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Original Article

SEED GERMINATION BIOLOGY OF RARE TULIPA SPECIES IN NORTHERN AND CENTRAL KAZAKHSTAN

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ABSTRACT

Kazakhstan has one of the highest diversities of *Tulipa* species, most of which are threatened. The threat of their extinction necessitates the use of biotechnological approaches, such as *in vitro* micropropagation, which allows the preservation of valuable genotypes without harming natural populations.

In the process of increasing climate change and anthropogenic impact, these endemic species are at high risk of extinction. In this regard, the study and conservation of biodiversity of endemic plant species is considered a global priority throughout the world.

The aim of the work was to study the effect of temperature and growth regulators on germination of *T. auliekolica*, *T. turgaica* seeds for their introduction into *in vitro* cultures.

In vitro germination was carried out on ½ MS media with and without the addition of GA₃ (13 and 52 mg/l). Germination was recorded for 60 days, germination (%) and T₅₀ were calculated.

According to the results of the tetrazolium test, the viability of *T. turgaica* and *T. auliekolica* seeds was 95% and 100%, respectively. The studied species showed different temperature preferences; for *T. auliekolica* seeds, the optimal temperature was 4°C, and for *T. turgaica*, 10°C. In addition, *T. auliekolica* seeds germinated faster than *T. turgaica* seeds. The results showed that temperature significantly affects seed germination. Seeds of both species germinated only at low temperatures (4 and 10°C), at 20°C, seed germination was absent in both species. The obtained data are of significant practical importance for the creation of effective methods for *in vitro* cultivation of these rare *Tulipa* species. Selection of optimal GA₃ concentrations in combination with temperature regimes significantly increases the success of *in vitro* propagation, which plays a key role in programs for the conservation of biodiversity of endemic plants.

Key words: biodiversity conservation, endemic and rare species, growth regulators, *in vitro* culture, micropropagation, *Tulipa*.

INTRODUCTION

At present, the anthropogenic impact on the biosphere has become global, and the scale and rate of impact continue to increase year by year [1, 2]. In the process of increasing anthropogenic impact on natural complexes, endemic species occurring in nature in small numbers or in very limited areas are particularly endangered [3].

Endemic plants are more vulnerable to anthropogenic threats and climate change, and are at high risk of extinction because of their low levels of genetic diversity due to inbreeding and genetic drift [4]. Conservation of these species is a serious problem worldwide, and the use of *in situ* conservation methods alone does not guarantee their preservation. Habitat destruction due to anthropogenic factors is the main cause of endemic species extinction. Human activity due to the development of the agro-industrial complex, industrialization or urbanization leads to the fragmentation of the distribution areas of most endemics, the fragmentation of populations and their complete disappearance [5]. In this regard, the conservation of biodiversity of endemic plant species is seen as a global priority worldwide [4, 6].

Kazakhstan, which is part of the Central Asian Genetic Center, is the center of domestication of many endemic species, which account for about 12% of the total number of vascular plants [7]. A significant number of endemics (about 20%) are represented in the genus *Tulipa* [8]. The main primary centers of origin of representatives of the genus *Tulipa* are located in the foothills of the Pamir-Altai and Tien Shan

ranges, later diversification occurred in Western Europe and in the east through Western China, therefore Kazakhstan is considered one of the key territories for the distribution of tulips in the world [9]. Of the 34 wilds Kazakh *Tulipa* species, 12 are endemic, and 18 are listed in the Red Book of the Republic of Kazakhstan [10].

In the territory of Northern and Central Kazakhstan, 9 species of *Tulipa* have been established to grow, of which 3 species are endemic – *T. albertii*, and 2 relatively recently discovered of *Tulipa* species – *T. turgaica* and *T. auliekolica* [11, 12, 13]. Despite the high popularity of tulips worldwide, the taxonomy, ecology and biological characteristics of most endemic species have not been studied, although endemic tulips are an important genetic resource and play an important role in ecosystems [14].

Tulips reproduce *in situ* mainly by seeds, but the dormancy period of seeds results in a low germination rate. Dormancy is a biological adaptation of seeds, regulated by many factors.

Temperature is the main environmental factor responsible for various changes in seed dormancy, and the dormancy period can be disturbed by temperature fluctuations or warm/cold stratification [15]. Hormones also influence the process of seed dormancy and germination.

Seed germination is the first stage in the life of a plant and is of great importance for maintaining the population size and diversity. The transition from dormancy to germination begins when a dry seed comes into contact with water and ends

when the root emerges through all the membranes of the developing embryo.

This process is sensitive to the influence of many factors, including the levels of exogenous and endogenous phytohormones, the content of internal storage proteins, water in seeds, seed age, as well as abiotic stress conditions, among which an important role belongs to temperature and light conditions. Temperature is the main environmental factor responsible for the various changes in seed dormancy, and dormancy can be disrupted by temperature fluctuations or warm/cold stratification [16, 17, 18].

It is known from literary data that tulip seeds are characterized by a deep complex morphophysiological type of dormancy. The reason for such dormancy is the underdevelopment of the embryo and a strong physiological mechanism of inhibition of germination (Su et al. 2020).

Low positive temperatures (the optimum of which is in the range from 0° to 10°C) contribute to the removal of physiological dormancy of the embryo, the consequence of which is its relatively rapid intrafamily growth.

Phytohormones, among them exogenous gibberelins such as GA₃, GA₄, GA₇, are widely used for dormancy termination and seed germination. Gibberellic acid plays a key role in dormancy termination and germination. To initiate seed germination and increase seed germination, it is necessary to select the exact concentration of the phytohormone used [19, 20].

The aim of this study is to investigate the effect of temperature and growth regulators on the germination of *T. auliekolica*, *T. turgaica* seeds. The germination requirements of these *Tulipa* reproduce in situ mainly by seeds, but the dormancy period of seeds results in a low germination rare species have not been studied previously, and we have attempted for the first time to optimize the seed germination protocol for the endemic species *T. auliekolica* and *T. turgaica* of Northern and Central Kazakhstan.

MATERIALS AND METHODS

Collection of source material of endemic Tulipa species of Northern and Central Kazakhstan (T. auliekolica, T. turgaica) for introduction into in vitro culture

The objects of research were seeds of endemic species of tulip plants - *T. auliekolica* and *T. turgaica*, collected in their natural habitats in Northern and Central Kazakhstan. To collect material of endemic *Tulipa* species, expeditions were made to the territory of Northern and Central Kazakhstan. The collections were made by scientists of the Astana Botanical Garden under the leadership of S.A. Kubentayev at the stage of mature seeds (June-July) in compliance with the principles of preserving natural populations - no more than 10% of seeds from the total number per individual.

The collected material underwent primary processing and was identified by Kubentayev, S.A. in the Botanical Garden of Astana (Kazakhstan). Herbarium specimens were stored in the Herbarium Fund of the Botanical Garden of Astana.

Morphological characterization of Tulipa plants

The analysis of morphological characters of *T. auliekolica* and *T. turgaica* plants was carried out by six quantitative characters in 10-fold repetition. Morphological characters such

as: plant height, bulb diameter, boll length, seed size, embryo length, embryo/seed length ratio were studied.

Seed size and embryo length were measured in triplicate using an Axioscope 5 microscope. Additionally, the ratio of embryo length to seed length was calculated as an important indicator of maturity and potential germination.

Determination of seed viability

Seed viability was determined using 10 g/L -1 solution of 2,3,5-triphenyltetrazolium chloride (2.3.5-TTC). Twenty seeds each were used in three repetitions.

The assessment was carried out visually under an Axioscope 5 microscope: completely stained red – viable; stained at least 2/3 of the basal part of the cotyledon – conditionally viable; unstained – non-viable.

Seed sterilization

Seeds were pre-incubated in soap solution with stirring on a laboratory rocker for 30 minutes, then washed three times with distilled water. Sterilization was carried out with 0.01% sulphur solution for 20 minutes followed by threefold rinsing with sterile distilled water [21].

Production of sterile seedlings under in vitro conditions

Sterile viable seeds were placed on media with half the composition of Murashige and Skoog mineral salts (MS) with the addition of 13 and 52 mg/l gibberellic acid (GA₃), ½ MS and distilled water were used as a control. Gibberellic acid was added after autoclaving by membrane filtration (0.22 µm filter). The pH of the media was adjusted to 5.8 before sterilization. For each variant, 20 seeds were placed in three repetitions. Seeds were considered germinated when the seed stalk appeared 1-2 mm long. Seed germination was recorded daily until the seeds stopped germinating. The observation was carried out for 60 continuous days. The percentage of germination (G) was calculated using the formula:

$$G = (N_p/N_t) \times 100\%, (1)$$

where N_p is the number of germinated seeds, N_t is the total number of seeds.

The time of 50% seed germination (T₅₀) was calculated using the following formula:

$$T_{50} = t_i + ((N+1)/2 - n_i)/(n_j - n_i) \cdot (t_j - t_i), (2)$$

where N is the final number of germinated seeds, n_i and n_j are the number of seeds germinated by the time points t_i and t_j, respectively (n_i < N/2 < n_j).

Temperature test

The seeds were cultivated at different temperature conditions: 4°C, 10°C and 20°C.

RESULTS

Collection of source material of endemic Tulipa species of Northern and Central Kazakhstan (T. auliekolica, T. turgaica)

As a result of expedition trips, plant material was collected (*T. auliekolica*, *T. turgaica*). The species *T. turgaica* is part of the fescue-wormwood steppe with the dominance of *Festuca valesiaca* Gaudin and *Artemisia schrenkiana* Ledeb, the species *T. auliekolica* is part of the wheatgrass-forb communities with the dominance of *Elytrigia repens* (L.) Nevski. The coordinates of the location of the populations of *T. auliekolica* are Kostanay region, Auliekolsky district, 10 km from the

turnoff to Karamendy on the road Karamendy - Auliekol (N 64.395259; E 51.983026) and *T. turgaica* - Kostanay region, Amangeldy district, the outskirts of the village of Tasty, near the mausoleum of Keiki-batyr (N 66.026724; E 50.486579)

Morphological characteristics of T. auliekolica and T. turgaica plants

Morphometric analysis revealed significant differences between the species *T. turgaica* and *T. auliekolica* (Table 1).

Table 1 - Comparative morphometric characteristics of the species *T. auliekolica* and *T. turgaica*

Sign	<i>T. auliekolica</i>	<i>T. turgaica</i>
Plant height, cm	11,0 ± 0,6	14,5 ± 0,8
Bulb diameter, cm	2,0 ± 0,3	1,6 ± 0,2
Capsule length, cm	3,5 ± 0,3	5,2 ± 0,4
Seed size, mm	5,0 ± 0,2	3,55 ± 0,3
Embryo length, mm	4,5 ± 0,3	2,8 ± 0,4
Embryo/seed length ratio	0,90 ± 0,05	0,79 ± 0,07

The species *T. turgaica* is characterized by a greater plant height of 14.5±0.8 cm, while in *T. auliekolica* this figure was 11.0±0.6 cm. At the same time, the bulb diameter in *T. turgaica* was smaller - 1.6±0.2 cm compared to *T. auliekolica*, which reached 2.0±0.3 cm. Significant differences were also noted in the sizes of the seed capsules: in *T. turgaica* their length was 5.2±0.4 cm, while in *T. auliekolica* it was 3.5±0.3 cm (Figure 1).



Figure 1 - Capsule and seeds of tulip species *T. auliekolica* (A); *T. turgaica* (B)

The seeds of *T. auliekolica* were significantly larger in size, 5.0 ± 0.2 mm, while in *T. turgaica* this figure was 3.55 ± 0.3 mm. The embryo of *T. auliekolica* was well developed, its average length was 4.5 ± 0.3 mm. The ratio of the embryo length to the seed length was 0.90 ± 0.05, indicating a high degree of seed maturity. In *T. turgaica* this ratio was lower, 0.79 ± 0.07. In addition, in some seeds of this species the embryo was not visually determined.

Determination of seed viability

Determination of the viability of seeds of the species *T. auliekolica* and *T. turgaica* allowed a quick and high-quality assessment of the percentage of live seeds. The principle of the method is based on the presence of dehydrogenase activity in viable seed tissues during respiration. Dehydrogenase can catalyze a colorless solution of 2,3,5-triphenyltetrazolium chloride to form the red dye formazan.

Tetrazol test showed high viability of seeds of both species. For *T. auliekolica*, 100% of seeds gave a positive reac-

tion (full staining), while for *T. turgaica* this indicator was 90%, the remaining 10% were conditionally viable seeds. It is worth noting that among the uncolored seeds of *T. turgaica*, seeds with a visually indistinguishable embryo predominated. The data obtained indicate high physiological activity of the seeds and confirm their suitability for subsequent in vitro culture studies.

Obtaining sterile seedlings in vitro.

To obtain sterile seedlings, seeds of both species were placed on ½ medium with the addition of different concentrations of gibberellic acid, and cultivation was carried out under different temperature conditions (from 4 to 20°C) (Figure 2).

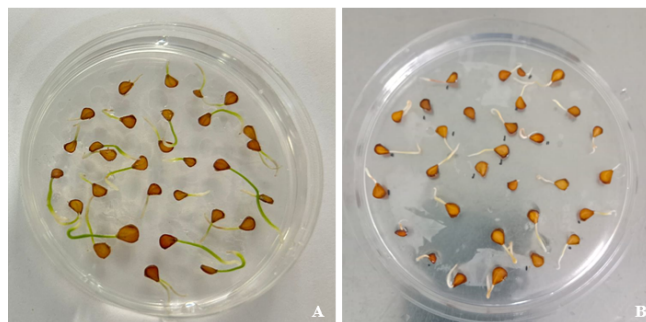


Figure 2 - Germination of *T. auliekolica* (A) and *T. turgaica* (B) seeds on ½ MS + 13 mg/L GA₃ medium

Analysis of the effect of temperature factors and phytohormones on the rate of seed germination showed species differences (Table 2).

Table 2 - Seed germination (%) depending on cultivation conditions

Cultivation conditions	<i>T. auliekolica</i>	<i>T. turgaica</i>
4°C		
½ MS	63 ± 2,1	12 ± 1,5
H ₂ O	60 ± 3,1	10 ± 1,2
½ MS + 13 mg/l GA ₃	72 ± 3,5	30 ± 2,8
½ MS + 52 mg/l GA ₃	68 ± 2,8	25 ± 2,1
10°C		
½ MS	51 ± 2,5	60 ± 3,2
H ₂ O	48 ± 2,1	55 ± 3,1
½ MS + 13 mg/l GA ₃	53 ± 2,8	65 ± 3,5
½ MS + 52 mg/l GA ₃	51 ± 2,3	58 ± 2,9
* Data on seed germination (%) at 20°C are not provided due to the absence of germinated seeds in both species.		

For *T. auliekolica*, a constant temperature of 4°C was found to be optimal, at which maximum germination (72%) was achieved on the ½ MS + 13 mg/l GA₃ medium. Increasing the GA₃ concentration in the cultivation medium to 52 mg/l reduced germination to 68%. On the control variants of ½ MS and water, the germination percentage was 63% and 60%, respectively. Increasing the temperature to 10°C led to a decrease in germination by 23-25%, and at 20°C the seeds did not germinate at all. For *T. turgaica* the picture was different: maximum germination (65%) was observed at 10°C on a medium with 13 mg/l GA₃, while at 4°C this figure did not exceed 30%. As with the species *T. auliekolica*, a tem-

Table 3 - Time to reach 50% germination (T50) under different conditions

Temperature conditions	Media	Germination rate T50, days	
		<i>T. auliecolica</i>	<i>T. turgaica</i>
4°C	½MS (control)	42,3	45,1
	H ₂ O (control)	44,8	47,2
	½MS + GA ₃ 13 мг/л	35,0	37,0
	½MS + GA ₃ 52 мг/л	37,5	38,4
10°C	½MS (control)	39,6	40,2
	H ₂ O (control)	43,0	42,5
	½MS + GA ₃ 13 мг/л	37,5	35,4
	½MS + GA ₃ 52 мг/л	38,8	39,0

perature of 20°C completely inhibited the germination of the species *T. turgaica*. Analysis of the time parameters of germination revealed interspecific differences in the rate of germination, assessed by the T50 indicator - the time to reach 50% of the maximum germination (Table 3).

For the analysis, ½MS nutrient medium and water were used as a control variant. The control with water showed delayed germination, which confirms the importance of macro- and micronutrients in the medium for successful seed germination. The concentration of 52 mg/L was less effective than 13 mg/L: in *T. auliecolica* plants, germination at 4°C (T50) increased from 35.0 to 37.5 days, while in *T. turgaica* at 10°C (T50) it increased from 35.4 to 39.0 days.

It was found that for the species *T. auliecolica* the minimum germination time (35 days) was recorded at a constant temperature of 4°C. An increase in temperature to 10°C increased T50 to 37.5. For *T. turgaica* the optimum germination temperature was 10°C (35.4). At 4°C a slowdown in germination was observed (38.0).

DISCUSSION

In the context of global anthropogenic impact on ecosystems, the problem of preserving endemic plant species is becoming especially urgent. Preserving rare and endemic plant species is a global problem, since traditional methods of in situ conservation do not provide their reliable protection.

In this regard, biotechnological approaches, in particular, in vitro cultivation methods, which allow to multiply and preserve valuable genotypes, are of particular relevance. The studied species *T. auliecolica* and *T. turgaica*, being narrow-areal endemics of Northern and Central Kazakhstan, are of special value as unique elements of steppe ecosystems.

In this work, the effects of temperature and growth regulators on seed germination of *T. auliecolica* and *T. turgaica* were investigated.

The aim of this study is to investigate the effect of temperature and growth regulators on seed germination of *T. auliecolica*, *T. turgaica*. The conditions of seed germination of these *Tulipa* species have not been studied before, and in this work for the first time an attempt to develop an optimal germination protocol for endemic species of *T. auliecolica* and *T. turgaica* in Northern and Central Kazakhstan.

The studies revealed significant differences in tempera-

ture preferences and response to gibberellic acid for these endemic species. The optimum temperature for *T. auliecolica* was 4°C (germination rate 72%), while for *T. turgaica* it was 10°C (germination rate 65%).

The high germination rate of the tulip seeds at these temperatures indicates their adaptation to early spring vegetation, which is typical for many steppes *Tulipa* species [22]. Of particular importance is the identified mechanism of thermodormancy (no germination at 20°C), which ecologically protects species from germination under unfavorable conditions [18]. According to IPCC data, average annual temperatures in Central Asia may increase by 2-3°C by 2050, posing serious threats to species with a narrow ecological amplitude [1].

The results obtained confirmed the key role of gibberellic acid in seed germination. The mechanism of action of GA₃ in this case can be explained by its ability to activate the processes necessary to overcome morphophysiological dormancy characteristic of many species of the genus *Tulipa*. The obtained results confirmed the key role of gibberellic acid in seed germination. The mechanism of action of GA₃ in this case can be explained by its ability to activate processes necessary to overcome morphophysiological dormancy, characteristic of many species of the genus *Tulipa*.

As our results show, the optimal concentration of GA₃ (13 mg/L) significantly increases seed germination of both species. This effect is particularly pronounced in combination with optimal temperature regimes for each species - 4°C for *T. auliecolica* and 10°C for *T. turgaica*. It is worth noting that a higher concentration of GA₃ (52 mg/l) demonstrated a less pronounced stimulating effect, which may be due to hormonal imbalance. According to literature, excessive concentrations of exogenous gibberellins can disrupt the fine regulation of endogenous phytohormones, in particular, cause a compensatory increase in the content of abscisic acid, the main inhibitor of germination [23].

The effect of GA₃ on mitigating dormancy and ensuring germination is consistent with current understanding of the role of gibberellins in overcoming seed dormancy in species [10].

The conducted research made it possible to determine the optimal conditions for germination of seeds of rare endemic species *T. auliecolica* and *T. turgaica*, growing in Northern and Central Kazakhstan. The obtained data are important for the development of effective *in vitro* reproduction protocols,

which opens up prospects for the conservation and restoration of populations of these unique representatives of the flora of Kazakhstan in the context of increasing anthropogenic

CONCLUSION

The conducted research allowed to determine the key factors affecting seed germination of endemic species *T. auliekolica* and *T. turgaica* of Northern and Central Kazakhstan.

It was found that the optimum temperature conditions for seed germination differed between species: for *T. auliekolica*, the most favorable temperature was 4°C, while *T. turgaica* demonstrated maximum germination at 10°C. The use of gibberellic acid (GA₃) at a concentration of 13 mg/l contributed to the acceleration of germination, while a higher concentration (52 mg/l) had a less pronounced effect.

Morphological analysis revealed differences between species, including seed size, embryo length and other parameters, which may be related to their adaptation to different environmental conditions. The tetrazolium test confirmed high seed viability (*T. auliekolica* – 100%, *T. turgaica* – 95%), indicating a good physiological condition of the embryos. This is an important condition for successful germination and subsequent micropropagation.

The obtained results have important practical significance for the development of effective methods for *in vitro* cultivation of these rare species. Optimization of GA₃ concentrations in combination with species-specific temperature conditions allows to significantly increase the efficiency of their *in vitro* cultivation, which is especially important for programs for the conservation of biodiversity of natural populations of rare, endemic species.

Thus, this work contributes to the conservation of endemic *Tulipa* species in Kazakhstan and can serve as a basis for developing strategies for their protection using biotechnological approaches.

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БИОЛОГИЯ ПРОРАСТАНИЯ СЕМЯН РЕДКИХ ВИДОВ ТЮЛЬПАНОВ В СЕВЕРНОМ И ЦЕНТРАЛЬНОМ КАЗАХСТАНЕ

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АННОТАЦИЯ

Казахстан является важной территорией произрастания тюльпанов, включая редкие эндемичные виды *Tulipa turgaica* и *Tulipa auliecolica*. Угроза их исчезновения требует применения биотехнологий, таких как микроразмножение *in vitro*, позволяющих сохранять ценные генотипы без вреда для природных популяций.

В процессе усиления изменения климата и антропогенного воздействия эти эндемичные виды подвергаются высокому риску исчезновения. В связи с этим изучение и сохранение биоразнообразия эндемичных видов растений считается глобальным приоритетом во всем мире.

Проращивание *in vitro* проводили на среде ½ MS с добавлением GA₃ (13 и 52 мг/л) и без него. Всхожесть регистрировали в течение 60 дней, рассчитывали всхожесть (%) и T₅₀.

По результатам тетразолиевого теста жизнеспособность семян *T. turgaica* и *T. auliecolica* составила 95% и 100% соответственно. Изучаемые виды показали разные температурные предпочтения: для семян *T. auliecolica* оптимальной температурой была 4°C, а для *T. turgaica* — 10°C. Кроме того, семена *T. auliecolica* проросли быстрее, чем семена *T. turgaica*. Результаты показали, что температура существенно влияет на прорастание семян. Семена обоих видов проросли только при низких температурах (4 и 10°C), при 20°C прорастание семян отсутствовало у обоих видов.

Полученные данные имеют важное практическое значение для создания эффективных методов культивирования *in vitro* этих редких видов тюльпанов.

Подбор оптимальных концентраций GA₃ в сочетании с температурными режимами существенно повышает успешность размножения *in vitro*, что играет ключевую роль в программах по сохранению биоразнообразия эндемичных растений.

Ключевые слова: сохранение биоразнообразия, эндемичные и редкие виды, регуляторы роста, культура *in vitro*, микроразмножение, *Tulipa*.

ӨОК 581.1

СОЛТҮСТІК ЖӘНЕ ОРТАЛЫҚ ҚАЗАҚСТАНДА СІРЕК КЕЗДЕСЕТІН ҚЫЗҒАЛДАҚ ТҮРЛЕРІ ТҰҚЫМЫНЫҢ ӨНУ БИОЛОГИЯСЫ

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ТҮЙІН

Қазақстанда қызғалдақ түрлерінің алуан түрлілігі жоғары, олардың көпшілігі жойылу қаупінде. Олардың жойылу қаупі табиғи популяцияларға зиян келтірместен құнды генотиптерді сақтауға мүмкіндік беретін *in vitro* микроөбейту сияқты биотехнологиялық тәсілдерді қолдануды қажет етеді.

Климаттың өзгеруі мен антропогендік әсердің күшеюі барысында бұл эндемикалық түрлердің жойылу қаупі жоғары. Осыған байланысты эндемикалық өсімдік түрлерінің биоалуантүрлілігін зерттеу және сақтау бүкіл әлемде жаһандық басымдылыққа ие.

Жұмыстың мақсаты - *T. auliecolica*, *T. turgaica* тұқымдарының өнуіне температура мен өсу реттегіштерінің әсерін зерттеу, оларды *in vitro* культурасына енгізу.

In vitro культурасына енгізу GA₃ (13 және 52 мг/л) қосылған және қосылмаған ½ MS орталарында жүргізілді. Өсіру 60 күн бойы бақыланды, өну (%) және T₅₀ есептелді.

Тетразолиум сынамасының нәтижесі бойынша *T. turgaica* және *T. auliecolica* тұқымдарының өміршеңдігі сәйкесінше 95% және 100% құрады. Зерттелген түрлер әртүрлі температуралық артықшылықтарды көрсетті; *T. auliecolica* тұқымдары үшін оңтайлы температура 4°C, ал *T. turgaica* үшін 10°C болды. Сонымен қатар, *T. auliecolica* тұқымдары *T. turgaica* тұқымдарына қарағанда тез өнген. Алынған нәтиже температураның тұқымның өнуіне айтарлықтай әсер ететінін көрсетті. Екі түрдің тұқымдары тек төмен температурада (4 және 10°C) өніп шықты, 20°C-та тұқымның өнуі екі түрде де болмады.

Алынған мәліметтердің осы сирек кездесетін қызғалдақ түрлерін *in vitro* жағдайында өсірудің тиімді әдістерін жа-

сау үшін маңызды практикалық маңызы бар.

Оңтайлы GAз концентрациялары мен температуралық режимдермен үйлестіре таңдау эндемикалық өсімдіктердің биоалуантүрлілігін сақтау бағдарламаларында шешуші рөл атқарып, *in vitro* көбеюдің табыстылығын айтарлықтай арттырады.

Кілтті сөздер: биоәртүрлілікті сақтау, эндемикалық және сирек кездесетін түрлер, өсу реттегіштері, *in vitro* культурасы, микрокөбейту, *Tulipa*.