants tumor growth with N-nitrose-N-menthyl urea. //Reports of Academy of sciences of USSR- 1944p.233, №1, p. 242-244.
4. A.M. Serebryany, N.N. Zoz, N.A. Semenova, I.s.Morozova, P.V. Koshenkov, M.M. Babaev. Antitumor activity of phosphoric acid derivatives// Izvestiya Academy of sciences of USSR, boil. -1979, №4, p. 610-611.
5. V.A.Zinchenko. Regularities and mechanism of formation and overcoming of tumor cells radio resistance. Abstr. of Doctor's degree thesis.- Kyiv, 1999, p.35.
6. A.I. Potopalsky Amitozyn as a promising preparation of wide spectrum of action for diagnostics, treatment and prophylaxis of neoplasms//III Congress of oncologists of Commonwealth of Independent States, May, 26. 2004. p. 415.
7. N.M. Emmanuel. Kinetics of experimental tumor processes. Moscow. "Nauka" Publ. 1977. p. 409.
8. Inventor certificate №1050151 T Combating "coronated gal" tumors of vine/ A.I. Potopalsky, Z.Yu. Tkachuk, N.B. Lemanova.22.06.83.