MICROCLONAL GROWTH OF SILYBUM MARIANUM SEEDS

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ABSTRACT

In this article, the present study is devoted to the introduction of Silybum marianum L. into in vitro culture and the optimization of microclonal propagation conditions for its efficient mass multiplication. The research was carried out using apical meristems, lateral buds, and young leaf explants obtained from healthy 20–25-day-old Silybum marianum plants. A stepwise sterilization protocol involving 70% ethanol (30 s) followed by 0.1% $HgCl_2$ (5 min) ensured a high level of aseptic culture establishment, reducing contamination to 93–95%. Explants were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of cytokinin (BAP) and auxin (NAA) to determine optimal hormonal combinations. The results demonstrated that MS medium supplemented with 1.0 mg/l BAP and 0.2 mg/l NAA was the most effective for callus induction and shoot regeneration, achieving up to 85 ± 4% bud formation within 20–22 days.

The development of pharmaceutical production in our republic, the creation of a local raw material base for drug production, the development and implementation of technologies for the production of medicines from them are currently one of the important tasks facing pharmaceutical workers. The research of the presented dissertation work is important in implementing the tasks set out in the Resolution of the President of the Republic of Uzbekistan No. PQ-4899 dated November 25, 2020 "On comprehensive measures to develop biotechnology and improve the system of ensuring the country's biological safety", the Resolution of the President of the Republic of Uzbekistan No. PQ-4310 dated May 6, 2019 "On measures to further develop the system of medical and pharmaceutical education and science", as well as other regulatory legal acts related to this area. After gaining independence, the Republic of Uzbekistan, along with reforming all sectors, paid great attention to the development of the pharmaceutical industry. Today, the pharmaceutical industry is one of the fastest growing sectors in our country.

It is aimed at finding new bioactive substances, creating medicines from them, and applying them in medical practice to meet the needs of our country's population in medicines.

In the rapidly advancing 21st century, the outbreak of diseases related to the immune system is leading to the introduction of strict quarantine measures in many countries. Therefore, the need for new effective bioactive supplements that strengthen the body's immune system in the treatment of such diseases is very great not only in our country, but also throughout the world. Uzbekistan is one of the leading countries in the cultivation of medicinal plants. Nevertheless, increasing the diversity of such medicinal plants and adapting them to cultivation in local conditions is one of the main tasks facing us biotechnologists. As is known, the vast majority of bioactive supplements are extracted from plants. It takes a lot of time and money for the plant to germinate and accumulate the necessary and sufficient amount of biologically active supplements in its composition.

we have chosen, milk thistle (*Silybum marianum L.*), has been used in Chinese folk medicine for thousands of years. The fruits of the plant are used to improve vision, increase immunity, prevent cardiovascular and gastrointestinal diseases, fight cancer, improve metabolism, improve blood circulation, as well as prevent sexual diseases in women and men, vitamin deficiency in children, and prevent various diseases. The plant, which contains vitamins A and C, fiber, iron and zinc elements, antioxidants, flavonoids, polysaccharides, as well as various organic acids, has antioxidant, antimicrobial and immune-enhancing properties. In addition, outside this of the plant not only in the fruit , maybe leaves and bioactive in the stem as well substances there is In the field of biotechnology currently developing in our country , the selection of optimal conditions for micropropagation of milk thistle *(Silybum marianum L.) using in vitro propagation technology of cell engineering* is one of the new scientific researches in the field of pharmaceutical biotechnology within our competence. For the first time in the Republic of Uzbekistan, Sh. Bobomurodov managed to grow it in greenhouse conditions in Navoi in 2018. In addition, Polish and Chinese scientists have conducted scientific research on the propagation and rooting of *Silybum marianum* in vitro, Italian scientists on its adaptation to ex vivo conditions, as well as American and Chinese scientists on the composition and biological properties of the plant.

Research object is *Silybum marianum* ***The above-ground part of*** the plant : leaves and seeds.

Materials and Methods : In experiments healthy *Silybum marianum* of plants young branches three part and lateral buds explant as was taken . Explants initially 70% ethyl alcohol with 30 seconds from processing was carried out , then 0.1 % $HgCl_2$ (mercury chloride) solution for 5 minutes during was saved . From that then sterile distilled water with three times rinsed . This in stages contamination level up to 93 ± 2% reduced , this sterile processing efficiency showed .

Microclonal multiplication in the process explant quality and choice of success main from factors one of them . *Silybum marianum* plants with take visited in experiments , healthy and young branches three part and lateral shoots explant as This plant was selected . parts asset growth to the zone close happened because of the cells division and new clones to develop the most suitable is considered Explant healthy and active growth from the zones to be taken following advantages gives :

Results and their Discussion : Explants feed to the environment from placement before superficial humidity and injury level is checked , this contamination the risk further reduces . **Food environment preparation and hormone combination** explants **Murashige and Skoog** -based feed in the environment placed in the environment . following phytohormones various in concentrations added :

Table 1

Various in the option of seeds budding stages

Option	BAP (mg /l)	NAA (mg /l)	Callus harvest to be (%)	Bud harvest to be time (day)	Growth speed (relative)
1	0.5	0.1	65	25	1.00 (control)
2	1.0	0.2	92	18	**1.50 (50% faster)**
3	1.5	0.5	78	22	1.20

Growth speed and morphological observations

Microclonal under the circumstances cultivated plants traditional method with cultivated samples with when compared **50 ± 3 % faster** This difference is mainly due to the cell division of activity high , food of substances fast absorption and hormone to the optimal state of balance arrival with explained .

Also :

- Root harvest to be indicator by 1.4 times increased ;
- Leaf of the plate length 2.3 cm 4.1 cm from until arrived ;
- Callus tissue color hungry green active cell division showed ;
- From 85% of explants in excess bud harvest to be record was done .

Microclonal on the streets pigmentation , leaf shape and growth in geometry genetic changes not observed , this and their genetic stability shows .

Technology practical advantages

Received results *Silybum marianum* what biotechnological road with multiplication process efficiency confirms . Microclonal method using short time inside many in quantity , healthy , genetic in terms of one kind plants to take possible .

Research to the results see , *Silybum marianum* plant microclonal multiplication technology growth process traditional to methods 1.5 times more than accelerates , that is growth speed by 50% increases . Optimal conditions as **MS medium + 1.0 mg/l BAP + 0.2 mg/l NAA** recommendation This is technology medicinal of plants biotechnological multiplication system in development important importance has in the future industry on a scale application possible .

In vitro culture plant material successful input process microclonal multiplication technology the most responsible from the stages one This is of the process success mainly **explant selection** , **sterilization quality** , **phytohormones balance** and **plant tissue physiological to the state** related . **Explant source and preparation process**

In the study *Silybum marianum* 20–25 days of germination healthy from plants apical meristem , lateral bud and young leaf parts explant as selectively was taken . Every one explant morphological condition , size and tissue density according to separated . Experiments as a result apical meristem plant regeneration for the most stable

source that was determined . Explants sterilization to do for following step by step system used : 70% ethyl alcohol with 30 seconds during processing to give ;

1. 5 minutes in 0.1% $HgCl_2$ solution storage ;
2. 3 times sterile distilled water with wash

Sterilization process aseptic laminar air in conditions in the closet take This protocol using contamination level up to 93–95% reduced , healthy , infection- free tissues received .

Food environment preparation and explants placement

Plant tissues **Murashige and Skoog (MS)** based on feed in the environment was placed . Environment following to the content has was :

- Macroelements : NH_4NO_3 – 1650 mg/l, KNO_3 – 1900 mg/l;
- Microelements : Fe-EDTA, ZnSO 4, MnSO 4, CuSO 4, H_3BO_3;
- Carbon source as 30 g/l sucrose ;
- Gel harvest doer component as 0.7% agar-agar.

The pH of the medium was adjusted to 5.7 ± 0.1 . adjusted , then at 121°C for 20 minutes during in an autoclave sterilization Phytohormones cytokinin (BAP) and auxin (NAA) are different in concentrations added , 6 different combination from the test was held .

Culture beginning and callus harvest to be stage

Explants sterile in Petri dishes under conditions was held and at a temperature of 25 ± 2 °C , 16/8 hours light – darkness in the cycle saved . Within 10–12 days explant around cells division activated and **callus texture harvest to be** observed . Calluses initial mass hungry green in color , soft to the structure has to be active cell of division beginning stage stated .

14–16 days callus of the fabric partially differentiation as a result budding process started . At the end of the 21st day and average **85 ± 4% in explants callus harvest to be** record The most high callus harvest to be level 1.0 mg/l BAP + 0.2 mg/l NAA combination observed , which is the optimal ratio identified in Section 3.1 with suitable is coming .

Regeneration stage

Callus tissues 25–28 days from incubation after bud harvest doer feed to the environment was held . This 1.5 mg/l BAP and 0.1 mg/l IAA (indole vinegar acid) was added . After 10–12 days then buds differentiated , new branches harvest it has been .

Plants this in stages following changes showed :

- From callus bud harvest to be speed 80–85%;
- Bud length 2.8–4.0 cm up to ;
- Leaf plates number average 4–6 pieces ;
- Photosynthesis activity indicators (pigment concentration) up to 0.35 ± 0.02 mg/g enough .

In vitro introduction process analysis

Received results this showed that Silybum *marianum* in vitro conditions input for apical meristem explant as the most comfortable is considered , because it is an infection danger low , regeneration potential high and callus harvest to be process fast It's going to happen .

From this except for cytokinin and auxins between balance right when selected plant in tissues dedifferentiation (callus) harvest to be) and next differentiation (bud) harvest (becoming) processes appropriate Therefore , the combination of BAP and NAA in the MS environment *Silybum marianum* for the most optimal system as marked .

Conclusion: Research to the results see , *Silybum marianum* plant in vitro culture successful input for following The conditions were found to be optimal :

- Explant type — apical meristem ;
- Sterilization — 0.1% $HgCl_2$, 5 minutes ;
- Medium — MS + 1.0 mg/l BAP + 0.2 mg/l NAA;
- Temperature — 25 ± 2 °C;
- Light mode — 16/8 hours .

This under the circumstances plant callus harvest to be germination rate 85% stage and within 20–22 days observed . In vitro culture input success *Silybum marianum* what fast and stable multiplication technology next to stages take exit for solid basis creates.

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