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BIOTECHNOLOGICAL APPROACHES FOR CONSERVATION OF THE ENDANGERED SPECIES *Crambe koktebelica* (Junge) N. Busch AND EFFECT OF ASEPTIC *in vitro* CULTIVATION ON ITS BIOCHEMICAL PROPERTIES

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The aim of the study was to establish efficient protocols of seed surface sterilization with further multiplication *in vitro* for threatened species *Crambe koktebelica* (Junge) N. Busch and to show the effect of biotechnological approach (*in vitro* cultivation) of biodiversity conservation on plants biochemical properties. Seed surface sterilization was carried out according to the original method with further microclonal multiplication of aseptic sprouts from lateral buds on the Murashige and Skoog (MS) medium supplemented with different concentrations of growth regulators. Fatty acid content was determined using Gas chromatography-mass spectrophotometry of fatty acid ethers. Antioxidant activity was determined using 2.2-diphenyl-1-picrylhydrazyl radical scavenging method. Total soluble protein content was measured using Bradford method and polyfructan content determination was based upon ketosugars ability to color in the acidic environment with resorcinol. Plants that were grown under *in vitro* and *in vivo* conditions and seeds were used in this research. Efficient protocol of surface sterilization that resulted in 45% of aseptic seed material 50% of which has sprouted was elaborated for *C. koktebelica* as well as fast microclonal multiplication methods that provided with up to 5.25 ± 0.50 new formed plantlets from 1 lateral bud (on the MS medium that contained 1 mg/L of 6-benzylaminopurine). It was also shown that aseptic cultivation benefits to saturated fatty acid accumulation and increases protein content but on the other hand it reduces unsaturated fatty acid amount and polyfructan content as well as antioxidant activity of plant material. Obtained data confirms the prospect of biotechnology approach to biodiversity conservation and suggest the necessity of father *in vitro* cultivation effect on biochemical composition of plant study.

Key words: *Crambe koktebelica*, biodiversity conservation, *in vitro* cultivation.

The *Crambe* L. genus belongs to *Brassicaceae* family and consists of about 44 species. These species are annual, biennial or perennial and have diverse application: as vegetable or forage plants, as oilseed, as the source of biofuels (seeds have up to 60% of erucic acid), in Food Industry for making pastry, in paint and varnish industry, in chemical industry [1]. Nowadays all studies on species of *Crambe* genus were focused on *C. abyssinica* cultures which is proven to be a valuable biofuel source due to: it doesn't hybridize with any known oilseeds which eliminates gene flow issues [2]; seeds have high content of slowly-drying oil with low Iodine number, rich with erucic acid; it has high crop capacity and low demands for soil quality; it is drought-resistant and has short vegetation period [3].

A number of *Crambe* genus species are threatened so they require conservation measures. *In vitro* techniques need a small

number of plants and result in a relatively high propagation coefficient even for the species which have problematical *in situ* and *ex situ* reproduction and do not depend on the climatic conditions. These methods provide a long time conservation of plant species outside their natural habitats (in seed banks, under introduction conditions, in *in vitro* collections and cryobanks) with thorough study possibility and can be a significant addition to the global plant biodiversity conservation system [4]. *In vitro* culture conditions clearly differ from *in vivo* conditions: no seasonal temperature, humidity and photoperiod duration changes, exclusion of fungal and bacterial contamination, etc. At the same time, microclonal propagation process, an important step for multiplying the initial number of individuals, could lead to some gene expression changes due to the use of exogenous hormones (kinetin and auxins) [5–6]. *In vitro* and *in vivo* cultivated plants biochemistry comparison could lead to better understanding of the aseptic conditions influence on plant secondary metabolites synthesis and accumulation processes and could also estimate the efficiency of *in vitro* culture methods application for fast gaining of raw materials with high content of secondary metabolites.

The *Brassicaceae* family plants biochemistry study is a topical task due to high economic value of this family. A number of this family species is known to play a significant role in the global oil and biofuel production [7]. Very-long-chain FA analysis is an important task for the researches as these acids are components or precursors of numerous specialized metabolites synthesized in specific cell types [8]. FA are also essential for plant natural processes and are widely used in medicine [9].

Biodiesel is a kind of biofuel which is manufactured mostly from vegetable oil (rapeseed, palm and coconut oils). It has some benefits over traditional types of fuel — it isn't toxic, it decomposes under natural conditions in relatively short terms, has almost no sulfur and benzene and is obtained from renewable materials. Taking into account all the benefits of the biofuel its active investigation and application in the USA, Japan, China, Canada and in the EU countries is understandable. International Energy Association predicts the rise of biofuel production up to 150 million tons of the oil energy equivalent till 2030. Ukraine is in beneficial conditions for biofuel production from agricultural materials. Total biodiesel volume that can be manufactured in Ukraine could reach 500 thousand tons that can ensure 60% of our country fuel needs and 10% of petrol needs [10].

It is known that quantitative and qualitative composition of lipids and FA as the main structural components of vegetative membranes is known to play an appreciable role in a vegetative organism's adaptive response to the stress of any nature [11]. The analysis of FA content could be useful for searching species that would be valuable for agriculture due to their high resistance to low temperatures and for understanding of the plant adaptation process to unfavourable environmental conditions.

The aim of the study was to establish efficient protocols of seed surface sterilization with further multiplication *in vitro* for threatened species *Crambe koktebelica* (Junge) N. Busch and to show the effect of biotechnological approach (*in vitro* cultivation) of biodiversity conservation on plants biochemical properties.

Materials and Methods

C. koktebelica — endemic species of the flora of Ukraine [12, 13] was used for this research. The most conserved in *Crambe* genus *C. koktebelica* is listed in the Red data book of Ukraine (Rare) [13], IUCN Red List of Threatened Species (Data Deficient (EU), Not Evaluated (EU 27) [14], European Red List of Vascular Plants (Data Deficient (EU), Not Evaluated (EU 27) [15] and Convention on the Conservation of European Wildlife and Natural Habitats [16].

Seed surface sterilization is the first step in establishing aseptic culture. Seeds were first washed under running water for couple min, then hard external covering of seeds was removed. After that explants were put into 70% ethanol for 60 seconds, and then into diocidum for 3 min. After establishing aseptic conditions explants were washed with water for 5 min three times. Prepared seeds were placed in hormone-free solid MS medium [17] at 24 °C with a 16-h photoperiod.

To study different growth regulators effect on microclonal propagation process 1–1.5 month old aseptic plants were cultivated on the solid MS medium complemented with different concentrations of 6-Benzylaminopurine (BAP) (0.3 — 1 mg/l), 1-Naphthaleneacetic acid (NAA) (0.5 — 2 mg/l) or kinetin (0.1–1.5 mg/l). Plant response to the growth regulators presence in the medium observations has been carried out in 30 days after transferring plants to the

fresh medium. Microclonal propagation was done by dividing plants that were formed on sprouts. Each experiment was repeated twice with three replicates.

For GC/MS analyses seeds and fresh apical leaves from plants grown under *in vitro* (on hormone-free solid MS medium [17] at 24 °C with a 16-h photoperiod and recurrent transplantation on the fresh medium every 30 days) and *in vivo* (plant material was gathered in may (average monthly temperature 21 °C)) conditions were used. *In vivo* plants were grown in Gryshko National Botanical Garden and provided by prof. Rakhmetov D. B. Seeds and *in vitro* plants were obtained from seed bank and *in vitro* collection of the Institute of Cell Biology and Genetic Engineering of National Academy of Sciences of Ukraine and also provided by research assistant (M. S. Kalista) of National Museum of Natural History of the National Academy of Sciences of Ukraine.

Gas Chromatography-Mass Spectrophotometry of fatty acid ethers. FA extraction and methylation were conducted stepwise accordingly to Garces R. [18]. 50 mg of seeds and 200 mg of fresh leaves were used for extracts preparation. Seeds samples were ground in pounder and leaves were cut with defatted scissors. Then material was moved to glass tubes with spin caps and teflon gaskets. Reaction mixture which consisted of methanol: toluene: sulfuric acid (volume ratio 44:20:2) was added to the plant material first. Then, 1.7 ml of hexane was added (methanol, toluene, hexane — HPLC-grade, Sigma-Aldrich, Germany; sulfuric acid — chemically pure, Alfarus, Ukraine). Tubes were kept in water bath at 80 °C for 2 hours and then after cooling down to room temperature were gently shaken which led to separation of the liquid into two phases. Upper phase which contained concentrated methyl fatty acid ethers was gathered. The acidity of the solution was adjusted to neutral pH with saturated solution of 1M sodium phosphate. FA composition was determined using GC/MS system Agilent 6890N/5973inert (Agilent Technologies, USA) with capillary column DB-FFAP (length — 30 m; inner diameter — 0.25 mm; stationary phase thickness — 0.25 μm). Chromatographic fractionation occurred in gradient mode from 150 to 220 °C with a temperature gradient of 2°/min. Helium was used as a carrier gas with flow rate of 1 ml/min. Identification was done using mass spectrum library NIST 02 and standard bacteria methyl fatty acid ethers solution (Supelco). Heptadecanoic acid ($C_{17:0}$) (chemically pure, ABCR, Germany) was used as an inner standard.

All data were expressed as a mean ±SD.

For saturation degree estimation in leaves and seeds the index of saturation (double bound index — DBI) was used [19]:

$$DBI = \sum Pjn/100,$$

where Pj — the amount of FA (mol%), n — the number of double bounds in every unsaturated FA. The unsaturation index (K) — the ratio between total amount of unsaturated FA (UFA) and total amount of saturated FA (SFA) is also used. Acil-lipid ω9, ω6 and ω3 desaturases activity, which catalyzes formation of double bonds into the carbon chain of Oleic (C18:1), Linoleic (C18:2) and α -Linolenic (C18:3) acids respectively was determined by stearic- (SDR), oleic- (ODR) and linoleic- (LDR) desaturases ratio. These ratios were calculated by amount (mol% of total FA content) of C18 components:

$$SDR = (C18:1) / (C18:0 + C18:1),$$

$$ODR = (C18:2 + C18:3) / (C18:1 + C18:2 + C18:3),$$

$$LDR = (C18:3) / (C18:3 + C18:2),$$

where C18:0, C18:1, C18:2 i C18:3 — mol% amount of Stearic (C18:0), Oleic (C18:1), Linoleic (C18:2) and α-Linolenic (C18:3) acids [20].

Antioxidant activity, total soluble protein and polyfructan content. Fresh apical leaves from plants grown under *in vivo* and standard *in vitro* conditions were weighed (200 mg), homogenized with distilled water (0.7 ml) and centrifuged at 10000 g for 10 min. 100 ul of supernatant was taken and mixed with 100 ul of 0.1% alcohol solution of resorcinol (chemically pure, Alfarus, Ukraine) and with 100 ul of concentrated hydrochloric acid (chemically pure, Alfarus, Ukraine). Extraction of polyfructan was held in water bath at +80 °C for 20 min and measured on spectrophotometer (550 nm) (Eppendorf, USA). Calibration was made using fructose [21]. Antioxidant activity of extracts was measured by DPPH radical scavenging method [22]. Bradford method was used for total soluble protein determination [23].

Statistical analysis of results was performed by expressing the mean difference (Student's t-test) [24].

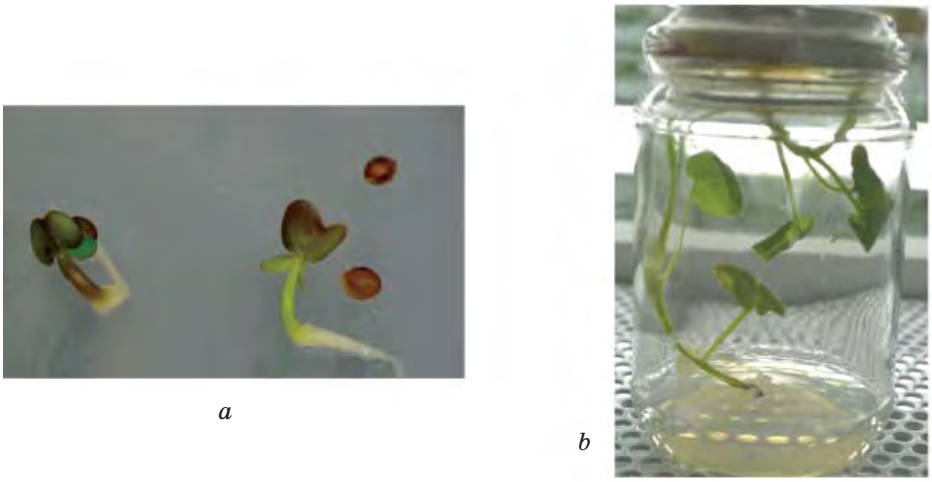
Results and Discussion

In 40–45 days after the sterilization procedure aseptic seeds started to sprout. Applied surface sterilization method provided with 45% of aseptic seed material 50% of which has sprouted (Fig. 1). Aseptic plantlets that were formed were cultivated on the solid MS medium at 24 °C with a 16-h photoperiod and regularly sub-cultivated by transferring apical or lateral buds to fresh medium.

Different growth regulators effect on lateral buds microclonal propagation was studied. The highest number of new formed plantlets was gained on the MS medium that contained 1 mg/l of BAP (5.25 ± 0.50 plantlets from 1 lateral bud) and 1.5 mg/l of kinetin (5 ± 0.81 plantlets from 1 lateral bud) (Fig. 1). NAA presence in the medium caused callus tissue formation without microclonal propagation. Control plants were grown on MS medium without grows regulators (no callus tissue or new formed plantlets were formed during 30 days of observation).

FA content. FA can be divided by the unsaturation degree into 2 groups: saturated (SFA) and unsaturated (USFA) (monounsaturated and polyunsaturated). GC/MS of the samples from seeds and leaves of grown *in vitro* and *in vivo* showed the presence of such SFA as Lauric acid (C12:0, Palmitic acid (C16:0) and Stearic acid (C18:0). Monounsaturated FA (Oleic acid (C18:1 Δ9, ω9), Paullinic acid (C20:1) and Erucic acid (C22:1)) and polyunsaturated FA (Linoleic (18:2 Δ9, 12, ω6), α-Linolenic (18:3 Δ9, 12, 15, ω3), eicosadienoic acid (C20:2) were detected in seed samples but no traces of long carbon chain FA (C20:1, C20:2, C22:1) were found in leaf samples. Total amount of FA in seed and leaves was quite different (Table). *C. koktebelica* seeds had high content of total FA (890 ± 99 mg/g). Total amount of FA in leaf samples was approximately equal in both aseptic and not aseptic plants. USFA total amount in seeds was much higher than SFA (K — 44 ± 5.82) but DBI was rather low (DBI — 4 ± 0.05, $P < 0.05$). Extracts from *C. koktebelica* plants grown *in vivo* had higher total amount of USFA then SFA (K — 4.41 ± 1.43) and *in vitro* leaf samples on the contrarily had almost equal amount of USFA and SFA (K — 1.24 ± 0.42). Though, these results were not significant. The DBI was higher for *in vivo* cultured plants then for plants grown *in vitro* ($P < 0.05$, Table).

Calculated SDR, ODR and LDR coefficient showed relevant desaturases (ω9, ω6, ω3) activity level. The activity of ω9 — desaturase that provides first double bound input was the lowest among others desaturases activity in aseptic plant samples (0.49 ± 0.04, $P < 0.05$), and it's highest activity was determined in seed samples (0.98 ± 0.005, $P < 0.05$). ODR coefficient was very high in leaves of both aseptic and not aseptic plants that indicate high activity of ω6-desaturase. LDR coefficient was high in plants grown *in vivo* (0.83 ± 0.06, $P < 0.05$) but low in aseptic plants (0.43 ± 0.02, $P < 0.05$) and the lowest in seeds which indicates low activity of ω3-desaturase. According to SDR, ODR and LDR coefficients that were calculated activity of ω9-, ω6- and ω3-desaturases in leaves grown in different conditions and seeds were different. Samples from seeds showed high ω9-desaturase and low ω6- and ω3-desaturases activity while samples from leaves, on the contrary, showed high ω6- and ω3-desaturases and low ω9-desaturase activity.



a *b*

Fig. 1. **The *Crambe koktebelica*:**
aseptic seeds sprouting (*a*) and *in vitro* cultivation (*b*)

Total amount of FA, saturation degree, unsaturation index and acil-lipid ω9, ω6 and ω3 desaturases activity in *Crambe koktebelica* seeds and leaves of plants grown *in vitro* and *in vivo*

Parameters	*C. koktebelica*	
	In vitro leaves	*In vivo* leaves (control)
FA, mg/g	3.64 ± 0.70	3.46± 0.09*
SFA, mg/g	1.58 ± 0.01*	0.70 ± 0.20*
USFA, mg/g	2.51 ± 0.69	2.76 ± 0.29
K	1.24 ± 0.42	4.21 ± 1.43
DBI	1.46 ± 0.22*	2.07 ± 0.17*
SDR	0.43 ± 0.04*	0.57 ± 0.17*
ODR	0.94 ± 0.01*	0.95 ± 0.02*
LDR	0.43 ± 0.02*	0.83±0.06*

Notes: * $P < 0.05$ (compared with the control; abbreviations see in Materials and Methods; significant values see in the text).

Further FA ethers gas-spectrums of the samples from seeds and leaves study showed that Lauric acid was present in all seed and leaves samples. While seeds and in vivo plants had insignificant amount of C12:0 (seeds — 0.22 ± 0.02 mol%; in vivo grown plants — 0.28 ± 0.05 mol%, $P < 0.05$) a part of lauric acid in leaves samples from aseptic plants was higher (4.91 ± 0.82 mol%). Palmitic acid had the biggest share of saturated FA content. It's content was low in seeds — 2.04 ± 0.31 mol%, but leaf samples, on the contrarily, had high content of C16:0 (aseptic plants — 38.36 ± 8.82 mol% and not aseptic plants — 18.19 ± 7.50 mol%) (Fig. 2). Despite that data was not significant we can assume that the amount of palmitic acid in aseptic plats increased comparing to not aseptic ones.

Consumption of palmitic acid (C16:0) increases risk of developing cardiovascular diseases so plants with its high content are better to be used to produce soaps, cosmetics, and release agents. Therefore, green mass of *C. koktebelica* plants that were cultivated under *in vitro* conditions is not fitted for food industry or for animal feeding but we can assume that plants which were cultivated *in vivo* more fit to for animal feeding as they had about twice less of palmitic acid then the aseptic ones.

Leaf samples had higher content of C18:0 then seed samples (seeds — 0.31 ± 0.05 mol%, aseptic plants — 3.70 ± 0.97 mol%; not aseptic plants — 2.13 ± 0.72 mol%). Taking into account calculated standard deviations we can assume that there is no significant difference in C18:0 content between in vitro and in vivo grown plants.

Unsaturated FA has preventative and therapeutic ability in cardiac diseases, rheumatoid arthritis, bronchopulmonary pathology, diabetes, viral infections, mental disorders [25]. In seeds of studied species the biggest share of all FA had Oleic acid but in leaves its content was low. Samples from seeds had 30.07 ± 2.28 mol% of C18:1 but samples from aseptic plants leaves had 2.84 ± 0.32 mol%; compared to its content in plants cultivated in the botanical garden (3.56 ± 2.36 mol%). We assume that there is no significant difference in oleic acid content between *in vitro* and *in vivo* grown plants.

Samples from seeds had high content of monounsaturated omega-9 fatty acids — Gondoic acid (C20:1) and Erucic acid (C22:1) while leaves from both aseptic and not aseptic plants had no traces of these acids. Those acids are harmful to human and animal health, so their absence in leaves samples indicates the possible use of *Crambe* species green mass as forage crops. At the same time, the use of their seeds as forage crops is not advisable due to high content of C22:1. The Erucic acid content in seed samples was rather low for *C. koktebelica* (11.72 ± 3.27 mol%) compared with well studied oilseed crop *C. abissinica* (62.50%) [26]. Gondoic acid content in seeds was high (18.98±0.21 mol%) while *C. abissinica* seeds had only 2% of C20:1 [26].

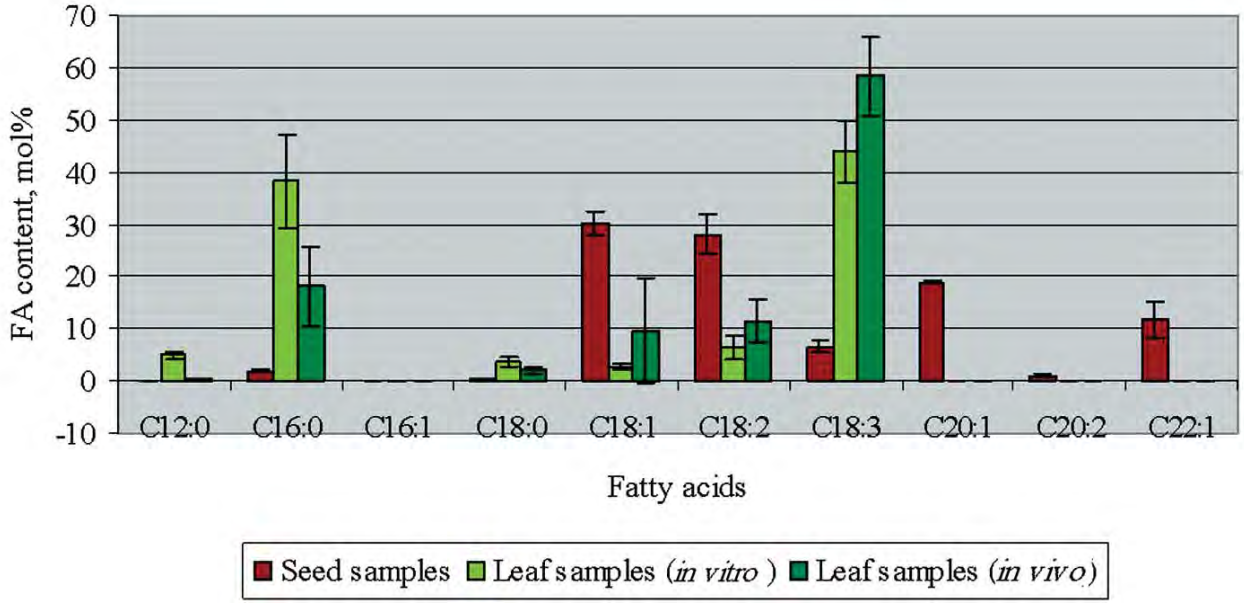


Fig. 2. **Comparison of *Crambe koktebelica* seeds and plants grown *in vitro* and *in vivo* FA content**

Some polyunsaturated FA were detected in seeds and leaves samples of studied Crambe species. Linoleic and α-Linolenic acids are essential fatty acids so foods with high content of these acids should be included in human diet [24]. The biggest amount of C18:2 was detected in *C. koktebelica* seeds samples (28.14 ± 3.73 mol%). Leaves samples from *in vivo* grown plants had higher amount of C18:2 (11.42 ± 4.26 mol%) than *in vitro* cultivated plants (6.40 ± 2.45 mol%). But, since the data is not signifacant we cannot report a great difference in C18:2 content between aseptic and not aseptic plants. α-Linolenic acid content was the highest among SFA and UNFA in leaves samples from plants that were cultivated in both *in vitro* and *in vivo* conditions but not aseptic plants had higher content of C18:3 (58.39 ± 7.50 mol%) then aseptic ones (43.77 ± 5.89 mol%). In seed samples C18:3 content was much lower (6.50 ± 1.09 mol%) though the data was not significant.

Eicosadienoic acid (C20:2) was detected only in seed samples from *C. koktebelica* (1.05 ± 0.29 mol%) though this FA wasn't present in *C. abissinica* [25], but *C. tataria* also had similar to our data content of C20:2 (0.4%) [27].

Palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and erucic acid composition of vegetable oils affects biodiesel properties and plants with high content of these FA are often suggested for cultivation as a source of biofuel. *C. koktebelica* is a wild crop which didn't undergone specific cultivation or hybridization for FA content improvement, therefore it shows some potential in comparison with commonly used oilseeds [28].

Antioxidant activity, total soluble protein and polyfructan content. In vitro leaf samples had significantly higher total soluble protein content (8.10 ± 0.97 mg/g of fresh weight) comparing to *in vivo* samples (2.38 ± 0.29 mg/g). It is interesting that for the most commonly used species of *Brassicaceae* family that were grown *in vivo*, total protein content was lower than for *Crambe* species (4,8 mg/g of fresh weight for broccoli) [29]. Exogenous phytohormones which are used for *in vitro* microclonal propagation and cultivation are proven to have the ability to activate expression of soluble and stress proteins synthesis genes [30–31]. This ability could be responsible for such great difference in total soluble protein content between aseptic and not aseptic plants.

AOA for *C. koktebelica* both *in vitro* and *in vivo* samples was very high comparing to the ascorbic acid solution (1 mg/ml — 98.22%). Leaves samples of not aseptic plants showed very high AOA (110%) which is higher then

ascorbic solution that was used as a reference and than aseptic plans AOA (82%).

While *in vitro* plant samples total soluble protein content was higher comparing to *in vivo* plants their polyfructan content on the contrary was quite low: aseptic plants — 4.50 ± 1.86 mg/g and not aseptic plants — 9.51 ± 0.12 mg/g.

The calculated results of AOA, total soluble protein and polyfructan content were not statistically significant.

So, efficient protocol for seed surface sterilization that resulted in high number of aseptic spouts and fast multiplication method (up to 5.25 ± 0.50 new formed plantlets from 1 lateral bud in 30 days period) were elaborated for endangered species *C. koktebelica*. GC/MS of the samples from seeds and leaves study reviled an impact of aseptic conditions on the FA composition of studied *C. koktebelica* species. Quantitative FA analysis shows difference between plants grown in aseptic and not aseptic conditions and also suggests that aseptic conditions benefits to SFA accumulation and increases protein content but on the other hand it reduces USFA amount and polyfructan content so as AOA of plant material. The study of ω9-, ω6- and ω3-desaturases activity showed that seed samples had high ω9-desaturase activity but very low ω6- and ω3-desaturases activity. Samples from leaves on the contrary showed high ω6- and ω3-desaturases activity and low ω9-desaturase activity.

Quantitative analysis of FA content of *C. koktebelica* leaves from *in vitro* and *in vivo* plants hasn't reviled significant difference in amount of each detected FA and that has showed the minimal impact of *in vitro* cultivation and propagation on FA composition of *C. koktebelica* plants. The dominant FA in leaves samples was α-Linolenic acid and *in vitro* cultured plants had its highest content. The dominant FA of all present in seed samples was Oleic acid. *C. koktebelica* seeds had high content of erucic acid which indicates that these species could be used as a source of biofuel. On the contrary Erucic acid hasn't been detected in both aseptic and not aseptic plant samples. To confirm the potential interest of *C. koktebelica* in some niches of the lubricating, emulsifying and refrigerating fields, some characteristics of these plants such as some components of unsaponifiable water (sterols and aliphatic alcohols) and main physical properties (smoke point, saponification number, viscosity) must be determined.

C. koktebelica both *in vitro* and *in vivo* samples showed high radical scavenging activity, comparing to the ascorbic acid solution. Aseptic plants also showed high amount of total soluble protein.

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БІОТЕХНОЛОГІЧНІ ПІДХОДИ ДО ЗБЕРЕЖЕННЯ ЗНИКАЮЧОГО ВИДУ *Crambe koktebelica* (Junge) N. Busch ТА ВПЛИВ АСЕПТИЧНИХ УМОВ КУЛЬТИВУВАННЯ *in vitro* НА БІОХІМІЧНІ ПОКАЗНИКИ РОСЛИН

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Метою дослідження було створення ефективних протоколів поверхневої стерилізації насіння зникаючого виду *Crambe koktebelica* (Junge) N. Busch в асептичній культурі та дослідження впливу застосування методів біотехнології (культивування *in vitro*) для збереження біологічного різноманіття на біохімічний склад рослин. Введення в асептичну культуру *in vitro* було проведено за розробленим авторами методом. Дослідження мікроклонального розмноження із бічних бруньок рослин дослідного виду проводили на живильному середовищі Мурасіге-Скуга, доповненому регуляторами росту в різних концентраціях. Вміст жирних кислот визначали методом газової хроматографії-мас-спектрофотометрії ефірів жирних кислот, отриманих із насіння та наземних органів рослин *C. koktebelica*, культивованих *in vitro* та *in vivo*. Антиоксидантну активність було визначено за 2.2-дифеніл-1-пікрилгідрацил методом. Вміст загального розчинного протеїну визначали методом Бредфорда, а вміст поліфруктанів — за здатністю кетоцукрів забарвлюватись у кислотному середовищі за наявності резорцину. В роботі використано рослини, вирощені в умовах *in vitro* та *in vivo*, і насіння *C. koktebelica*. В результаті було розроблено ефективний метод поверхневої стерилізації насіння, який давав змогу отримати 50% асептичних насінин, з яких у культурі *in vitro* проростало 45% насінин. Було визначено склад живильного середовища, що забезпечував ефективне мікроклональне розмноження рослин в асептичній культурі *in vitro*. На середовищі Мурасіге-Скуга, доповненому 1 мг/л 6-бензиламінопурину, з однієї бічної бруньки утворювалось 5,25 ± 0,50 пагонів. Також встановлено, що асептичні умови вирощування рослин *C. koktebelica* сприяють збільшенню вмісту насичених жирних кислот та загального розчинного протеїну, однак призводять до зменшення вмісту поліфруктанів та знижують антиоксидантну активність. Отримані результати підтверджують ефективність використання методів біотехнології для збереження біорізноманіття та зумовлюють необхідність глибшого вивчення механізмів впливу культивування *in vitro* на біохімічний склад рослин.

Ключові слова: *Crambe koktebelica*, збереження біорізноманіття, *in vitro*.

БИОТЕХНОЛОГИЧЕСКИЕ ПОДХОДЫ К СОХРАНЕНИЮ ИСЧЕЗАЮЩЕГО ВИДА *Crambe koktebelica* (Junge) N. Busch И ВЛИЯНИЕ АСЕПТИЧЕСКИХ УСЛОВИЙ КУЛЬТИВИРОВАНИЯ *in vitro* НА БИОХИМИЧЕСКИЕ ПОКАЗАТЕЛИ РАСТЕНИЙ

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Целью исследования было создание эффективных протоколов поверхностной стерилизации семян исчезающего вида *Crambe koktebelica* (Junge) N. Busch в асептической культуре *in vitro* и исследование влияния применения методов биотехнологии (культивирование *in vitro*) для сохранения биологического разнообразия видов на биохимический состав растений. Введение в асептическую культуру *in vitro* было проведено в соответствии с разработанным авторами методом. Исследование микроклонального размножения из пазушних почек растений иследуемого вида проводили на питательной среде Мурасиге-Скуга, дополненной регуляторами роста в разных концентрациях. Содержание жирных кислот определяли методом газовой хроматографии-масс-спектрофотометрии эфиров жирных кислот, полученных из семян и вегетативных органов растений *C. koktebelica*, выращенных *in vitro* и *in vivo*. Антиоксидантная активность была определена по 2.2-дифенил-1-пикрилгидрацил методу. Содержание общего растворимого протеина определяли методом Брэдфорда, а содержание полифруктанов — по способности кетосахаров окрашиваться в кислотной среде при наличии резорцина. Растения, выращенные в условиях *in vitro* и *in vivo*, и семена были использованы в данной работе. В результате был разработан эффективный метод поверхностной стерилизации семян, обеспечивающий 50% асептических семян, из которых в культуре *in vitro* прорастало 45%. Был установлен состав питательной среды, обеспечивающий эффективное микроклональное размножение растений в асептической культуре *in vitro*. На среде Мурасиге-Скуга, дополненной 1 мг/л 6-бензиламинопурина, из одной латеральной почки розвивалось 5,25 ± 0,50 побегов. Также было установлено, что асептические условия выращивания растений вида *C. koktebelica* способствуют увеличению количества насыщенных жирных кислот и общего растворимого протеина, однак приводят к уменьшению количества полифруктанов и снижают антиоксидантную активность. Полученные результаты подтверждают еффективность использования методов биотехнологии для сохранения биоразнообразия и обуславливают необходимость более глубокого изучения механизмов влияния культивирования *in vitro* на биохимический состав растений.

Ключевые слова: *Crambe koktebelica*, сохранение биоразнообразия, *in vitro*.