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RESEARCH ARTICLE

In Vitro Biological Activity of Acetone, Antioxidants, And Establishing of Its Similarity with Phytohormones, Using QSAR Method

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Abstract

Studies have shown the prospect of using acetone in the cultivation of explants of different origins. At the same time, it is important to determine the optimum concentrations of this solvent and the specific selection of plant objects.

It is perspective to use Cs Chem office programs to optimize nutrient medium in order to establish synergism and antagonism of chemical compounds characterized by biological activity.

Keywords: Acetone; Phytohormones; *In Vitro* Technology; Pattypan Squash; Tomato; Cucumber; Epidermis; Explants; QSAR (Quantitative Structure-Activity Relationship) Program

Introduction

Finding new ways to enhance the regenerative capacity of plant organisms is an urgent problem in plant biotechnology. These ways include: optimization of nutrient medium, search and use of biologically active substances (vitamins, amino acids, antioxidants, etc.) as growth promoters not previously used in *in vitro* technology, which contributes to the possibility of studying the laws of heredity and variability in vegetative propagation, physiological and biochemical formation of plant organisms.

Growth regulators allow enhancing or attenuating the expression of plant traits and properties within their genotype response rate. Therefore, nutrient medium optimization plays an important role in enhancing the regeneration process *in vitro*.

From 1986 to 2002, the Cs Chem office program was developed by Cambridge Soft Corporation; it includes such routines as the CS Chem Draw graphic editor, which displays chemical compound formulas, and the CS Chem 3D editor, which creates a three-dimensional molecule image [1,2].

The program can simulate the behavior of molecules with changes in temperature, pressure; it contains methods of molecular mechanics as well as semi-empirical methods of quantum mechanics. With these methods it is possible to optimize the conformation of the molecules, to determine the heat of formation of the molecule and the electron energy, to find the configurations of the molecular orbitals (higher-occupied, lower-free) and their energy.

Computer programs have previously been used in pharmacology and medicine to find the connection of a chemical structure with the biological activity of substances (bond, structure, activity) in order to find the most effective compounds for the development of new drugs [3,4].

The rapid development of quantum-chemical studies of biologically active substances has allowed to distinguish in science an independent field called "quantum pharmacology".

Quantum chemistry-based approaches are the bridge that combines empirical studies of the relationship of activity structure with the theoretical understanding of the mechanism of biological action of chemical compounds [5].

Describing the structure is possible by using local characteristics (the presence of a certain type of functional groups, setting the value of constants of substituents) as well as integral, general properties of substances. The characteristics can be experimental or found during calculations with varying degrees of accuracy.

Biotechnological studies have not studied the interaction of biologically active substances in nutrient medium. And solving the problem of mass screening is possible only by putting (before the traditional tests) a system of high-performance tests that allow to predict the biological effect of various compounds and optimally plan their further use in the system *in vitro*.

The biological activity of acetone is thoroughly presented in [6-9], where acetone is shown as a biologically active substance that can be used in nutrient medium as a plant growth promoter.

It is known that enriched with carbon dioxide air was used to increase crop yields. In this case, the plants increased resistance to pests and diseases, and in the leaves the content of chlorophyll went up. This is due to the fact that the usual carbon dioxide content in the air (0.03%) is not optimal, and therefore its increase in the atmosphere leads to an increase in photosynthesis energy and has a positive effect on the ontogeny of plants. But when its maximum permissible concentration is reached, and due to the closure of stomata, the metabolism of plants can slow down.

The dependence of ontogeny of seedlings and explants on the presence of a stable composition of additional compounds in the nutrient medium has been proved.

The mechanism of acetone activity, in our opinion and other authors, is associated with the fact that it dissolves the cell wall of explants, promoting the release of endogenous hormonal substances in the nutrient medium and sterile CO_2 [10].

Acetone can also be used in genetic engineering as an explant growth promoter and as an antiseptic capable of suppressing infection.

But acetone is also a toxicant, because, by increasing its concentration in the nutrient medium, it inhibits the ontogeny of plants, which is associated with the activation of stomata gaps and closing of stomata.

The biggest impact of toxicants falls on the process of photosynthesis and is observed on inhibited biometric parameters of plants [11].

Therefore, the purpose of our studies is to study the effect of different concentrations of acetone on the ontogeny of explants of pattypan squash of the Cucurbitaciae genus: Cucurbita pepo L "Orange", Cucurbita cucumber and Lycopersicon esculentum tomato "Novachok"; to study the process of regeneration from explants in the presence and the absence of the epidermis; to establish the similarity of the chemical compounds of cytokinin, kinetin and synthetic antioxidants ionol and kinetin by the QSAR method.

Previously, we have performed calculations of the selected parameters of the electronic structure of molecules for acetone and phytohormones (ethylene, 6-benzylaminopurine, indolylacetic, naphthylacetic, gibberellic, abscisic acids); optimized configuration of the molecule to search for a specific biological activity descriptor; the characteristics of the descriptors were determined and the hypothesis for acetone dissolution of cell wall and release of endogenous phytohormones into the nutrient medium *in vitro* was tested.

Materials and Methodology

For the experiment we selected 30 seeds of Cucurbita pepo L pattypan squash "Orange" for each variant. The experiment was performed in 3 replicates. The seeds were pretreated with a 20% $KMnO_4$ solution (2 min.) and $CaOCl_2$ solution (15 min.). The plants were grown on the Knop's nutrient medium with the addition of acetone at the rate of 5, 10, 15, 20, 25, 40, 60, 80, 100 and 110 ml per 1 liter of solution. The seedlings were cultured at 16-hour photoperiod at 24 °C.

The obtained data showed that when studying the effect of acetone on the length and mass of the sprout, the concentration of 5-25 ml/l practically did not affect the studied parameters, and the maximum manifestation of the length of the sprout was noted at the concentration of acetone of 60 ml/l. (Figure 1) .

According to the indicators of cotyledon mass and sprout at a concentration of 40 ml/l, the highest rates of the absolute values were observed (Figure 2).

At a concentration of acetone in an amount of 40-80 ml/l, the dry weight indicator of the sprout of pattypan squash was the highest.

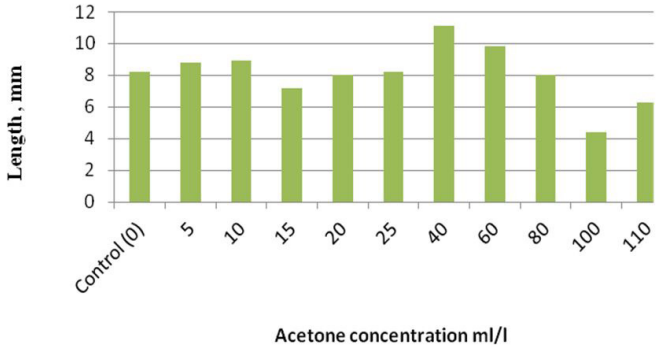

Figure 1: Dependence of sprout length (mm) of pattypan squash "Orange" on acetone content (ml/l)

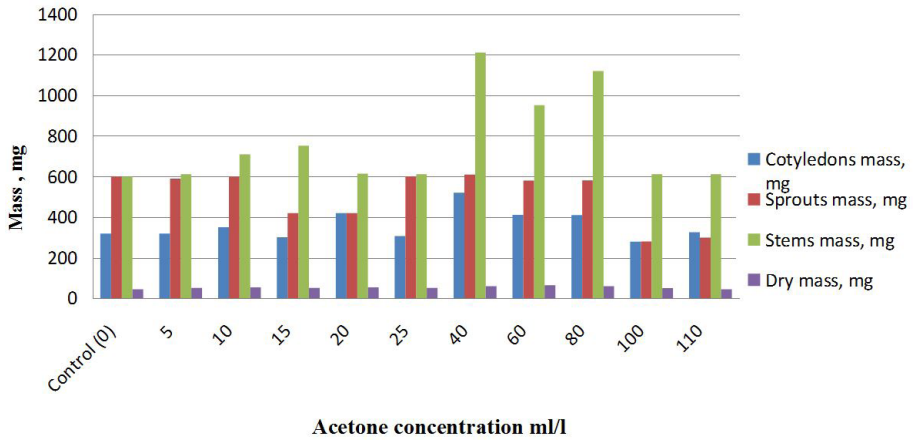

Figure 2: Influence of acetone on the mass of cotyledons, sprouts, stem, dry mass of pattypan squash "Orange"

Thus, the results of the studies suggest that acetone, depending on the concentration, stimulated or inhibited the manifestations of the studied parameters in the pattypan squash seedlings. The optimal concentrations that stimulated the growth and development of seedlings were of 40-80 ml/l of acetone, depending on the studied parameter. The area of the cotyledons practically did not respond to changes in the concentration of acetone.

One of the hypotheses that explains the activity of acetone is its ability to dissolve the epidermal membrane and the release of natural endogenous phytohormones into the nutrient medium. In this regard, we conducted an experiment on the removal of the epidermis from tomato and cucumber explants in order to compare the process of explant regeneration on a different nutrient medium *in vitro* (Table 1).

Crops	Variant	Nutrient medium
Tomato	1	MS + 1mg/l Indoleacetic acid (IAA) + 2mg/l 6-benzylaminopurine
	2	MS + 2mg/l IAA + 4mg/l 6-benzylaminopurine
	3	MS + 3mg/l IAA + 6mg/l 6-benzylaminopurine
Cucumber	1	MS + 1mg/l IAA + 0,1mg/l 6-benzylaminopurine
	2	MS + 2mg/l IAA + 0,2mg/l 6-benzylaminopurine
	3	MS + 3mg/l IAA + 0,3mg/l 6-benzylaminopurine

* (MS) – Murashige and Skoog medium

Table 1: Characteristics of nutrient medium with phytohormones

Previous studies have shown that acetone has the greatest influence on the initial stages of ontogenesis. Adult, formed plants are not sensitive to non-toxic concentrations of acetone. The curve constructed according to the experimental data, as a rule, passes through the maximum values of the biometric indicator and gradually passes into the toxic zone (the values are due to the toxic effect on the plant organism). Therefore, we attempted to study the regenerative potential of explants in the absence of the epidermis.

This is explained by the fact that the epidermis contains a highly developed agranular endoplasmic reticulum. As is known, it is the most characteristic sign of the structure of terpinoidogenic cells, which synthesize secretory terpinoids, which are significantly different from each other. Thus, agranular endoplasmic reticulum in plants can take part in the biosynthesis of certain terpenoids of hormonal nature – gibberellins, fusicoccins, etc.

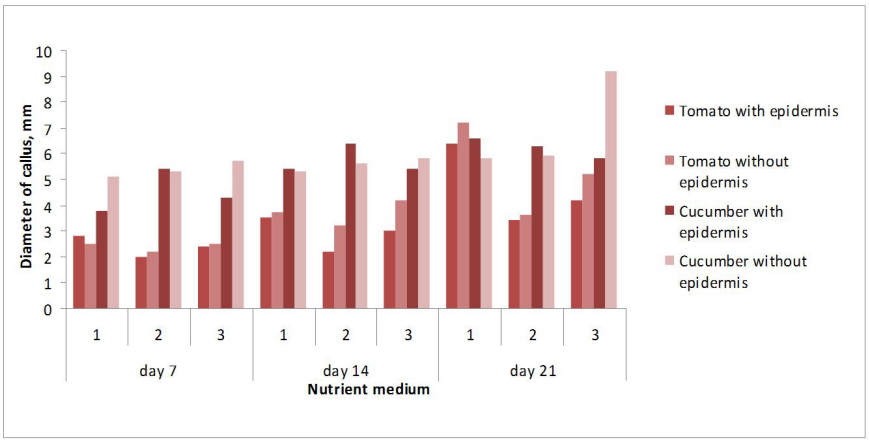

Figure 3: Influence of different concentrations of phytohormones on callus diameter of tomato and cucumber

Studies have shown that the absence of the epidermis affects the features of in vitro explant growth processes (Figure 3).

It was found that the size of the callus largely depended on the absence of the epidermis and the concentration of phytohormones in both tomato and cucumber. The callus diameter in tomatoes was higher in explants of the hypocotyl and cotyledons without epidermis. According to our studies, the factor of epidermal presence in explants did not affect risogenesis and sprout formation.

The conducted studies indicate the features of regeneration of explants in tissue culture *in vitro* in the absence of the epidermis and indicate the possibility of its future use in genetic engineering to increase the yield of transformants.

With the help of QSAR computer programs, we have also carried out a research to study the similarity of the chemical structure of substances such as phytohormone cytokinin kinetin (6- furfurilaminopurin), synthetic antioxidants ionol (3,5-dibutyl-4-hydroxytoluene) C15 H24 O and fenoxane (C17H25 03 K).

Summary of PM3 Calculation
MOPAC 97 00
C10 H9 O (**kinetin**)
Geometry Optimised Using Eigenvector Following (EF).
SCF field was achieved
Heat of formation =56.630861 kcal=236.94352 KJ
Electronic energy = - 14659.370603 EV
Core-Core Repulsion = 12232.933610 EV
Dipole = 2.41533 Debye Symmetry: C1
No. of filled levels = 40
Ionization potential = 8.692027 EV
Homo lumo energies (EV) = $-8.692^{-0.445}$
Molecular weight = 215.214
SCF Calculation = 63
Computation time = 1 Minute and 52.434 Seconds

Summary of PM3 Calculation
MOPAC 97 00
C15 H24 O (**ionol)**
Geometry Optimised Using Eigenvector Following (EF).
SCF field was achieved
Heat of formation =-77.736196 kcal=-235.24824 KJ
Electronic energy = - 17150.805281 ev
Core-core repulsion = 14707.918957 ev
Dipole = 1.31867 debye symmetry: c1
No. Of filled levels = 45
Ionization potential = 8.794623 ev
Homo lumo energies (ev) = $-8.795^{0.317}$
Molecular weight = 220.354
Scf calculation = 48
Computation time = 2 minutes and 22.039 seconds

Summary of PM3 Calculation
MOPAC 97 00
C17 H25 O3 K (**fenoxane**)
Geometry optimised using eigenvector following (ef).
SCF field was achieved
Heat of formation =-0.026391 kcal=-0.11042 kJ
Electronic energy = - 25829.223760 ev state: doublet a
Core-core repulsion = 22552.315035 ev
Dipole = 9.23868 debye symmetry: c1
No. Of filled levels = 55
And no of open levels=1
Charge on system = 1
Ionization potential = 12.835883 ev
Homo (somo) lumo energies = -12.096(-10.266)
Molecular weight=316.481
SCF calculation = 8
Computation time = 41 minutes and 18.730 seconds

We have conducted a study of the manifestation of biometric parameters of tomato "Novachok" on different variants of the nutrient medium. The basic medium was Murashige and Skoog (MS). There were 4 variants formed: 1- control (MS); 2 - MS + kinetin (1.39 * 10^{-5}M; 3 - MS + ionol (5.9 * 10^{-6}M); 4 – MS + kinetin (1.39 * 10^{-5}M) + ionol (5.9 * 10^{-6}M). The duration of cultivation lasted for 8 weeks (Figure 4).

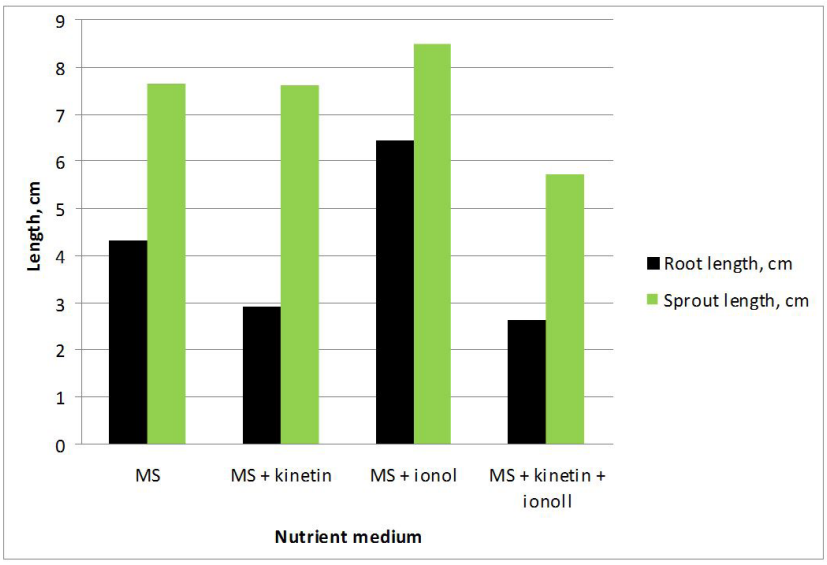

Figure 4: The manifestation of biometric indicators of tomato, depending on the composition of the nutrient medium

In the study of root length and sprout height of seedlings of tomato, it was found that the maximum results were obtained on MS + ionol. The control advantage was 32.97% and 10.04% respectively. In the variant MS + kinetin + ionol an inhibition was observed on all studied features. Thus, antioxidants have influenced the manifestation of the characteristics of tomato *in vitro* differently, indicating the lack of synergism of the studied biologically active substances and showing the presence of antagonism between these compounds and the inability to use them together as growth regulators in nutrient medium *in vitro*.

The prospect of the acetone use in the cultivation of explants of different origin has been proved. At the same time, it is important to determine the optimum concentrations of this solvent and the specific selection of plant objects.

We consider it perspective to use Cs Chem office programs to optimize nutrient medium for the purpose of establishing synergism and antagonism of chemical compounds characterized by biological activity.

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