использован сорт картофеля Невский. Научно-исследовательские эксперименты проводились в ТОО "Кокшетауское опытно-производственное хозяйство". Полевые опыты по контролю и учету проводились в соответствии с методикой исследования растений картофеля и методикой полевого эксперимента. Полученные данные подверглись математической обработке с использованием методики полевого эксперимента Б.А. Доспехова. Масса картофеля в контрольном варианте согласно учетным работам составил 817 г с площади 0,072 га. В варианте, обработанном регулятором роста "Зеребра Агро", составила 1320 г, а регулятором роста" Агростимулин " - 850 г. Самый высокий показатель наблюдался в варианте, обработанном препаратом "Humika" - 1390 г. В вариантах, в которых использовался регулятор роста "Агростимулин", наблюдались хорошие показатели по количеству стеблей и массе стеблей, масса товарного клубня составила в среднем 67,5 г. Урожайность 11,6 т/га. При использовании регулятора роста "Humika " урожайность картофеля в среднем составляла 19,0 т/га, что на 69,6% выше контрольного варианта. Товарность показала 79%. Наибольшую урожайность показали варианты, обработанные регуляторами роста Humika – 19 т/га, "Зебра агро" - 18 т/га и Энрайвел – 16,2 т/га.

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BIOTECHNOLOGICAL FEATURES OF MICROCLONAL REPRODUCTION OF *TULIPA* L. SPECIES

ANNOTATION

The research work is devoted to the problem of conservation of rare and endangered species of *Tulipa* L. using the tissue cultivation method. Tulips are bulbous geophytes with high decorative value all over the world. Anthropogenic impact on this species has led to a decrease in the population of wild tulips. The paper presents the results of a study of the effect of the hormonal composition of the nutrient medium on the morphogenesis of Tulipa L. explants in vitro. During the period of introduction into sterile culture, single-soil nodular segments of adult plants were used as the primary explant, internodes and leaves of one-month explants were used during the period of micropropagation. The study suggests the optimization of sterilization plant materials, nutrient media and adaptation of newly restored plants using bulbs and seeds of selected taxa.

In a modified Murashige-Skuga nutrient medium, micro leaves and internodes of shoots formed from calluses were grown in the dark. The study used cuttings of bulbs of an adult plant. The disinfection method was carried out using a complex chemical treatment. The effectiveness of sterilization, consisting of fungicidal, bactericidal processes, was determined by the viability of explants after sterilization. It is noteworthy that ethanol treatment, starting with a simple soap wash, significantly increased the viability of explants. In addition, the study examines the results of in vitro

cultivation, in which the level of viability after cultivation was observed, as well as the development of morphological indicators of germination.

Key words**:** *types of tulips, bulbs, phenophase, culture medium, clonal microprogation.*

Introduction. There are 36 species of Tulipa L in Kazakhstan, 20 of which are listed in the Red Book. The vast majority of more than 60% of the endemic species of the tulip genus have a limited range. Tulips (*Tulipa* L.) have significant decorative and aesthetic value all over the world [1]. Currently, the number of species of the genus is unknown due to difficulties in the taxonomy of the genus, confusion in classifications, high rates of interspecific hybridization and polymorphism [2]. In Kazakhstan, many species of Tulipa L are listed in the Red Book. For example, among the rare and endangered tulips: Greig's Tulip (*Tulipa greigii* Regel), Kaufman's tulip (*Tulipa Kaufmanniana* Regel), Albert's Tulip (*Tulipa albertii* Regel), Borshchov's Tulip (*Tulipa borszczowii* Regel), False-flowered tulip (*Tulipa dasystemonoides* Vved.), Korolkov's Tulip (*Tulipa korolkowii* Regel), Lehmann's Tulip (*Tulipa lehmanniana* Mercl.), the Erect tulip (*Tulipa orthopoda* Vved.) are listed in the Red Book. The species *Tulipa* L. is an economically valuable cultivated plant due to its high decorative indicators. In recent years, the habitats have been declining as a result of excessive plowing of natural meadows, cattle breeding and ruthless pulling of flowers by local residents [3, 4].

The method of tissue cultivation is used to solve the problem of preserving the number of rare species of *Tulipa* L. [5]. The microclonal reproduction of the species has not yet been sufficiently studied [6, 7]. The culture of cell tissues of *Tulipa* L. species is of great interest to researchers, since it allows reproducing unique species, hybrid genotypes, selecting valuable mutant forms and preserving rare species in collections [8, 9]. In the field of microclonalization, research is intensively developing in the direction of microclonal reproduction of *Tulipa biebersteiniana* Schult &Schult fil., *T. gesneriana* L. and *Tulipa kaufmanniana* Regel, whereas data on tissue culture of other *Tulipa* L. species few.

There is also no data in the literature on tissue culture of *Tulipa orthopoda* Vved. and *Tulipa bifloriformis*. In this regard, we were faced with the task of developing a method of microclonal reproduction of these two species and studying their morphogenetic features in vitro.

Material and methods. The objects of the study were Tulipa orthopoda Vved species. and Tulipa bifloriformis. Single nodular segments of adult plants were used as the primary explant at the stage of introduction into sterile culture. In the micro-reproduction itself, nodal segments, internodes and leaves of monthly micro-plants obtained in a non-hormonal environment were used.

The basis of the Murasi-Skuga nutrient medium was made up of vitamins and trace elements, phytohormones added in accordance with the methodology [10, 11, 12]. Of the phytohormones, cytokinins (6 BAP (6-benzylaminopurine), (isoprene-2-yl) adenine, zeatin, tidiazuron (TDZ), kinetin) and auxins (2,4-dichlorophenoxyacetic acid (2,4-D), IBC (indolbutylic acid). To prevent the development of microflora, antibiotics were added to the nutrient medium: cefotaxime, carbenicillin, gentamicin. A modified WPM medium without organic additives was used as a control. The pH of the medium is adjusted to 5.6–5.8 before sterilization. Autoclave treatment for 20 minutes at 1.1 atm. The material was grown at a temperature of 25 ± 1C, with a photoperiod of 16 hours and illumination of 2-3 thousand lux. The number of repetitions in each variant was 30. Statistical processing was carried out using the Microsoft Excel program [13-16].

Research results and their discussion. In our work, we investigated various stages of microclonal reproduction of Tulipa bifloriformis and Tulipa orthopoda Vved species. The entire period of micropropagation can be divided into 3 stages. The first stage is the explantation of primary plant tissue. At this stage, a plant with high viability, free from infection, and actively reproducing is obtained. At the second stage, micro-propagation itself is carried out, that is, an increase in the number of organogenic structures in the explant, induction of processes from them and rooting of propagated shoots in a nutrient medium. This stage is characterized by the use of heterogeneous compounds of various hormones and other biologically active substances, various explants in large quantities, as well as the use of various growing conditions. The third stage is the planting of micro-plants from sterile conditions into the soil.

Tissue culture of *Tulipa orthopoda* Vved. The initial material was obtained from one vegetative bud of the parent plant Tulipa orthopoda. It is known that field material is characterized by a high level of microflora infection, which makes it difficult to obtain sterile material. To reduce the degree of contamination, part of the plant is dug out in early spring and planted in a laboratory on specially

prepared soil. At the initial stage of flowering, cuttings are cut, sterilized, cut into single bud segments of shoots and planted in a nutrient medium of MS with the addition of 6BAP at a concentration of 2.0-5.0 mg / l. It should be noted that growing the mother plant in the laboratory made it possible to obtain a sterile culture with almost no problems, and all the planted buds turned into sprouts. If, in subsequent transitions, a bacterial infection occurred in the form of a cloudy white areola in the nutrient medium at the base of the explants, we immediately applied the following methods: sterilization with antiseptics, periodic cultivation of micro-plants in a nutrient medium supplemented with antibiotics.

To study the process of morphogenesis in vitro, segments of single-soil shoots, shoot tips and interarticular spaces were used as an explant, which were isolated from monthly microclonal plants grown in a non-hormonal environment under sterile conditions (table 1).

Table 1 – Optimization sterilizing agents and their concentrations

Sterilizing agents	Concentration	Time
Soapy water	-	30 min
Fungicide "Luna"	10%	3 hours
Calcium	11 %	30 min
Hypochlorite is a solution of hydrogen peroxide	3%	3 sec
Alcohol	90%	3 sec
Sterilizing agents	Concentration	time

The preparation of the plant material for sterilization consisted of the following stages: 1) the bulbs were washed in soapy water at room temperature for 30 minutes 2) the bulbs were placed for 3 hours in a solution of the fungicide "Luna" 3) after half an hour in a solution of calcium hypochlorite 4) before planting in the laminar flow box, it was kept for about 3 seconds in a solution of hydrogen peroxide, then in alcohol 90%-3 seconds.

The explants were placed in a nutrient medium supplemented with hormones (Table 2). After 30 days, the explants were transferred together with the extracted structures to a new hormone-free environment to determine regenerative capacity and obtain regenerants, where cultivation continued for another 25 days under optimal conditions.

Table 2 – Quantitative ratio of the nutrient medium depending on the hormonal composition

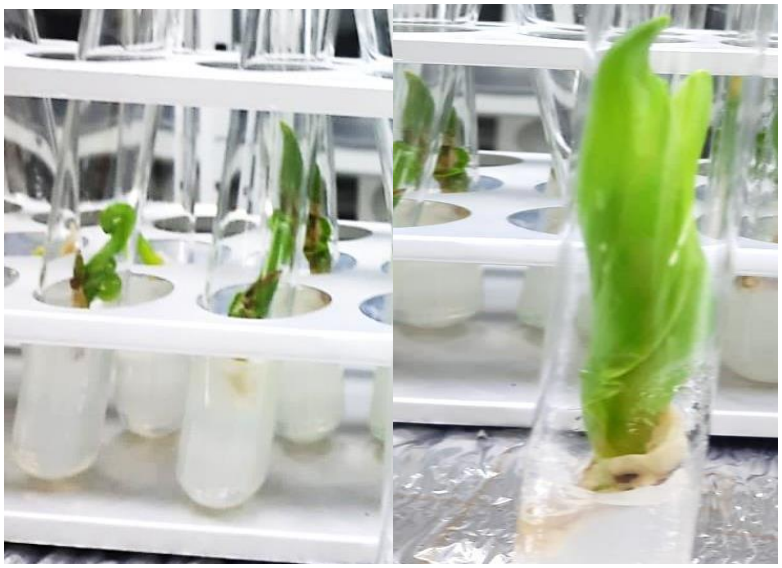
Components	Content Per 1 liter of distilled water
1	2
Macrosols KNO_3 $CaCl_2 \cdot 2H_2O$ NH_4NO_3 $MgSO_4 \cdot 4H_2O$ KH_2PO_4	50 ml
Microsols H_3PO_4 $MnSO_4 \cdot 4H_2O$ $ZnSO_4$ Kl $Na_2MoCl_4 \cdot 2H_2O$ $CuSO_4 \cdot 5H_2O$ $CoCl_2 \cdot 6H_2O$	10 ml
Mesoinosite	0,1g
Glycine	0,0005g

1	2
Vitamin	0,5ml
IAA	0,1ml
6 BAP	0,5ml
Agar	8g
Sucrose	30g

During the first and second planting, the development of nodal segments and the growth of the resulting shoots were noted. In the nutrient medium with the addition of 6 BAP, the formation of lateral shoots from the lower axillary buds with the formation of microgrowths was observed.

Callus formation at the ends of nodal segments of plants was observed in 100% of explants in almost all experimental variants, with the exception of the variant with kinetin 5.0 mg/l. The calluses were 2-3 mm in diameter, brownish-green in color, dense, shiny. The formation of adventitious buds in the first pass was observed in media with the following hormones: 6 BAP 0.5 ml/l, IAA 0.1 ml/L. The number of buds in one explant varied from 1 to 2.Thus, it was found that additional cultivation of internodes with kidneys obtained during the first planting for 20 days in a non-hormonal environment practically does not affect the morphogenetic activity of explants. The formation of calluses was observed in all explants grown in media with the addition of 6 BAP 0.5 ml/l.As a result of the study, an effective method for obtaining a sterile culture of Tulipa orthopoda was proposed. The effect of a wide range of hormones on the processes of Tulipa orthopoda morphogenesis in tissue culture has been studied. This species is *Tulipa* L. It has a low proliferative and organogenic ability when using single-stem segments of shoots and internodes as an explant [17-20].

Tissue culture of *Tulipa bifloriformis*. In February, shoots of Tulipa bifloriformis were collected. To cause swelling of the kidneys, the sprout is placed in water and kept at 25^0C, 2000-3000 lux illumination for 2-3 weeks. Then the parts of the sprout are cut off and sterilized with 0.1% diacid solution for 20-30 minutes. After sterilization was completed, the material was washed three times with autoclaved distilled water. Isolated pods with stem parts were placed in a laminar box in a medium containing 6 BAP at a concentration of 0.5-2 mg/l. The material was grown under 2000-3000 lux illumination, a photoperiod of 16 hours and a temperature of 25 ± 1 C. Every two weeks, explants containing calluses were removed from necrotic areas and moved to a new medium of a similar composition. Two months after planting, many buds and calluses with shoots formed on the explants. The sprouts were grown in a nutrient medium with a low cytokinin content, and then transplanted into a non-hormonal medium.



A B

Figure 1 – Leaf sprouts sprouted from bulbous parts of tulip species using benzoaminopurine (0.5 mg/ l) (A), development of organogenesis ready for adaptation (B)

Micropropagation was performed using leaves and internodes as an explant. It was found that the regeneration of shoots occurs in a nutrient medium even in the absence of growth hormones in both types of explants studied. Induction of shoot formation occurs in 50% of leaves and 100% of internodes. However, two more plantings were carried out in a non-hormonal environment so that the resulting regenerants were suitable for transplanting into the soil according to morphological parameters.

Table 3 – Meffect of the hormonal composition of the nutrient medium on the organogenesis of leaves and internodes of *Tulipa bifloriformis*

Hormones – ml/l	Number of explants, %	
	With sprouts	
	l	*i*
Indolylacetic acid 0.1	15	43,5
BAP 0,5	85,4	83,9
BAP 0,5 + IAA 0,1	89,7	97,5
Kinetin 0,5	75,6	45,7

Explants: l – leaf, i – internodes

15 experimental variants of nutrient media were tested to increase the rate of reproduction (Table 3). Regenerating plants were obtained from internodes and leaves as a result of reproduction and subsequent regeneration of calluses. After 4 weeks of cultivation, a dense, shiny, yellow-green callus with internodes was formed in all experimental variants. In most of the media used, induction of adventitious kidneys was observed in the callus at the end of the first pass. The most intensive process of shoot formation in nodal intervals was observed in a medium with 0.5 ml/l. The number of random kidneys on the explant was 3-8. The formation of adventitious buds on callus in nutrient media containing only cytokinins occurred only in the second planting after growing callus in a non-hormonal environment. In these experimental versions, the ability of calluses to form growths was 80%, respectively. At the third stage, the transplantation of *Tulipa bifloriformis* microplants into the soil was studied. The plants have successfully adapted to the soil. Thus, as a result of studies of *Tulipa bifloriformis*, a sterile culture was obtained, optimal nutrient media for leaf and internode explants were selected, and a regeneration method was developed.

Conclusion. Studies conducted using methods of culturing cells and tissues of *Tulipa* L. have shown the possibility of preserving rare species. The low proliferative and organogenic ability of Tulipa orthopoda tissue culture was found when using interstitial spaces as explants. For this species, the most effective in vitro micropropagation method was recommended for reproduction by apical shoots, axillary buds from meristematic node tissues when grown in a non-hormonal environment. As a result of the work carried out, an effective method for obtaining a sterile culture of *Tulipa orthopoda* was proposed, a sterile culture was obtained, and a method for the regeneration of *Tulipa bifloriformis* was developed. For the latter, the optimal environment was chosen for each type of explant used at the breeding stage.

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ТҮЙІН

Зерттеу жұмысы ұлпа дақылы әдісін қолдана отырып, *Tulipa* L. түрлерінің сирек кездесетін және жойылу қаупі бар түрлерін сақтап қалу мәселесіне арналды. Қызғалдақ - бұл бүкіл әлемде сәндік құндылығы жоғары пиязшық тәрізді геофиттер. Бұл түрге антропогендік әсер жабайы қызғалдақтардың популяциясының азаюына әкелді. *Tulipa* L. эксплантарының in vitro морфогенезіне қоректік ортаның гормондық құрамының әсерін зерттеу нәтижелері берілген. Стерильді культураға енгізу кезеңінде бастапқы эксплант ретінде ересек өсімдіктердің бір бүршікті түйінді сегменттері пайдаланылды, микрокөбейту кезеңінде бір айлық микроөсімдіктердің түйін аралықтары және жапырақтары пайдаланылды. Зерттеу стерилизациялық өсімдік материалдарын, қоректік орталарды оңтайландыруды және таңдалған таксондардың баданалары мен тұқымдарын пайдалану арқылы жаңадан қалпына келтірілген өсімдіктерді бейімдеуді ұсынады.

Модификацияланған Мурасига-скуга қоректік ортасында, каллустың түзілуінен өсіп шыққан өскіндердің микрожапырақтары мен түйінаралықтары қараңғыда өсірілді. Зерттеу барысында ересек табиғи өсімдіктің пиязшықтарының кесінділері қолданылды. Зарарсыздандыру әдісі кешенді химиялық өңдеулер арқылы жүргізілді. Фунгицидтік, бактерицидтік процестерден тұратын стерилдеудің тиімділігі эксплантардың стерилдеуден кейінгі өміршеңдігімен анықталды. Атап айтар болсақ, жай сабындық жуудан бастап этанолмен өңдеу эксплантардың өміршеңдігін айтарлықтай арттырды. Сонымен қатар, зерттеу өскіннің морфологиялық көрсеткіштерін дамытумен қатар, өсіруден кейінгі өміршеңдік деңгейі байқалған in vitro өсіру нәтижелерін зерттейді.

РЕЗЮМЕ

Исследовательская работа посвящена проблеме сохранения редких и находящихся под угрозой исчезновения видов Tulipa L. с использованием метода культивирования тканей. Тюльпаны-луковичные геофиты, имеющие высокую декоративную ценность во всем мире. Антропогенное воздействие на этот вид привело к сокращению популяции диких тюльпанов. В работе представлены результаты исследования влияния гормонального состава питательной среды на морфогенез эксплантатов Tulipa L. in vitro. В период введения в стерильную культуру в качестве первичного эксплантата использовались однопочвенные узловые сегменты взрослых растений, в период микроразмножения использовались междоузлия и листья одномесячных эксплантов. Исследование предлагает оптимизацию стерилизационных растительных материалов, питательных сред и адаптацию вновь восстановленных растений с использованием луковиц и семян выбранных таксонов.

В модифицированной питательной среде Мурасига-Скуга микролистья и междоузлия побегов, образовавшиеся из каллусов, были выращены в темноте. В исследовании использовались черенки луковиц взрослого растения. Метод обеззараживания проводился с помощью комплексной химической обработки. Эффективность стерилизации, состоящей из фунгицидных, бактерицидных процессов, определялась жизнеспособностью эксплантатов после стерилизации. Примечательно, что обработка этанолом, начиная с простой мыльной промывки, значительно повысила жизнеспособность эксплантатов. Кроме того, в исследовании изучаются результаты культивирования in vitro, в которых наблюдался уровень жизнеспособности после культивирования, а также развитие морфологических показателей прорастания.

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