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BIOLOGICAL ACTIVITY OF LECTINS EXCTRACTED FROM LEGUMES' CALLUSES

Abstract. Nowadays cell and tissue cultures *in vitro* are widely used in many biological experiments, particularly in production of natural biologically active food supplements and protein substances for different purposes. Legumesare characterized by high level of proteins and protein compounds such as lectins. Studying different patterns of hormonal regulation of lectins*in vitro*makes possible understand the physiological mechanisms of growth and development of legumes. In this regardselecting the source of explants and optimizing concentration of minerals and hormones in culture media allowed us to obtain morphogenic and non-morphogenic calluses. Usage of 2,4-D helped to increase frequency of morphogenic calluses more than 5 times in contrast with NAA. Biological activity of lectins, extracted from differenttypes of calluses, was determined visually by intensity of hemagglutination and their titer. Itisproved, thatlectinactivity of legume specimens depends on genotype and type of calluses, which is determined by ingredients of culture media. The level of lectinsis controlled and regulated by concentration of auxins and cytokinins in culture media. Allmorphogenic samples of calluses demonstrated higher activity thannon-morphogenic ones. Andmaximum doze of lectins was observed at concentration of 1 mg/l 2.4-D and0.5 mg/l of kinetin. The highest lectin activity was marked for two representatives of morphogenic type of calluses – "Aktatti" and "Juravushka". Realex periments demonstrated that determination of agglutinative activity in plants and the level of lectins in calluses allows to see fluctuations in lectin activity in cells and tissues. Also this data might be used to study the hormonal processes of differentiation, proliferation and early development of legumes and to improve current methods of lectin extraction.

Key words: *Phaseolusvulgaris*, lectins, callus culture, activity, hormonal dependence.

Introduction. One of the main functions of lectins is participation in processes of cell division, stretching and differentiation and, consequently, regulation of morphological and physiological processes in plants.

Some hormones such as ABA, IAA, BAP, GAregulate synthesis of lectins. Biological role of these substances is associated with the seeds formation, the stage of rest and the stage of awakening [1, 2]. Mechanism of action of plant growth regulators is controlled by changing in activity and synthesis of different proteins. There is information that lectins have an ability to attach molecules of phytohormones. So, lectinin wheat wheat germ agglutininhas a high affinity to many phytohormones such as auxins, cytokinins and gibberellic acid [3]. It is supposed, that complex of lectins and phytohormoes takes participation in storing of hormones and regulation of plants growth [4, 5].Recently it is not doubt that there is a specific lectin-dependent system to regulate some functions inside animal cells. Probably the similar system might be founded in plants too[6]. In scientific articles there are proves that lectins with phytohormones may be involved in regulation of growth processes in vegetating plants. This suggestion is based on the facts that various lectins can interact with hormones. Independenceofkinetin in different culturemedi at he level of lectin in calluses usually varies [7].

Also it was revealed, that cooperation between K on A with IAA and its derivatives and lectins in lima bean with cytokinins is possible because of hydrophobic binding sites in lectins [8].

It is shown that PBAcan result in a rapid changing in hormonal system of beans, demonstrating an ability of PBA complex and phytohormones to regulate proliferation in roots. Obtained results prove that variousphy to lectinsare in volved in regulation such an important process as a plant growth [9].

Thus, oneofthe most prospective ways to study physiological mechanisms of growth regulation of legumes is a chance to observe different patterns of hormonal regulation of quantitative content in legumes *invitro.*

The aim of the research is to determine biological activity and lectin concentration in beans calluses, having differences ii morphogenetic способности, to find sources of lectins and study the process of lectin regulation.

Materials and methods. To get calluses specimens «Aktatti», «Juravushka», «Kamelia», «Red Goya» were used. Primary callus cultures were cultivated on a special media Murasige-Skuga, containing 2.4-D (2-8 mg/l) and kinetin (0.25 mg/l).The role of explants was played by epikotyls of 7-14-days aseptic seedlings. Calluses were grown at 23-25 C in the light, during 16-hours photoperiod with light intensity equal to 10 000 lux. Passageoccurredonceper 28 days. Lectins were extracted from calluses in according to the method invented by us in the previous researches [10]. The content of lectins was determined right after extraction and dialysis. Extracted mass was weighted and calculated in mg per 100g of wet weight. The lectin activity was defined by reaction of hemagglutination with rabbit blood.

The lectin activity was determined by its titer and measured in units of inverse $[мг/мл]^{-1}$. It means the minimal concentration of protein leads to hemaggutinaton. For this purpo selectins were diluted five times [11]. To determine the impact of phytohormones on lectins accumulation, calluses were grown on a culture media, containing 1 mg/l of2.4-Dand 0-10 mg/lof kinetin. The content of lectins was fixated after 21 days. To exclude mistakes and errors this experiment was repeated three times. Microphotographies were given by MoticDM 143 SERIES, a microscope.

Results and discussion. Inmanyscientific articles it was proved that formation of one or another morphological type of callus tissue might be regulated by different phytohormons, by changing compounds in a media, conditions of cultivation, and also by using species and specimens with a high potential *in vitro*.The last factor is very important for the process of cell and tissue cultivation in some legumes whichhave different traits appearing at the level of morphogenetic processes[12-14]. In the previous researches it was shown, thatforselectedspecimens of legumes the most intensive process of callus formation occurred on a modified Murasige-Skuga media [15].Formation of morphogenic and non-morphogenic type of calluses depended on the type and concentration of phytohormones. The presence of 2 mg/l of 2.4-DиIAAin a media resulted in formation of 87% and 15 % of morphogenic calluses respectively. Increasing concentration of 2.4-D provoked a degradation of morphogenetic calluses and in presence of 8 mg/l of 2.4-D necrosis was observed. It was supposed that high concentrations of2.4-Dincreased the speed of ethylene formation, contrariwise it decreased the speed of cell stretching. Probably high concentrations of auxins influence on suppression of growth of dicotyledonous plants and associate with the synthesis of ethylene [16]. Analysis of extracts showed their differences in doses of lectin. The concentration of lectin in morphogenetic calluses was significantly higher than in non-morphogenic ones and varied from 37.3 mg/100g of raw mass in ″Juravushka″ to 26,0 mg/100g in ″Kamelia″ (figure 1).

Non-morphogenic calluses are characterized by low concentration of lectins (about 18.4-25.2 mg/100 g of wet weight)in all studied samples. It was assumed that these differences in lectin concentration between morphogenic and non-morphogenic calluses might be related to hormones in the media, because of morphogenic type was formed on culture media withIAAand low concentrations of2.4-D. According to literature sources it is known that synthesis of lectins is regulated by abscisic acid and high concentrations of 2.4-D decrease the content of ABA [17-19].

The next stage of this research was to determine lectin activity in morphogenic and non-morphogenic calluses of different legumes. In the first serial of experiments the intensity of hemagglutination was assessed visually using bands from 1 to 10. It was found that agglutinating activity in all specimens depended both on genotype and type of callus tissue. In contrast with non-morphogenic type morphogenic calluses demonstrated higher activity which varied from 10 to 13 bands. Two specimens″Aktatti″ and ″Juravushka″ had 13 bands, ″Kamelia″ had 11 bands, ″Red Goya″ had 8 bands, but non-morphogenic calluses got not more that 5 bands. Also in ″Aktatti″ hemagglutination was observed during the first minute after start, in ″Juravushka″ the same indicator was observed in two minutes. It is necessary to mention

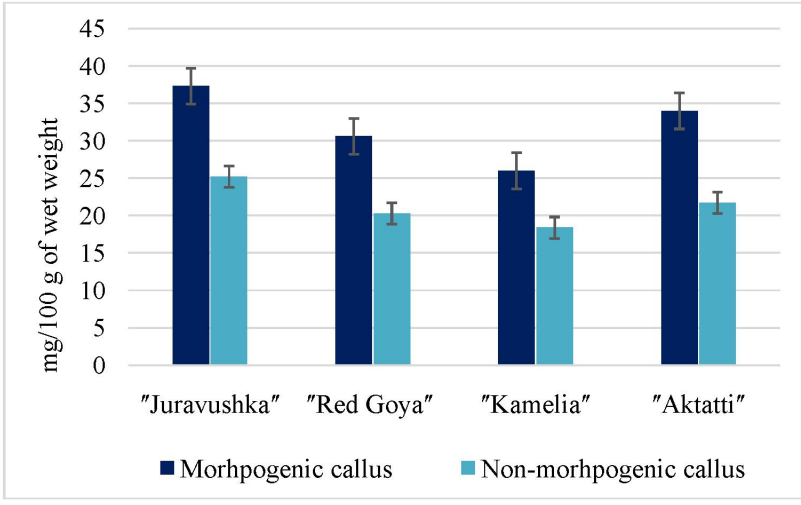


Figure 1 – The presence of lectins in callus tissues of various legume samples

that in this case the effect of hemagglutination was very strong and could be observed even after 5 dilutions. Shaking samples did not provoke destruction of red blood cells, liquid also remained clear and transparent. All samples were differed from the timing of hemagglutination. In "Kamellia" (morphogenic type of calluses) this reaction started later than in other specimens, but it was very strong and could be observed after several dilutions. "Red Goya" (a non-morphogenic one) demonstrated very low lectin activity, as the reaction of agglutination started 55 minutes later and was observed after the first dilution only.

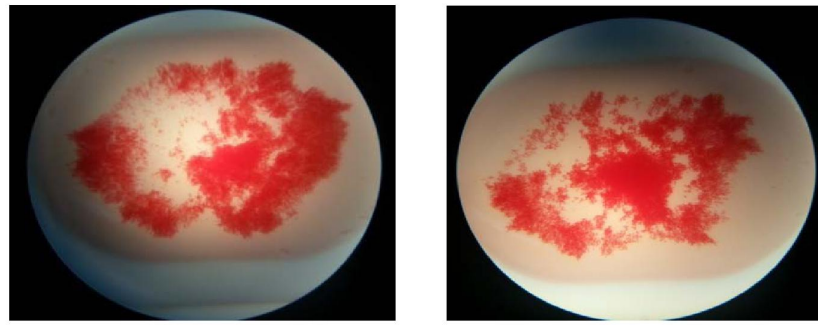
15 minutes later after the reaction of hemagglutination in "Aktatti", "Juravushka" and "Kamelia" was more perceptible. Red blood cells formed stable structures and liquid was perfectly transparent. In 20 minutes in all dilutions reaction was still perceptible, but not sustained. Agglutinates did not break down into pieces and liquid was still clear. In 30 minutes after shaking it was observed that agglutinates disintegrated, liquid was muddy. That was the first sign that hemagglutination was very weak (figures 2 and 3).

In other cases reaction was moderate and agglutinates accumulated at the edges of holes. In one hour there was not any visible changing.

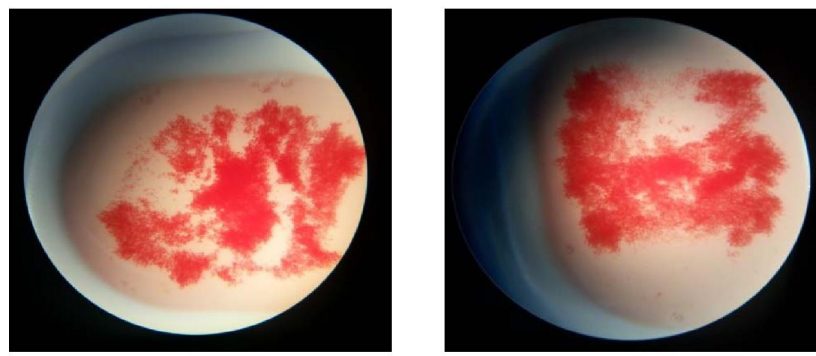
Further experiments of studying hemmaglutinative activity were performed by measurement of lectin titer, i.e. maximum dilution of minimum concentration in its solution it which red blood cells still can make agglutinates. As a result of these experiments it was proved that morphogenic calluses have high titer of lectin (27.7-30.8 $[mg/ml]^{-1}$. The highest activity was detected for "Aktatti" and "Juravushka". "RedGoya" and "Kamelia" demonstrateda little bit smaller activity than the previous ones. Non-morphogenic calluses had the smallest titer (not more than 10-11 $[mg/ml]^{-1}$. Probably these distinguishes can depend on types and concentration of phytohormones, used for callus growth induction. Obtained data does not contradict with the results of similar experiments with wheat [20, 21].

As mentioned earlier, lectins take participation in different processes such as cell growth and differentiation. Therefore, regulation of lectin concentration in callus cultures might be under impact of various phytohormones. Concerning, the influence of auxins and cytokinins on induction of lectin formation in morphogenic legume "Juravushka" was studied. Asauxinsitwasused2.4-D(1 mg/l), the role of cytokinins played kinetin (0-10 mg/l). As a result it was stated that the highest concentration of lectins is observed on a culture media containing 1 mg/l of 2.4-Dand 0,5mg/lof kinetin (figure 4).

During the cell growth cycle (7 weeks) the concentration of lectins was determined by passaging 1.5 g of callus on a culture media containing 1 mg/l of 2.4 D and 0.1 mg/l of kinetin. This procedure was repeated every 10 days. The highest concentration was marked in its initial stage of growth. This can be explainedby more intensive cell division, which is common for the phase (figure 5).

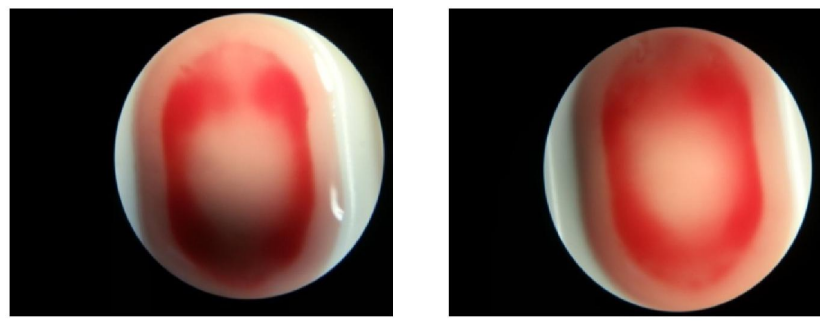


"Juravushka" "Red Goya"

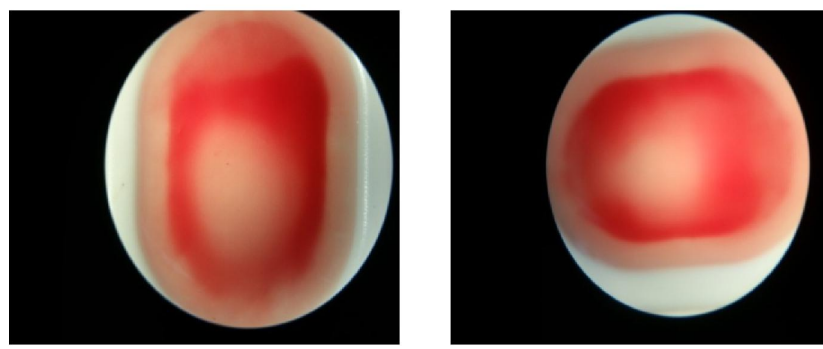


"Kamelia" "Aktatti"

Figure 2 – Microphotograph of hemagglutination of morphogenic calluses (x20)



"Juravushka" "Red Goya"



"Kamelia" "Aktatti"

Figure 3 – Microphotograph of hemagglutination of non-morphogenic calluses (x20)

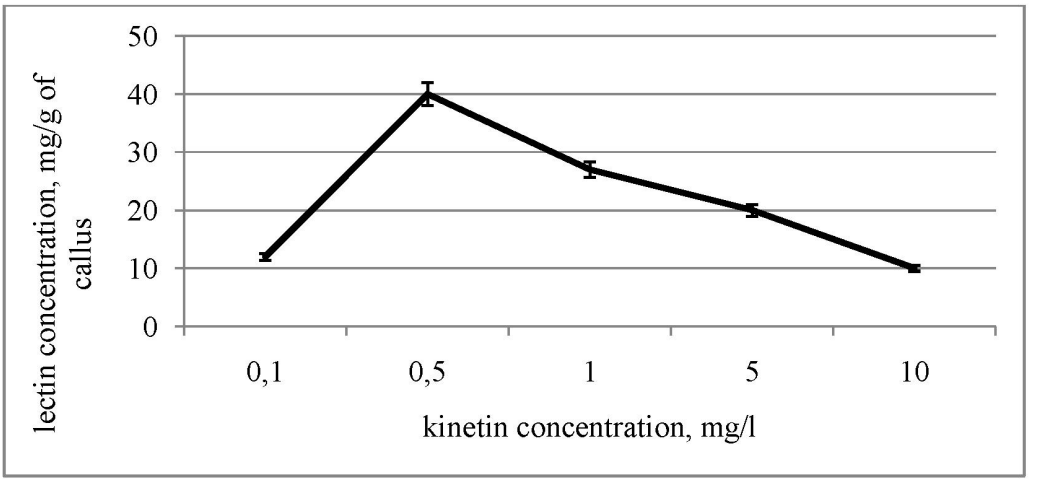


Figure 4 – Lectin concentration in calustissuein dependence on doze of kinetin

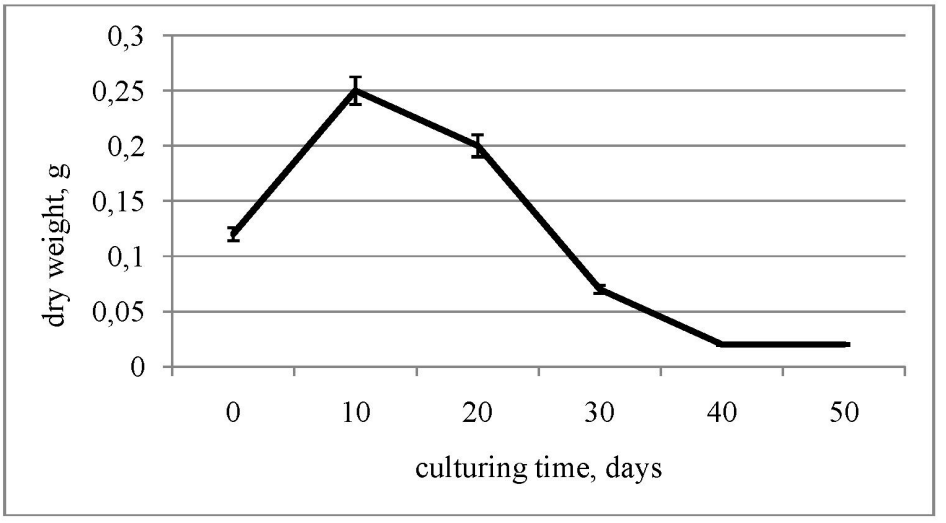


Figure 5 – Dependence between lectin concentration and growthcycle of calluses

Thus, this research on studying biological activity of lectins showed that accumulation, concentration and activity of lectins strictly depend on hormones in culture media. So, in the future calluses may be used as a biotechnological source to extract lectins for different purposes.

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ҮРМЕ БҰРШАҚ КАЛЛУСТЫҚ КУЛЬТУРАСЫНАН БӨЛІНІП АЛЫНҒАН ЛЕКТИНДІК БЕЛОКТАРДЫҢ БИОЛОГИЯЛЫҚ АКТИВТІЛІГІ

Аннотация. Қазіргі уақытта *invitro* клетка культуралары мен ұлпалары биологиялық зерттеулер үшін кең қолданылуда. Көп зерттеулер табиғи биологиялық активті заттар мен түрлі спектрде әрекет ететін белоктық компоненттер алу мақсатында жүргізілуде. Биохимиялық құрамы бойынша бұршақ тұқымдастары басқаларға қарағанда белок және белоктық компоненттердің көп болуымен ерекшеленеді. Солардың бірі - лектиндік белоктар. *In vitro*клетка культураларында лектиндердің сандық құрамының гормональдық реттелуі сияқты әр түрлі жолдармен зерттеу арқылы - өсу процесін реттеу, бұршақ тұқымдастардың дамуы сияқты физиологиялық механизмдерді дәлелдеуге болады. Осындай жолмен эксплантты іріктеп алу үшін, қоректік ортаның минералды және гормональдық құрамын оңтайландыру мақсатында үрмебұршақтың морфогенді және морфогенді емес каллус ұлпалары алынды. 2,4-Д қолдану кезіндегі морфогендік каллустардың пайда болу жиілігі НСҚ қолданғандағымен салыстырғанда бес есе жоғары болды. Әр түрлі каллустардан бөлініп алынған лектиндердіңбиологиялық активтілігі гемагглютинация реакциясының қарқындылығын сырт көзбен бақылау және лектиндерді титрлеп өлшеу жолдарымен анықталды. Бізге белгілі үрмебұршақтың әрбір сорт үлгілеріндегі лектиндік активтілік қасиеті каллус ұлпасының түрі мен генотипіне байланысты. Ал ол, өз кезегінде қоректік ортаның гормональды құрамымен анықталды. Лектиндік белоктардың құрамы қоректік ортадағы ауксин мен цитокининнің концентрациясымен реттеліп отырады. Морфогендік түрлі каллустардың барлық үлгілерінің активтілігі морфогендік емес каллустармен салыстырғанда айтарлықтай жоғары болды. Ал лектиндердің максималды (ең көп мөлшері) жинақталуы - 2,4-Д – 1 мг/л, кинетин – 0,5 мг/л концентрацияда байқалды. Ең жоғары лектиндік активтілік «Актатти» және «Журавушка» атты морфогенді типті каллус үлгілерінде анықталған.

Жүргізілген зерттеулер нәтижелері көрсеткендей, белгілі фитогемаглютеиндеуші активтілікті және өсімдік каллус культураларындағы лектиндердің құрамын анықтау жұмыстары лектиндік активтіліктің түрленгіштік деңгейін клеткалық және ұлпалық деңгейде анықтап, табуға мүмкіндік береді және дифференциа-

ция процесіндегі гормональдық реттелуді жете зерттеу үшін келешегі бар тәсіл болып табылады. Сондай ақ, бұл жұмыстар бұршақ тұқымдастардың өсіп өнуі мен дамуын реттеуге және әр түрлі биотехнологиялық жолмен лектиндерді алу тәсілдерін жетілдіруге мүмкіндіктер береді.

Түйін сөздер: *Phaseolusvulgaris*, лектиндер, каллустық культура, белсенділік, гормонға тәуелділік.

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БИОЛОГИЧЕСКАЯ АКТИВНОСТЬ ЛЕКТИНОВ, ВЫДЕЛЕННЫХ ИЗ КАЛЛУСНЫХ КУЛЬТУР ФАСОЛИ

Аннотация. Культура клеток и тканей *invitro* в настоящее время находит применение в широком диапазоне биологических исследований, в частности получения природных БАВ и белковых компонентов различного спектра действия и применения. По биохимическому составу бобовые культуры отличаются высоким содержанием белков и белковых компонентов, к которым относятся, например, лектины. Путем изучения различных путей гормональной регуляции количественного содержания лектинов в культуре *invitro* возможно установление физиологических механизмов регуляции роста и развития бобовых культур. В связи с этим путем подбора источника экспланта, оптимизации минерального и гормонального состава питательных сред, получены морфогеннные и неморфогеннныекаллусные ткани фасоли. Частота образования морфогенных калллусов при использовнии 2,4-Д была в пять раз выше, по сравнению с НУК. Биологическая активность лектинов, выделенных из различных типов каллусов определялась визуально по интенсивности реакции гемагглютинации и путем измерения титра лектинов. Установлено, что лектиновая активность сортообразцов фасоли зависит от генотипа и типа каллусной ткани, который определяется гормональным составом питательной среды. Содержание лектинов регулируется концентрацией ауксинов и цитокининов в питательной среде. У всех образцов активность морфогенного типа каллусов была значительно выше посравнению с неморфогенным, а максимальное накопление лектинов наблюдалось при концентрации 2,4-Д – 1 мг/л, кинетина – 0,5 мг/л. Наибольшая лектиновая активность отмечена для морфогенного типа калллуса сортообразцов «Актатти» и «Журавушка».

Проведенные исследования показывают, что определение фитогемаглютинирующей активности и содержания лектинов в каллусных культурах растений позволяет установить вариабельность уровня лектиновой активности на клеточном и тканевом уровнях и может служить перспективным подходом для изучения гормональной регуляции процессов дифференциации, роста и развития бобовых, а также усовершенствовать биотехнологические подходы получения лектинов.

Ключевые слова: *Phaseolusvulgaris*, лектины, каллусные культуры, активность, гормональная зависимость.