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INTRODUCTION OF EXPLANTS AND REPRODUCTION ON NUTRIENT MEDIUM OF DONOR MATERIAL *IN VITRO* VARIETIES OF *CALLISTEPHUS CHINENSIS (L.) NESS.* FOR ITS FURTHER USE IN LANDSCAPING

The relevance of the research topic. On the recent methods of biotechnology are increasingly used in plant breeding and seed production. Herbaceous plants such as strawberries, potatoes, a vegetable, some medicinal and others are capable of vegetative propagation the traditional methods of culture, successfully introduced in both in vitro and can achieve a high rate of reproduction.

Modern plant biotechnology – the sum of the technologies developed in molecular and cell biology of plants – a new stage in the development of the technology of plant breeding.

With these improved characteristics may occur at the level of individual genes and individual genes that determine a specific trait, can be identified. They may be the final selection, they can be isolated, insert, delete, or modify the genotype or variety.

Goal. Identify the features of the manifestation of economically valuable features and decorative properties of Callistephus chinensis and the inclusion of the best varieties in the biotech link, their adaptation to the conditions of the Forest-Steppe of Ukraine and their further use in landscaping. **Methods.** Laboratory – determination of seed germination; mathematical and statistical - for processing the reliability of the obtained research results. **Results.** The nutrient medium for growing plant tissues and cells, by analogy with the medium for culturing animal tissues, should contain all that the tissues in the plant organism receive from xylem and phloem currents of substances. However, in practice it has been found that vegetable juices cannot serve as a complete nutrient medium for growing isolated tissues and cells. This manifests the specificity of the receipt, transportation and especially the redistribution of nutrients in the plant.

Based on the analysis, research was conducted to study the possibility of mass off-season vegetative propagation of plants of Callistephus chinensis in vitro. Practical recommendations on the selection of sterilizer, sterilization, nutrient medium and for the adaptation period of the best genotypes of this culture have been developed.

As a result of the conducted researches the methods of selection of the initial plant material of Callistephus chinensis (Callistephus Chinensis (L.) NEES) and its surface sterilization, modification of existing aseptic culture methods have been studied and mastered. The morphogenetic potential of explants from different plant organs was investigated and selection of nutrient medium and study of the influence of plant growth regulators and physical parameters on the process of morphogenesis was carried out. The features of regeneration of isolated explants depending on the composition of the nutrient medium and selection of conditions for obtaining self-clones of Callistephus chinensis (Callistephus Chinensis (L.) NEES) were studied.

Key words: in vitro, plant biotechnology, Callistephus Chinensis, nutrient medium, rhizogenesis.

Introduction. The signing by Ukraine of the Association Agreement with the European Union requires a review of the strategic priorities for the development of the main sectors of the national economy, especially those that base their activities on the use of natural resources and influence the formation of the assimilation potential of the territories [1].

Biotechnology techniques have been increasingly used in plant breeding and seed production over the last decades. Herbaceous plants such as strawberries, potatoes, many vegetables, some medicinal plants and others capable of vegetative propagation by traditional methods of culture, successfully introduced in vitro and can achieve high rates of reproduction. However, accelerated reproduction of deficient genotypes in vitro makes sense only when in the process of microcloning, the heredity of the breeding individual remains intact [2].

Modern plant biotechnology – is the sum of the technologies developed from molecular and cellular plant; it is a new stage in the development of plant breeding technology.

Using these technologies, feature improvement can occur at the level of an individual gene, and the separate genes that define some feature can be identified. Genes can be sampled, they can be isolated, introduced, deleted or modified in the genotype of the plant or variety. The contribution of biotechnology to various fields, including floriculture, is to facilitate the traditional methods of plant propagation and to develop a new technologies that make it possible to increase the efficiency of agriculture. Genetic and cellular engineering methods were created resistant to pests, diseases, herbicides genotypes. The technique of plant healing from the accumulation of infections has been developed, which is especially important for vegetatively propagated crops (potatoes, etc.). One of the actual problems is the ability to manage the nitrogen fixation process, including including the possibility of introducing nitrogen fixation genes into the useful plant genome, as well as managing photosynthesis processes. Studies are underway to improve the amino acid composition of plant proteins, new plant growth regulators, microbiological plant protection products against pests and diseases, bacterial fertilizers are being developed [3].

Plant cells in vitro retain biosynthetic potential inherent in plants in vitro and can be a source of economically important cell metabolism products. This feature of cultured plant cells is used for for the creation of technologies for the industrial production of valuable substances [4].

In addition to the biosynthesis of important compounds, cultured cells are capable of biotransformation, that is, they can turn cheap recycling or waste products into valuable products. Another unique feature of cultured cells is their totipotency which enables the creation of unconventional technologies for agriculture that facilitate and accelerate the breeding process as well as plant reproduction.

The universal nature of modern biotechnology is manifested in the widespread use of cellular and genetic engineering methods, that are based on the development of molecular genetics, which provides great opportunities for the genetic reconstruction of living organisms in the directions desired for researchers. The main purpose of these studies is to obtain through genetic reconstruction the greatest possible diversity of organisms that could be used not only for the production of qualitatively new products, but also for the processing of various organic and inorganic substances [5].

Flowering plants in which the period of growth and development from sowing of seeds to harvesting takes place during one growing season are attributed to annuals. These plants also include flowering plants, which by their biological characteristics are perennial, but under different conditions of cultivation their ontogeny takes place over one year [6].

The genus Callistephus chinensis (L.) Ness. comes from the Far East of Russia, northern and northeastern regions of China, as well as Mongolia and Japan. Systematics of the genus Callistephus chinensis (L.) Ness. varied over a long period of cultivation. The aster is still preserved there in the wild, it grows mainly on rocks and clay-stony soils of the southern mountain slopes in the area of deciduous forests [7].

In botany literature, this plant species was mentioned and described under different synonyms, namely Aster hortensis L., Callistemma hortense Cass., Callistephus hortensis Cass., Diplopappus sinensis Less. [8]. This species was first described by Karl Linnaeus, it was initially attributed to the genus Aster L. In 1826 N. Cassini separated it into a separate species Callistemma Cass., which was later renamed Callistephus. The modern name of the species – Callistephus chinensis gave the aster the annual Neess (Ness) [9]. According to modern scientific ideas of the genus Callistephus Cass. belongs to the order Asterales Link, family Asteraceae Bercht. et J Presl [10].

The aster belongs to the Compositae family (asters) (Asteraceae). The botanists gave it the name Calistephus Chinese (Callistephus chinensis), however, worldwide this flower is called aster Chinese (Callistephus chinensis (L.) Ness.) or annual aster. It is an annual plant of the above mentioned family. In Greek, its name means "star". Ancients believed that asters emerged from the star dust that fell from the sky. Nowadays, this beautiful plant is rightly one of the most popular autumn flowers, the most favorite

garden culture on private plots in townspeople. It is appreciated for the generous autumn flowering, the variety of colors and forms of inflorescences, which, due to the fact that their central part consists of yellow tubular flowers, at the edges surrounded by long wavy, really resemble a wreath [11-13].

Astra belongs to the Angiosperm Class, Dicotyledonous, Asteraceae family (Compositae). Plants of this family are spread on all continents and climatic zones. The Aster family has 1300 genera and more than 20000 plant species [14].

Annual aster – a herbaceous plant with a wide-stemmed root system. It has a sufficiently large seedlings - within 1 cm with flat cotyledons. Most varieties have oval cotyledons, rosy asters – spherical, in varieties of Unicum – elongated. The stem of aster is green (usually in varieties with light-colored inflorescences) or reddish(in varieties with dark-colored inflorescences), hard, erect, often pubescent. There are longitudinal sulcuses all over the surface of the stem. The thickness of the stems in different varieties is different. According to the N.A. Petrenko classification (1973) [15,16], giant asters have a height of up to 100 cm. Tall asters grow up to 80 cm. Asters up to 60 cm tall belong to the middle-aged. Short-stemmed asters have a height of 35 cm, dwarfs – 25 cm.

Depending on the group, the branch of the bush in asters is different. Tall and undersized asters branches more than the medium-sized asters. According to the number of 1st order shoots, asters are divided into small branches – up to 4 shoots, such as Radio, Ostrich Feather, sufficiently branched – 5-7 shoots (most garden groups), heavily branched – up to 10 shoots (Pompon, Lilliput) and extremely branched - more than 10 shoots (Ambria, Waldersee). The branching of the stem starts from the top and has a pronounced sympodial character [17-19].

Materials and methods of research. In our studies, we used 20 varieties of Calistephus Chinese with various important features, origins and directions of use. The characteristics of the varieties are shown in table 1.

Nutrient medium are prepared in a separate room that has lab tables, reagent storage cabinets, refrigerators for the storage of concentrated solutions of nutrient medium, analytical, technical and torsion scales, pH meters, electric or gas cookers, water baths, magnetic mixers.

Table 1 – Characteristics of the starting material of the studied varieties of Calistephus Chinese, 2016–2018

№	Variety	Origin	Sortotype	Productivity, g / bush	Direction of use
1	King Size	Germany	Peonies-shaped	3,0-4,0	universal
2	Anastasia	IH NAAS		3,0-3,5	universal
3	Anastasia	IH NAAS		3,0-3,5	universal
4	Salmon Turm	Germany		2,5-3,0	universal
5	Oksana	IH NAAS		2,5-3,0	universal
6	Odarka	IH NAAS		3,5-4,0	on a cut
7	Hilda	Germany	Princess	4,5-5,0	on a cut
8	Princess (red)	IH NAAS		to 6	on a cut
9	Alexandra	Germany		4,5-5,0	on a cut
10	Raspberry layer	Russia	Pompony-shaped	to 6	universal
11	Winter Cherry	Western Europe		2,0-2,5	universal
12	Blue Moon	Western Europe		2,0-2,5	on a cut
13	Sophia	IH NAAS	Artistic	3,0-3,5	universal
14	Swan Lake	IH NAAS	Artistic	2,0	on a cut
15	Esmeralda	Germany	Spherical	3,0-3,5	on a cut
16	Velvet	IH NAAS		2,0-2,5	universal
17	The Gray Lady (blue)	Russia	Duchess	2,5-3,0	on a cut
18	Vesnyanka	IH NAAS	Rose-shaped	4,0	universal
19	Snizhana	IH NAAS	Laplata	3,0	on a cut
20	Amber	IH NAAS	American Bushes	3,5	on a cut

The room for sterilization of mediums, instruments and utensils is equipped with vertical (VK-60, VK-75) and horizontal (GK-100, AG-100) autoclaves, with drying cabinets for sterilization by dry heat.

For work in aseptic conditions use laminar box, which injected sterile air that passes through a bacterial filter. The laminates are placed in a separate room. If there are no laminar boxes, then equip a special room-box – operating room. The walls of such a room are tiled, the floor is covered with linoleum. Operating doors should be closed hermetically and there should be a tambour (pre-box) in front of them. The box should be supplied with sterile conditioned air. Boxing is equipped with bactericidal lamps. In the operating room placed that are covered with easy-to-wash material, medical cabinets, binocular microscopes, tools, alcohols.

Isolated tissues are cultured in a thermostated room (cultural room) with conditioned air, controlled temperature (22-28°C) and humidity (70-80%). Regulation of light mode is provided by time switch.

The laboratory premises are equipped with the equipment necessary for biochemical, histo- and cytogenetic and other studies related to basic plant tissue cultivation works.

Research results. The nutrient medium for growing plant tissues and cells, by analogy with the medium for culturing animal tissues, should contain all that the tissues in the plant receive from the xylem and phloem current substances. However, in practice it has been found that floral juices cannot serve as a complete nutrient medium for growing isolated tissues and cells. This manifests the specificity of the receipt, transportation and especially the redistribution of nutrients in the plant.

The basis for the selection of different mediums for the culture of plant tissues were nutrient solutions that were used in growing whole plants. The method's founders, R. Gautre and F. White, used the Knop nutrient mixture and the Uspenskykh solution, respectively.

These solutions were supplemented with sugars, trace elements, vitamins. R. Gautre also reduced the concentration of the mineral salts of the Knop original solution by half. F. White's medium, created in 1943 on the basis of the Uspenskykh solution, it was used first to grow isolated root culture and then unchanged to grow plant tissues.

With the development of the method of tissue culture and the introduction into the culture of new and new plant tissues of different species, it became necessary to change the composition of nutrient medium. The most complete study of tissue mineral nutrition was conducted by R. Heller in 1953. To achieve correctness, he refused from solid agar medium and grew tissues only in liquid nutrient medium. R. Heller studied in detail the importance of individual ions for the nutrition of tissues and the effect of their exclusion from the composition of the medium on the further tissue growth during transplantation. He proposed a nutrient medium whose composition was significantly different from the medium used for whole plants and from medium previously used for the cultivation of isolated tissues. This medium is very rich in K+ and P +. R. Heller also proposed a new composition of a mixture of trace elements.

As a result of a series of studies, the requirements of tissues for sources of carbohydrate nutrition, vitamins, and physiologically active substances were clarified.

Cultivation of plant organs, tissues and cells, most of which are modifications of the basic nutrient medium (White, Hamburg, Murashige and Skug). The composition of any nutrient medium for growing a culture of isolated plant tissue includes the following groups of substances: mineral salts - macro and microelements; carbohydrates; vitamins; amino acids; growth promoters of synthetic and natural origin; water; agar.

Due to the need to change the composition of the nutrient medium when finding the optimum for new, yet untested in culture tissue, it is worth to stop on the characteristics of the nutritional characteristics of isolated tissues.

Weighed microbiological agar was poured in half with distilled water and heated to 90-100°C.

Dissolved agar was added to the components of the nutrient medium, also dissolved in distilled water.

The medium was filtered, took the required volume, normalized to pH, poured into culture vessels and autoclaved.

To accelerate the work pre-prepared concentrated solutions of salts, which were then mixed in the preparation of the nutrient medium in accordance with the Murashige-Skug recipe (solutions 1-4).

In this regard, we aimed to study the impact of growth regulators on the development of Calistephus Chinese explants. Auxins and cytokinins were added at different concentrations to different medium, which are shown in table 2.

Table 2 – Composition supplements to the prescriptions of standard nutrient medium, 2016-2018

№	Medium	Growth regulators, concentration %	Medium code
1	MS+6BAP	0,5 1,0 1,5	MS–1 MS–2 MS–3
2	B_5+6BAP	0,5 1,0 1,5	B5-1 B5-2 B5-3
Note: MS - medium of Murashige and Skug (the composition of the medium in applications); B5 - medium of Hamburg (the composition of the medium in applications).			

This table shows the composition of additions to the prescriptions of standard nutrient medium used in the variants of the experiment. The basis of the nutrient substrate was introduced macro- and micronutrients according to the prescriptions of the medium Murasige-Skug and Hamburg.

Modified nutrient substrates by growth regulators of auxins and cytokinins to study concentrations and the ratio of exogenous regulators to the development of Calistephus Chinese explants.

Subsequent work has analyzed the growth and development of seeds of different varieties of Calistephus Chinese in different nutrient medium.

Analyzing table 3, we can conclude that the development of seeds of different varieties of Calisthephus Chinese in different mediums was uneven. The MS-2 and B5-2 variants of mediums were identified as the best substrates for explant development, on average by genotype. The MS-2 medium contributed to the seed development at the level of 96.0% and the B5-2 medium - 88.0%. These variants of the experiment had the same content of growth regulators, but their numbering depended on the time of introduction into the culture. Describing the composition of the nutrient medium, it should be noted that this similar balance of growth regulators had a positive hormonal effect on the development of Calisthephus Chinese explants.

Table 3 – Impact of growth regulators on the development of macrostructures, 2016-2018

Medium code	Number of planted material	Seed development		Stationary explant condition		Explant necrosis	
		pieces	%	pieces	%	pieces	%
1	2	3	4	7	8	9	10
MS-1	100	24	24	48,0	48,0	28,0	28,0
MS-2	100	96	96	2,0	2,0	2,0	2,0
MS-3	100	2,0	2,0	92,0	92,0	6,0	6,0
B_5-1	100	15,0	15,0	45,0	45,0	40,0	40,0
B_5-2	100	88,0	88,0	12,0	12,0	0,0	0,0
B_5-3	100	12,0	12,0	32,0	32,0	56,0	56,0

During the period of 10-12 days the volume of biomaterial planted on the nutrient substrate increased in 1.5-2.0 times. This indicates that the phytohormones introduced into the substrate accelerate the period of mitotic cycles passage.

However, in other variants of the experiment, the development of seeds of Calisthephus Chinese plants was not significant and ranged from 2.0 to 24%. At the same time, the steady state of the explant was almost the same for MS-1 and B5-1 mediums, in B5-1 medium this indicator was slightly lower and amounted to 32.0%. It should be noted that on MS-2 medium seed development, steady state of explant and necrosis of explant were at the same level - 2%. As for loss of the material (explant necrosis), the highest rates were in variants B5-1 and B5-3, respectively, with values from 40.0 to 56.0%. The average material loss rate was recorded on MS-1 medium and was 28%.

Therefore, as a result of the experiment, a nutrient medium was selected to activate the development of Calisthephus Chinese plants in vitro. That is, for the rapid growth and development of Calisthephus Chinese plants, the material requires the introduction of high average concentrations of auxins into the nutrient substrate.

It is well known that during the formation and growth of roots there are a number of caused by each other different biochemical, physiological and histological processes. Among anatomical factors, the formation of root primordia is facilitated by the cell's proximity to vascular tissues. The viability of rooted in vitro plants is largely determined by the rooting site. In some embodiments even with sufficient in vitro rooted test plants observed almost complete loss of plants under non-sterile conditions. In the process of rooting distinguish three main, specific for nutritional requirements and cultivation conditions, stages: induction, initiation, root growth. With a successful combination of the composition of the environment and the conditions of rooting and bringing them into line with the genotype of the plant, the duration of the first two stages is 10-15 days [19].

For accelerated root formation, the plants are planted in a nutrient medium for rhizogenesis. The nutrient medium should contain high concentrations of auxins. This is the basic rule of inducting root formation [20].

Under the influence of auxins (NOCs, hetero-auxins), the division of parenchyma cells is stimulated, which leads to differentiation of the root rudiments of the basal tissue [21].

To increase the activity of risogenesis, we excluded cytokines and added higher concentrations of gibberel acid to the nutrient medium. This made it possible to obtain an extension of the shoot even in the breeding medium.

In our studies, we used 5-7 mm biomaterial from the initial growth of the plant material. As can be seen from the data in table 4, the best medium for rooting plant material of Calisthephus Chinese was MS-2, since, in the breeding medium, we observed a single rhizogenesis.

Table 4 – Influence of indolylacetic and indolyl butyric acid on rooting Calisthephus Chinese plant material in vitro (on the average by genotype), 2016-2018

№	Medium	Growth regulators, concentration %	code
1	MS+IOK	0,5 1,0 1,5	MS–1 MS–2 MS–3
2	MS+IMP	0,5 1,0 1,5	MS–4 MS–5 MS–6
3	B_5+IOK	0,5 1,0 1,5	B5-1 B5-2 B5-3
4	B_5+IMK	0,5 1,0 1,5	B5-4 B5-5 B5-6

6-BAP was excluded from this medium and modified with indolylacetic acid - IOC (1.5-2.5%). The highest results were obtained with the introduction of 1.0 mg / l of indolylacetic acid. This allowed us to follow the relevant pattern. For 16-18 days (on average on repetitions) from 100 pieces of plants planted for rhizogenesis, 96% of the material formed roots. Concentrations of 1.5 and 2.5 mg / l gave a slightly smaller number of rooted plants (4 to 36, which is 12 and 78%) over a longer period (5 to 22 days).

That is, a concentration of indolylacetic acid up to 1.0 mg / l is optimal for the rhizogenesis of Chinese calisthephus plants (shorter period of time).

Therefore, the nutrient medium were improved with different concentrations of indolylacetic and indolyl butyric acid, and it was proved that the concentration of 1.0 mg / l is optimal for the rhizogenesis of Calisthephus Chinese plants for a shorter period of time.

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АЛДАҒЫ УАҚЫТТА КӨГАЛДАНДЫРУ МАҚСАТЫМЕН *CALLISTEPHUS CHINENSIS (L.) NESS.* СОРТЫНА ЖАТАТЫН *IN VITRO* ДОНОРЛЫҚ МАТЕРИАЛЫНЫҢ АЗЫҚТЫҚ ОРТАДА ТАРАЛУЫН ЗЕРТТЕУГЕ КІРІСПЕ

В. В. Полищук[1], С. Я. Турчина[1], А. Ф. Балабак[1], И. В. Козаченко[1], В. В. Мамчур[1], Л. М. Карпук[2], Т. В. Полищук[3]

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ВВЕДЕНИЕ ЭКСПЛАНТОВ И РАЗМНОЖЕНИЕ НА ПИТАТЕЛЬНОЙ СРЕДЕ ДОНОРСКИХ МАТЕРИАЛОВ *IN VITRO* СОРТОВ *CALLISTEPHUS CHINENSIS (L.) NESS.* С ЦЕЛЬЮ ДАЛЬНЕЙШЕГО ИСПОЛЬЗОВАНИЯ В ОЗЕЛЕНЕНИИ

Актуальность темы исследования. На протяжении последнего времени методы биотехнологий находят все больше применение в селекции растений и семеноводстве. Травянистые растения, такие как земляника, картофель, многие овощные, некоторые лекарственные и другие, способны к вегетативному размножению традиционными методами культуры, успешно вводятся в in vitro и могут достигать высоких показателей коэффициента размножения.

Современная биотехнология растений – сумма технологий, которые развиваются из молекулярной и клеточной биологии растений, – новая стадия в развитии технологии селекции растений.

С помощью этих технологий улучшение признаков может происходить на уровне индивидуального гена, а отдельные гены, определяющие определенный признак, могут быть идентифицированы. По этим показателям может быть проведен отбор, их можно изолировать, ввести, удалить или модифицировать в генотипе растения или в сорте.

Цель. Выявить особенности проявления хозяйственно-ценных признаков и декоративных свойств калистефуса китайского и включения лучших сортов в биотехнологическую звено, их адаптация к условиям Лесостепи Украины и дальнейшее использование в озеленении. **Методы.** Лабораторный – определение всхожести семян; математически-статистический – для обработки достоверности полученных результатов исследований. Для проведения работ в асептических условиях используют ламинарный бокс, в который нагнетается стерильный воздух, проходящий через бактериальные фильтры. Ламинар размещают в отдельной комнате. **Результаты.** Питательная среда для выращивания растительных тканей и клеток, по аналогии со средой для культивирования тканей животных, должна содержать все то, что ткани в растительном организме получают от ксилемного и флоемного потока веществ. Однако, на практике выяснилось, что растительные соки не могут служить полноценной питательной средой для выращивания изолированных тканей и клеток. В этом проявляется специфика поступления, транспортировки и особенно перераспределения питательных веществ в растении.

На основе анализа было проведено исследование по изучению возможности массового позасезонного вегетативного размножения растений калістефусу китайского через in vitro. Разработаны практические рекомендации по подбору стерилизатора, стерилизации питательной среды и адаптационного периода лучших генотипов этой культуры.

В результате проведенных исследований изучены и освоены методики отбора исходного растительного материала калистефуса китайского (Callistephus Chinensis (L.) NEES). Определена его поверхностная стерилизация, проведена модификация существующих методов получения асептической культуры. Исследованы морфогенетичний потенциал эксплантов из различных органов растений и проведен подбор питательных сред и изучение влияния регуляторов роста растений и физических параметров на процесс морфогенеза. Изучены особенности регенерации изолированных эксплантов в зависимости от состава питательной среды, и подбор условий получения самоклонов калистефуса китайского(Callistephus Chinensis (L.) Nees).

Ключевые слова: in vitro, биотехнология растений, калистефус китайский, питательные среды, ризогенезу.

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