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TRIAMELON — A NEW EFFECTIVE INDUCTOR OF ORGANOGENESIS IN PLANT TISSUE CULTURE IN VITRO

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It is set the first, that chemical compound Triamelon (Iodide tris (2,2-trimethylammoniummethyl phosphate)) showing before high growth regulatory activity on melons cultures can be used as an effective inductor of organogenesis in plant tissue culture of Solanaceae family in vitro.

ТРИАМЕЛОН — НОВИЙ ЕФЕКТИВНИЙ ІНДУКТОР ОРГАНОГЕНЕЗУ У КУЛЬТУРАХ ТКАНИН РОСЛИН IN VITRO
Л.О.Галкіна, В.А.Циганкова, А.Д.Синиця
Вперше встановлено, що хімічна сполука триамелон (йодид трис (2,2-триметиламоній-метилфосфат)), яка раніше показала високу рістрегулюючу активність на баштанних культурах, може використовуватись як ефективний індуктор органогенезу у культурах тканин рослин сімейства пасльонових in vitro.

ТРИАМЕЛОН — НОВЫЙ ЭФФЕКТИВНЫЙ ИНДУКТОР ОРГАНОГЕНЕЗА В КУЛЬТУРАХ ТКАНЕЙ РАСТЕНИЙ IN VITRO
Л.А.Галкина, В.А.Цыганкова, А.Д.Синица
Впервые установлено, что химическое соединение триамелон (йодид трис (2,2-триметиламмонийметилфосфат)), показавшее ранее высокую рострегулирующую активность на бахчевых культурах, может быть использовано как эффективный индуктор органогенеза в культуре тканей растений семейства пасленовых in vitro.

The most important problem of cell biology and biotechnology is the substitution of expensive natural plant growth regulators (phytohormones) by synthetic compounds [1-6]. The world-wide experiments in substitution of natural phytohormones with artificial preparations (2,4-D, NAA, NOAA, BAP) confirm a perspective this approach.

At the long time in the Institute of Organic Chemistry and Institute of Bioorganic and Petroleum Chemistry of National Academy of Sciences of Ukraine the synthesis and screening of plant growth regulators are the significant direction of scientific investigations.

For the biological screening of substances with growth-regulative activity synthesizing in these Institutes we proposed to use the cultures of isolated plant tissues of family Solanaceae as test-system for which conditions of induction callus tissue formation in vitro from differentiated cells, organogenesis and regeneration of plants from cells of callus tissues were determined in detail (i.e. compositions of nutrient media and which phytohormones or its combination produce each of these processes) [7-9].

Results of experiments. The influence of Triamelon concentrations (0,1-20 mg/l) on the induction of cell division (this test can be considered as specific one for cytokinin activity [3, 7]) was investigated. With this aim indicated quantities of Triamelon instead of kinetin were added to Linsmaier and Skoog medium (LS_1) and callus culture of core parenchyma of tobacco stem was cultivated. The same media without kinetin and minimum enriched with kinetin (LS_2) served as control samples. The index efficiency of plant growth regulators was estimated by increase of callus biomass counted for dry and humid mass. Results of experiment testify that Triamelon evinces the cytokinin activity at concentration 2 mg/l in the best way and under this conditions it is more effective than kinetin on 23%.

In test experiments with mezophyll protoplasts of tobacco the possibility to use Triamelon (at concentration 1-5 mg/l) instead of kinetin on both W-5 medium (ussually applied for formation of cells' colonies) and MS medium (for induction of callus tissues formation and producing of plants-regenerants) was also demonstrated.

The possibility to substitute of cytokinins by Triamelon was shown on 3 different media, that usually used for the formation and support of tobacco callus in regime of long term passing:

1) $RMKU_1$, where 0,1 mg/l of Triamelon mixed with 1 mg/l of NAA;

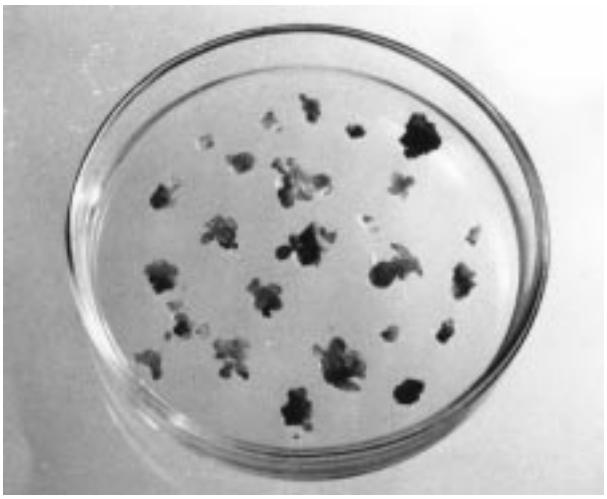

Figure 1. The stem bud formation and the appearance of leaves on the RMO_1 medium containing 1 mg/l of Triamelon and 2 mg/l of NAA.

2) RMP_1, where 0,1 mg/l of Triamelon mixed with 0,1 mg/l of 2,4-D;

3) $RMNO_1$, where 0,05 mg/l of Triamelon mixed with 3,0 mg/l of IAA and with 0,1 mg/l 2,4-D.

4) $RMNO_2$, where 0,05 mg/l of Triamelon mixed with 3,0 mg/l of NAA and with 0,1 mg/l 2,4-D.

At all combinations the growth and support of callus were observed. There has not been callus growth inhibition.

A next stage of researches was to test the action of synthetic compounds in the media using for induction of organogenesis. The root formation in cultured tobacco callus and at following passing — the formation of stem buds and then leaflets were observed on RMO_1 medium containing a mixture of 1 mg/l Triamelon and 2 mg/l NAA (figure 1).

The formation of buds was found on the $RMOP_1$ medium with 1,5 mg/l Triamelon and 0,1 mg/l NAA. When we used $RMKU_2$ medium with 2,5 mg/l Triamelon and 1 mg/l NAA plants-regenerants grew. Also it should be noticed that under the influence of light callus became green because indicated concentration of Triamelon induced the synthesis of chlorophyll and formation of chloroplasts.

The somatic embryogenesis on $RMKU_3$ medium at high concentrations (10-20 mg/l) of Triamelon and

Figure 2. Plant growth on the $RMOP_2$ medium containing 5 mg/l of Triamelon and 0,1 mg/l of NAA.

Figure 3. The regeneration of whole tobacco plant on the RMB_1 medium containing 5 mg/l of Triamelon

on RMP_2 medium at certain combination Triamelon with artificial auxin NAA was induced.

The good result for active growth of embryos and connecting with it further elongation of stem (i.e. transition from meristematic tissue to phase of active growth [7-14]) have been received at 2-4 consistent passages of embryogenic callus on the $RMOP_2$ medium, containing high concentrations (2,0-5,0 mg/l) of Triamelon. Then callus tissue was placed in the medium P without hormones (figure 2).

The appearance of plant-regenerant was observed on RMB_1 medium containing no auxin, but enriched with 5 mg/l Triamelon instead of 0,1-1,5 mg/l cytokinin BAP (figure 3). This test-tube plant-regenerant with the aim of root formation was placed in RM_{min1} medium (without phytohormones) or containing 0,01 mg/l Triamelon instead auxin NAA.

Screening of induction of callus formation and callus passing of wild species of tomato have been made on BK medium. In this medium to two auxins — NAA and 2,4-D we added 0,5 mg/l Triamelon instead of kinetin. The same procedure was investigated on RMOP medium containing 1 mg/l of Triamelon. To support the callus of cultural species of tomato we used RMKU medium with 0,2 mg/l Triamelon.

As a result of subsequent microclonal reproduction by shoots, the plants- regenerants grown in presence Triamelon possess more powerful development of vegetative and generative organs than the plants grown in presence cytokinin — kinetin. For example, biomass of elite sorts of tobacco is multiplied in 2-3 times; the width of leaf plate increases in 1,5 times and the distance between interbundles becomes short in 1,5 times; the content of nicotine in tobacco leafs increases on 10%; the plants grown using Triamelon are more steady to pathogens and unfavorable environmental factors.

Plant material. The plants of tobacco (Nicotiana tabacum, varieties R-1; SR-2, visconsinia), were used in test in vitro.

Before introducing in the culture the plant tissues (leaf or stem tissues) were sterilized with diocid (or hypochloride) and 70% ethanol and then washed with sterile distilled water.

Cultural media for plant tissues. Modified by us different nutrient media (RMKU, RMP, RMOP, RMO, RMNO) on the basis of elaborated by Murashige and Skoog (MS) medium, as well as Gamborg et al. (B_5) medium and Linsmaier and Skoog (LS) medium were used*. Names and compositions of the media were taken from ref. [9]. To prepare the nutrient media the initial concentrated solutions were used:

1) 10 times solution of macrosalts; 2) 100 times solution of microsalts (besides salt solutions of $CoCl_2$ x $6H_2O$ and $CuSO_4$ x $5H_2O$ were prepared separately by a consistent dissolving of 25 mg of each salt in 10 ml of water. Then they were united with solution of other microsalts. General volume was increased to 100 ml); 3) an initial solution of Fe-helat was prepared by a consistent dissolving 7,45 g of Na_2EDTA and 5,57 g of $FeSO_4$ x $7H_2O$ in water (the final volume was led to 1 l and heated to 100°C). To prepare 1 l of an agar nutrient medium we mixed 100 ml of the initial solution of macrosalts, 10 ml of microsalts, 5 ml of Fe-helat and corresponding quantities of agar, vitamins, hormones, hydrocarbons, etc.

Vitamin solutions were prepared just before experiments. Auxins were dissolved initially in a small quantity of ethanol and brought to the necessary volume with water under heating; cytokinin solutions were prepared by a dissolving of a corresponding cytokinin in a small volume of 0,5N HCl under heating with the following mixing with necessary amount of water.

Agar nutrient media (6-8 g of agar / l) brought to certain pH were autoclaved.

Isolation and cultivation of mesophyll protoplasts from leaf discs of tobacco were realised by methods in detail described in protocol part of the book [9].

Summary

In our experiments we showed that Triamelon can be used as substitute of phytohormones auxins and cytokinins (such as kinetin and BAP), and can be combined both with natural auxin IAA (indole-3-acetic acid) and with synthetic analogues 2,4-D and NAA (naphthaleneacetic acid) in the media used for cultivation of callus and for induction of plants shoots from undifferentiated callus cells and in the media used for active growth of embryos and further development plants-regenerants from embryogenic tissue in vitro.The data of this work also confirm that Triamelon can be used for selection of elite sorts of tobacco and tomato plants with the improved economic qualities, including for the receipt of virusless material.

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* Modified by us nutrient media designated through the text by figures index.