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Callus induction and maintenance in sunflower: Assessment for genotype conservation

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Abstract
In vitro technique for the conservation of sunflower germplasm provides a perspective for enhancing breeding programs. In this study, we investigated the induction of callus and evaluated direct plant regeneration from sunflower seeds of different genotypes using specific culture technology. For the experiment we used three commercial hybrids (genotypes G1, G2, G3) based on their technologies Imidazolinine (IMI), Sulphonylureic (SU) and Conventional (CONV) and were grouped as it follows: G1–IMI, G2–SU, and G3–CONV. Furthermore, for the in vitro culture Murashige & Skoog (MS) media with hormone combinations of auxins as the Alpha-naphthylacetic acid (NAA) and the 2,4-Dichlorophenoxyacetic acid (2,4-D) and cytokinins as the 6-Benzylaminopurine (BAP) were used. Callus induction was observed in all three genotypes using media with both 5 mg/l BAP and 2 mg/l 2,4-D. Genotype G3 showed the highest rate of induction to BAP (100%). Plant regeneration was successful only in the medium containing 5 mg/l BAP + 0.35 mg/l NAA, with the efficiency on genotype G1 of 30%. However, in the other hormone combinations, regeneration did not occur. Results suggest that the genotype has a strong dependency for the success of in vitro regeneration and highlight the importance of optimizing the phytohormonal concentrations to succeed plant regeneration.

Keywords: biotechnology, breeding, growth hormones, *Helianthus annuus*, MS media, regeneration

Introduction

The sunflower (*Helianthus annuus* L., 2n=34) breeding is an important objective in agriculture, posing challenges and opportunities for crop improvement. Globally, sunflower records in 2024 over 54 million tons on a total cultivated area of over 29 million hectares with an average yield of 1855 kg/ha. In Romania the year 2023 recorded over 1 million hectares were cultivated with a total production of just over 2 million tons with a yield of 1870 kg/ha, lower than in 2021, when the average yield per hectare was 2530 kg/ha [6] The evolution of the breeding programs, whether conventional or based on biotechnology has a key objective of selecting and maintaining individuals with desirable traits for further line development and hybrid creation

Sunflower breeding requires a long-lasting period for obtaining the inbred lines, an estimated interval of 6-8 generations. The development of germplasm, hybrids, and lines has brought revolutionary results by improving the plant's qualities such as an increased yield potential, resistance to diseases and pests, tolerance to challenging pedo-climatic conditions, and adaptability to mechanized cultivation. By the use of biotechnology [18] [14].

Advances in both classical breeding and biotechnological approaches have led to significant transformation in the development and preservation of rare and valuable genotypes. The creation of hybrids with the wild *Helianthus* species has introduced genes of interest for drought and cold resistance. The Leclercq method of breeding and reproduction based on cytoplasmic male sterility (CMS), has proved efficient in the exploitation of maternal and paternal traits and reducing the risk of genetic degeneration from repeated inbreeding [14,18].

Conservation and genetic selection has involved a set of challenges, bringing us to an overcome objective through modern biotechnological methods [5]. These include genomic selection, mutagenesis and variant selection, gene editing, immature embryo rescue, gynogenesis and androgenesis, marker-assisted selection, and in vitro tissue culture [30]. Among these, in vitro culture techniques offer a valuable perspective for preserving homozygous haploid genotypes, rescuing and reproducing non-viable embryos, and reducing space and logistic material with an advantage of economic demands compared to the classical methods [6]. The success of these cultures is determined by factors such as medium composition, hormonal balance, light, and temperature conditions [20].

The influence of phytohormones in the success of in vitro cultures is essential for inducing callus and regenerating plants. The main phytohormones used in micropropagation are: Alpha-naphthylacetic acid (NAA), a synthetic auxin that has the role of stimulating cell division, elongation and callus formation, but also inducing the formation of adventitious roots [10]. The cytokinin 6-Benzylaminopurine (BAP), which is synthetic in nature, plays the main role of inducing the formation of shoots and buds and stimulating the cell division in meristems [13]. Both NAA and BAP are used in combinations of different concentrations for an optimal auxin:cytokinin ratio that influences the direction of morphogenesis [7]. The 2,4-Dichlorophenoxyacetic acid is a very potent and effective synthetic auxin in inducing callus and promoting somatic embryogenesis, used in the first phase to obtain callus, and in the next stage for regeneration, the tissue is transferred to cytokinin media [2] [29].

In this study, we aimed to induce callus formation directly from seeds to obtain two types: differentiated and undifferentiated. These were subsequently developed into tissues for micropropagation, as part of a study on herbicide resistance in plant tissue, using genotypes from IMI, SULFO, and CONV technologies.

Material and Method

Chimicals

The Chemicals and reagents used were of analytical grade and purchased from Duchefa Biochemie (Haarlem, A. Hofmanweg 71, The Netherlands). Murashige and Skoog (MS) basal medium was used for culture, supplemented with 3% (w/v) sucrose and solidified with 1.6% (w/v) agar. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 20 minutes.

Germplasm

For the study three hybrids of sunflower from the three conventional technologies (CONV), Imidazolinone (IMI) and Sulphonylureic (SU) were used. The induction of callus has a purpose for producing vegetal material from explants to test herbicide resistance. The chosen hybrids were: CONV- ILINCA 115 from INCDA Fundulea, resistant at *Orobanche cumana* and *Plasmopara halstedii*, also resistant at drought and scorching heat [4]; For IMI, it was chosen the hybrid ES OASIS from LIDEA with genetical resistance at *Orobanche cumana* G-Race and it can be used as control for testing the resistance for *Plasmopara halstedii* [11]; And from the SU technology we have chosen the ALEXA SU hybrid from Saaten-Union, a mid-early hybrid with high resistance at the races A-F of *Orobanche cumana* and very resistent at diseases such as *Phomopsis* and *Sclerotinia sclerotiorum* [25].

Experimental design

The experiment had two objectives: I-Callus induction and II-Plant regeneration.The vegetal material consisted of 200 seeds per genotype: G1-IMI, G2-SU and G3-CONV.

For the callus induction (I) the seeds were grouped in 2 variants according to the phytohormone concentration: V1 - MS + 5mg/L BAP and V2 - MS + 2 mg/L 2,4-D. Each variant had 10 repetitions of 10 seeds each one and the period for the induction was 40 days.

For the plant regeneration (II), the successful callus induced seeds have been chosen to propagate the shoots and roots generation, for all of the three genotypes the plants were transferred on 3 variants with MS media as it follows: V1- 5mg/l BAP+0.35 mg/l NAA, V2- 0.5 mg/l NAA+0.5 mg/l BAP andV3-1mg/l NAA+ 2 mg/l BAP. The results for the plant regeneration were reported as percentage from the successfully callus induced seeds.

Data collection

The number of calluses induced seeds was taken for regeneration test and selected as percentage from the successful grown callus as such: 10 repetitions per genotype transferred from the initial medium to one of the 3 variants in a proportion as 50% from BAP and 50% from 2,4-D. Each variant had 3 repetitions per genotype, and each repetition had 10 callused plants.

Sterilization Protocol

The sterilization protocols was tested to evaluate its effectiveness in eliminating microbial contamination from the seeds and the influence of sterilization on seed germination: Seeds were rinsed in running tap water for 10 minutes to remove the dirt and debris, were mechanically hulled, followed by

immersion in 70% ethanol for 30 seconds, then in 0.1% mercuric chloride solution for 10 minutes, and rinsed 3 times in sterile distilled water.

Culture Conditions

Following sterilization, seeds were aseptically transferred to sterile culture dishes containing MS medium supplemented with the variant's concentration. Cultures were incubated in a growth chamber at 25 ± 2°C under a 16-hour photoperiod.

Assessment of Viability and Contamination

Seeds were monitored daily for signs of contamination (fungal or bacterial growth) and germination (as an indicator of viability) over a period of 14 days. The percentage of contaminated and viable seeds was recorded for each treatment group. After seed germination, the resulting explants were cut into small pieces using sterilized instruments (scalpel and tweezers) and inoculated.

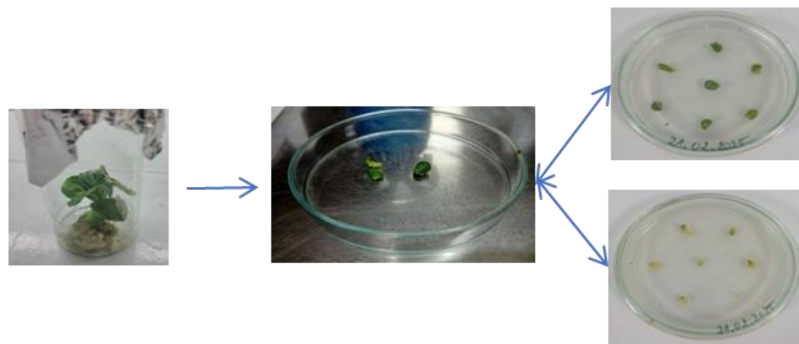

Figure 1. Callus induction protocol with cotyledon and stem explants on different hormonal balances

Results and Discussion

According to our results all three genotypes showed a rate of callus induction in all three genotypes (G1-IMI, G2-SU, G3-CONV) in both medium variants. After 40 days of trial, the seeds germination in vitro was registered. Furthermore, the average of callused materials counting the seeds from each repetition and defined the rate per medium and per genotype. The induction rate in the case of BAP was 100% for G3 and 90% for both G1 and G2, leading to an average of 93%. In the case of 2.4-D medium the highest rate was shown to be 94% for G3 and the lowest rate was shown for the G2 genotype in all the three genotypes, as can be observed in Table 1.

Table 1. Callus Induction from seeds in In Vitro Conditions

	V1-5 mg/l BAP (%)	V2-2 mg/l 2.4-D (%)
G1-IMI	90	90
G2-SU	90	90
G3-CONV	100	90
Average	93 ± 5.77	90 ± 0.0

Note: V1-5 mg/l BAP; V2-2 mg/l 2.4-D

Table 2. Successful regenerated plants resulted from the induced callus according to the variants with the hormonal balance

	V1 (%)	V2 (%)	V3 (%)
G1-IMI	30	0	0
G2-SU	0	0	0
G3-CONV	10	0	0
Average	13 ± 15,28	0 ± 0.0	0 ± 0.0

Note: V1-5mg/l BAP+0.35 mg/l NAA; V2-0.5 mg/l NAA+0.5 mg/l BAP; V3-1mg/l NAA+ 2 mg/l BAP

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The plant regeneration assessment led to a final observation that variant 1 of the medium with 5 mg/l BAP + 0.35 mg/l NAA was the only one capable of inducing plant regeneration. Genotype G1 has regenerated at a rate of 30%, while G3 offered a rate of 10%, proving a significant efficiency of this medium compared to the other concentration variants taken to test. Genotype G2 did not regenerate. The average regeneration rate was 13%, suggesting that this medium has an increased potential of selection, particularly for genotype G1. Medium with 0.5 mg/l NAA + 0.5 mg/l BAP, no regeneration was observed in any of the genotypes (0% for G1, G2, and G3). This hormonal combination proved to be ineffective for the sunflower callus tissues regeneration.

The medium with 1 mg/l NAA + 2 mg/l BAP, similarly to the previous variant, none of the genotypes regenerated (0%). This result highlights that the NAA and BAP combination at the tested concentrations, lower than for the first one, does not induce regeneration.

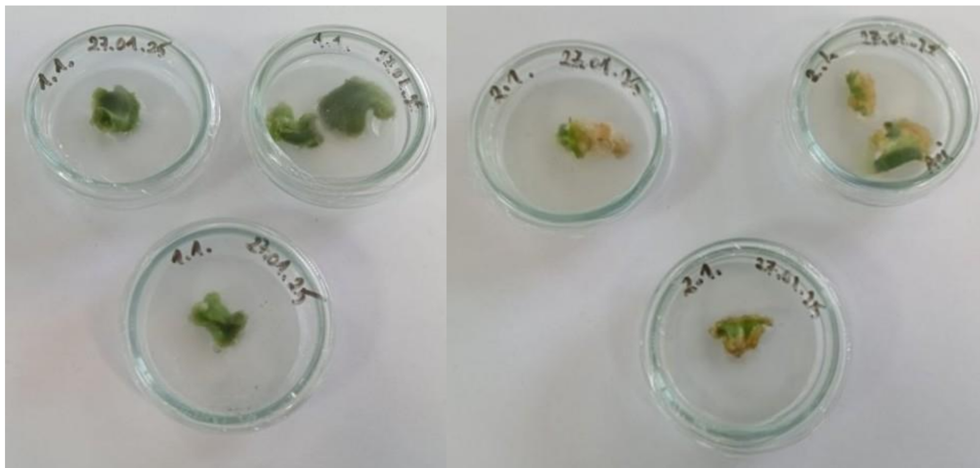

Figure 2. The color and consistency of the callus on V1 and V2.

Figure 3. The Induction of undifferentiated callus in V1

Regarding callus induction, we found the efficiency of the concentrations for both the BAP-supplemented and 2,4-D-supplemented media. The resulting undifferentiated callus was promising for material to be tested for regeneration. The resulting tissue could provide resources to monitor the regulation of phytohormone concentrations and be considered effective in obtaining in vitro regenerated plant material, ideal for use in various tests, including herbicide resistance testing, according to the readiness objective for a particular sunflower cultivation technology.

Regarding plant regeneration we were able to follow how differentiated and undifferentiated callus evolve, thus being able to ascertain the optimal concentrations of phytohormones that can provide optimal development and regeneration of vegetative organs. The concentration of the culture medium supplemented with 5mg/l BAP + 0.35 mg/l NAA was the only and most efficient concentration from which differentiated callus and subsequently vegetative organs resulted. Some challenges encountered in the study were the viability of the seeds and undifferentiated calluses, but also of the genotype that encountered difficulties in adapting.

As a future perspective, we will try to obtain undifferentiated calluses to streamline the reproduction of plant material directly from embryos to increase stability and adaptability in different experimental conditions.

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Various studies and researchers have explored the tissue culture strategies. For instance, optimal callus induction for wild H. annuus , H. occidentalis, H. tuberosus, and H. giganteus was achieved using MS medium with BAP and NAA in ratios of 2:1 and 5:1, respectively [31]. Regeneration of plants from hypocotyl-derived protoplast callus grown on V-KM medium supplemented with NAA and BAP in the dark, later transferring them to hormone-free MS medium for germination and field acclimatization [9]. This opens a further perspective for developing an acclimatization protocol in adapting the plants from the laboratory-controlled conditions to field conditions. Plant regeneration from sunflower anthers, using MS medium with 2.0 mg/l NAA and 1.0 mg/l BAP for callus induction, followed by elongation on a medium with reduced hormone levels. Variables such as light exposure, pre-cooling of seed caps, agar, and sucrose concentration significantly influenced embryogenesis [22]. A perspective that we are looking for applying it to regenerate plants from anthers and test the concentrations in the same conditions as our study. Genotype influence on callus production using hypocotyl and cotyledon tissues from five sunflower varieties. Callus induction varied across genotypes on MS medium with 1 mg/l 2,4-D, followed by transfer to MS medium containing 1 mg/l BAP and 0.5 mg/l NAA, revealing diverse regenerative capacities [17]. According to this study that we used in the experiment with lower ratios of BAP:NAA will keep searching for the conditions the researcher applied for and will try to repeat it. Callus induction from cotyledons grown on MS media supplemented with 2,4-D and kinetin [24] and the induction of callus under salinity conditions, the result obtained was the induction of callus with a rate of over 60% for three of the genotypes (Sakha 53, Giza102 and Par-1617-1), the effect of NaCl was necrosis and growth reduction, but the three mentioned genotypes showed the lowest percentage of necrosis [8]. Further experiments for us will include an assessment of callus tolerance at salinity and higher heavy metals concentrations. The efficiency of callus induction from immature embryos using 2,4-D supplemented MS medium to induce callus and BA and NAA supplementation for regeneration, the callus induction rate was high and variable depending on genotype [16]. This study confirmed our hypothesis that plant regeneration depends on genotype. Various sterilization protocols for explants in which he obtained the most effective combination for disinfection and induction of callus by treatment with benomyl (2g/l, 2 hours) followed by sterilization with H2O2 (10min) and NaOCl 2% (12 min, without pH adjustment). By adjusting the pH to 7 and 10 it reduced contamination with microorganisms, but affected the viability of the explants by degrading the callus [1]. A good sterilization protocol to have in perspective for sterilizing the laboratory instruments.

More recently employed immature embryo rescue to accelerate the development of inbred lines across three genotype categories—lines, populations, and hybrids. Embryo survival rates reached up to 81% in hybrids and 71% in inbred lines, depending on embryo harvest timing and medium composition, which included varied concentrations of BAP, NAA, and GA_3 [11]. This impacts our study in realizing the embryonic callus capacity in multiplication, differentiation and regeneration according to the culture medium. The effect of the heavy metals is a serious factor that needs to be taken in consideration due to the pollution increasing, the callus induction might reduce the negative effect by avoiding the intoxication of the seeds and the seedlings in soil [12].

Also, genetic studies and laboratory tests offer an impact perspective on the effects of substances tested for agricultural species, especially sunflowers. Both genotype and environmental conditions play an important role in defining empirical and observable characters, making it necessary to conduct a large-scale study and complex research on the effects of different substances in their interaction at the cellular level as well as at the level of genetic material [3, 25, 26, 27].

Conclusions

Callus induction succeeded for all three variants taken into study of sunflower genotypes using both BAP and 2,4-D, resulting in two types of calluses (differentiated and undifferentiated). However, plant regeneration was efficient with the hormonal combination of 5 mg/L BAP + 0.35 mg/L NAA, where genotype G1 showed the highest response. This suggests that regeneration capacity is influenced by genotype and hormone balance. In perspective, future research on herbicide resistance screening through callus-based assays and for refining *In Vitro* protocols aimed at conserving and regenerating resistant sunflower lines.

References

[1] Ahmadpoor, F., Zare, N., Asghari, R. and Sheikhzadeh, P. (2022), *Sterilization protocols and the effect of plant growth regulators on callus induction and secondary metabolites production in in vitro cultures Melia azedarach L*. AMB, Expr. 12, 3.

[2] Busi, R., Goggin, D.E., Heap, I.M., Horak, M.J., Jugulam, M., Masters, R.A., Napier, R.M., Ria, D.S., Satchivi, N.M., Torra, J., Westra, P., and Wright, T.R. (2018), *Weed resistance to synthetic auxin herbicides.* Pest Management Science, 74, 2265–76.

[3] Butnariu, M., Quispe, C., Herrera-Bravo, J., Fernández-Ochoa, Á., Emamzadeh-Yazdi, S., Adetunji, C.O., Memudu, A.E.., Otlewska, A., Bogdan, P., Antolak, H., Tamimi, K., Baghalpour, N., Mahroo, Bakhtiyari, J., Sen, S., Acharya, K, Segura-Carretero, A, Cádiz-Gurrea, M.D.L.L., Lim, S.H.E., Pentea, M., Sarac, I., Durna, Daştan, S., Abdull, Razis, A.F., Sunusi, U., Kamal, R.M., Setzer, W.N. and Sharifi-Rad, J. (2022), *A Review on Tradescantia: Phytochemical Constituents, Biological Activities and Health-Promoting Effects* Front. Bioscience (Landmark Ed), 27, 197.
[4] Compania Seminte Fundulea C. S. (2021), *Samanta românească (autohtona) floarea-soarelui Premium ILINCA 115 CSF, link: https://fundulea.ro/samanta-romaneasca-autohtona-floarea-soarelui-premium-ilinca-115/ .*
[5] Dagustu, N. (2018), *In Vitro Tissue Culture Studies in Sunflower (Helianthus spp.)* Ekin Journal of Crop Breeding and Genetics 4(1):13-21, 2018.
[6] Food and Agriculture Organisation Statistics, link: https://www.fao.org/faostat/en/#data/QCL/visualize .
[7] George, E.F., Hall, M.A. and Klerk, G.J.D. (2007), *Plant Propagation by Tissue Culture: Volume 1. The Background.* Springer Science & Business Media.
[8] Keshta, M. M. A., Hassan, N. M., Azzam, K. and Hassanin, O.S. (2011), *Embryogenic callus induction of some sunflower (Helianthus annuus, l.) genotypes under in vitro salt stress.* Journal of Plant Production, 2 327–33.
[9] Krasnyanski, S. and Menczel, L. (1993), *Somatic embryogenesis and plant regeneration from hypocotyl protoplasts of sunflower (Helianthus annum L.)*. Plant Cell Reports 12.
[10] Ku, Y.-S., Sintaha, M., Cheung, M.-Y. and Lam, H.-M. (2018), *Plant Hormone Signaling Crosstalks between Biotic and Abiotic Stress Responses.* Int J Mol Sci 19 3206.
[11] Lidea - ES OASIS CLP, link: https://lidea-seeds.ro/products/floarea-soarelui-1-1-1-oasis.
[12] Mate, C., Onișan, E., Petrescu, I., Simina, A. and Sărac, I. (2024), *The effects of cadmium, cobalt and copper on the germination of Helianthus annuus L. seeds.* JOURNAL of Horticulture 28.
[13] Mok, M.C. (1994), *Cytokinins and Plant Development - An Overview* Cytokinins. CRC Press.
[14] Onisan, E., Sarac, I., Petrescu, I. and Dorin, C. (2023) *New breeding methods in sunflower hybrid research and development.* Hybrid Research And Development, Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 1.
[15] Onisan, E., Petolescu, C., Tenche-Constantinescu, A., Petrescu, I., Horablaga, M.N., Simina, A., Gorinoiu, G., Camen, D., Popescu, S., Mate, C. and Sarac, I. (2025), *Application of Biotechnology for Sunflower Inbred Line Development: Immature Embryo Rescue.* Applied Sciences, 15, 1576.
[16] Ozyigit, I.I., Gozukirmizi, N. and Semiz, B.D. (2006), *Callus induction and plant regeneration from mature embryos of sunflower.* Russ J Plant Physiol, 53, 556–9.
[17] Ozyigit, I.I., Gozukirmizi, N. and Semiz, B.D. (2007), *Genotype dependent callus induction and shoot regeneration in sunflower (Helianthus annuus L.).* African Journal of Biotechnology Vol. 6 (13), pp. 1498-1502.
[18] Păcureanu-Joiţa, M., Vrânceanu, A.V., and Stanciu, D. (2007), *Cincizeci de ani de activitate în ameliorarea florii-soarelui la Fundulea.* AN. I.N.C.D.A. Fundulea, VOL. LXXV.
[19] Petolescu, C., Sarac, I., Popescu, S., Tenche-Constantinescu, A.M., Petrescu, I., Camen, D., Turc, A., Fora, G.C., Turcus V., Horablaga N.M. and Gorinoiu G. (2024), *Assessment of genetic diversity in alfalfa using DNA polymorphism analysis and statistical tools*. Plants, 13(20), p.2853.
[20] Punia M.S. and Bohorova N.E. (1992), *Callus development and plant regeneration from different explants of six wild species of sunflower (Helianthus L.).* Plant Science, 87, 79–83.
[21] Rogo, U., Fambrini, M. and Pugliesi, C. (2023), *Embryo Rescue in Plant Breeding.* Plants, 12, 3106.
[22] Saji K.V. and Sujatha M. *Embryogenesis and plant regeneration in anther culture of sunflower (Helianthus annuus L.).* Plant Science, 88, 65-68.
[23] Saaten-Union, Alexa Su (Floarea-soarelui), link: https://www.saaten-union.ro/floarea-soarelui/alexa-su/.
[24] Sankar, P.D., Subbraraman, N. and Hemavathy, A.T. (2006), *Callus induction studies in sunflower (Helianthus annuus l.*), Annals of Agricultural Research 27.
[25] Sarac, I., Petrescu, I., Onisan, E., Madosa, E., Iancu, P. and Bonciu, E. (2016), *The influence of diffrent concentration of cytokinin of mentha piperita.* Analele Universităţii din Craiova.
[26] Sarac, I., Bonciu, E., Butnariu, M., Petrescu, I. and Madosa, E. (2019), *Evaluation of the cytotoxic and genotoxic potential of some heavy metals by use of Allium test Caryologia*. International Journal of Cytology Cytosystematics and Cytogenetics, 37-43 Paginazione.
[27] Sarac, I., Onisan, E., Petrescu, I., Tenche-Constantinescu A., Morna, A.A., Timar, A.V., Camen, D., Ciulca, A. E., Vicas, L.G., Petolescu, C., Stroia, C. and Ganea, M. (2024), *Drosophila melanogaster as a Bioindicator in Comparative Copper and Lead Toxicology: Exploring the Health Implications of Heavy Metal Exposure.* Plant Sciences, 13-33.

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[28] Tenche-Constantinescu, A.M., Lalescu, D.V., Popescu, S., Sarac, I., Petrescu, I., Petolescu, C., Camen, D., Horablaga, A., Popescu, C.A., Berar, C. and Onisan, E., (2024), *Exploring the genetic landscape of Tilia spp. with molecular and statistical tools*. Horticulturae, 10(6), p.596.
[29] Todd, O.E., Figueiredo, M.R.A., Morran, S., Soni, N., Preston, C., Kubeš, M.F., Napier, R. and Gaines, T.A. (2020), *Synthetic auxin herbicides: finding the lock and key to weed resistance.* Plant Science, 300 110631.
[30] Vear, F. (2016), *Changes in sunflower breeding over the last fifty years. OCL,* 23 D202.
[31] Voronova, O. (2016), *Callus formation and plant regeneration in sunflower (Helianthus l., Asteraceae) in vitro tissue culture.* Applied Sciences, 67, 34-50.