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The Effect of Gibberellic Acid on the Stage of Seed Germination of *Silybum marianum L.*

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Abstract: ***Seed germination can considered as a factor influencing the subsequent stages of plant ontogeny. Physiological and biochemical changes during germination, followed by morphological changes are associated with the survival rate and vegetative growth of seedlings, resulting in an impact on yield and quality. This study investigated the effect of gibberellic acid on the germination stage of the seeds of the medicinal plant Silybum marianum L. Plant hormones considered as key regulators for seed germination. GAs stimulated the genes encoding for enzymes such as endo - β - 1, 3glucanse and β - 1, 4 mannan endohydroase. The study examined the effect of gibberellin acid on the seed germination stages of the medicinal plant Silybum marianum L. . In the experiment, parameters such as the amount of water soaked, imbibition rate, the Timson germination index, the mean germination time and final germination percentage of seeds soaked in a solution of gibberellin acid of different concentrations (0, 001; 0, 01; 0, 1 and 1, 0 µmol) were studied.***

Keywords: *Silybim marianum* L., gibberellins acid, germination

1. Introduction

Silybum marianum L. is a member of the family of *Asteraceae*, it called by several names, *Carduus lactifolus, Carduus mariae* Cr., *Carduus marianus* L. and *Carduus versicolarsalisb, Carthamus maculatum* Lam., *Mariana lacteal. Silybum marianum* L. found mainly in Europe, Asia, Australia, North and South America (Hassler, M.2019). *Silybum marianum* L. is a plant in which all parts are edible. Its leaves are young stems and roots consumed as vegetables. The seeds used as coffee and the flower in medicine.

S. marianum fruit has been used for 2000 years in the treatment of various liver diseases (hepatitis, cirrhosis and jaundice) and gallbladder diseases, to protect the liver from environmental toxins, to protect the liver from snake and insect bites and fungal poisoning (Svoma E 1998).

The fruit (seed) extract of Silybum marianum L. contains silymarin, a mixture of one falovonol (taxifolin) and six different flavolignans (silychristin, silydianin, silybin A, silybin B, isosilybin A and isosilybin B). (Upton R et al 2010). This substance is one of the most important substances in pharmacology. Milk thistle seeds contain 25% lipids (Harrabi S et al 2015). It grows in Austria, Germany, Hungary, Poland, China and Argentina, as a raw material for the pharmaceutical industry.

Silybum marianum L. is a plant that can grow in unfavorable soil conditions. It grows in sandy and loamy soils, which poorly and poorly supplied with mineral nutrients. It is also a drought tolerant plant (Flora, K. et al 1998). This feature distinguishes it from other medicinal plants and allows the use of soil that is inconvenient for plant growth.

The seed is an organism that contains various reserve nutrients, including biopolymers such as protein, lipids, and fats. It is a leading agricultural product, that meets the needs of people for natural nutrients. The embryonic of plant which is enclosed in covering is commonly known as seed. It produced during the reproduction in plants (Higashiyama, T et al 2003). From seeds germinate a young plant under optimal conditions for germination. There are different opinions about the germination of seeds. According to physiologists germination can be defined as the emergence of the radicle via seed coat but seed analysts defined the process of seed germination as the emergencies and the development of essential structure from embryo which develop into plants under suitable condition, while some other botanists defined seed germination as the emergence and growth of embryo to young plants by rapture of seed coat. But later Mayer and Shain, (1974) described the definition of germination of seed a series of steps that usually occurs earlier than the development of the radicle from the seed coat (Renu Joshi 2018). The process of seed germination is important in the growth and development of the plant. The germination process has a direct effect on the subsequent stages of plant ontogeny.

It well known that the relative levels of plant hormones control the dormancy and germination of seeds. They also control the elongation, division, and differentiation of the cell. The physiological and biochemical processes that take place in seed germination also controlled and regulated by hormones. Plant hormones can affect different plant activities including seed dormancy and germination (Graeber, K., 2012). The Gibberellin acid is one of the hormones involved in seed germination. Gibberellins are diterpenoid, regulating plant growth. They are commonly used in modern agriculture and were first isolated from the metabolite products of the rice pathogenic fungus, *Gibberella fujikuroi*, in 1938 (Yamaguchi, S., 2008). Gibberellins promote growth by increasing plasticity of the cell wall followed by the hydrolysis of starch to sugar, which reduces the potential in the cell, resulting in the entry of water into the cell causing elongation, hence, causing germination of seeds (Tura Bareke 2018). The gibberellin hormone plays an important role in regulating the synthesis of enzymes that are important in the plant germination process. GAs stimulated the genes encoding for enzymes such as endo - β - 1, 3glucanse and β - 1, 4 mannanendo hydroase (Zhihen Yuan et al 2014). Several studies have shown that moistening seeds with plant hormones before sowing improves their germination and increases their resistance to stress factors compared to untreated seeds in the early stages of germination. Our research team studied the effect of different concentrations of gibberellin solution on the seed germination stage.

2. Materials and methods

Experiments Medicinal *Silybum marianum L.* seeds used as the object of study. The experiment was performed at the Institute of Genetics and Experimental Biology of Plants in the laboratory "Genetics, selection and seed production of legumes, oilseeds and medicinal plants" The object of our study was *Carduus marianus* L. grown wild in Kashkadarya region (Uzbekistan), collected in 2020 (*Silybum marianum* L.) seeds were selected. Before sowing, the seeds are soaked in 70% ethyl alcohol for 1 minute for surface sterilization, after soaked in a 1% solution of potassium permanganate for 20 minutes. The seeds treated in the solution thoroughly washed in running water. The washed seeds were (was) soaked in gibberellin acid 1; 0.1; 0.01 and 0.001 micromolar solutions. The selected sterilized seeds were placed in two layers of filter paper in a 12 cm Petri dish, the seeds in each experimental variant 4 were held in return. The experiment performed on 10 selected seeds in each variant. The amount of water absorbed by the seeds during the swelling stage was determined relative to the dry weight using the weighing method. The experiment is determined by weighing every 4 hours until the seed mass remains unchanged.

The amount of water soaked in the seeds of medicinal plant and rate of hydration was determined on the basis of the following formula (Yu Tian, Bo Guan, Daowei Zhou et al 2014**)**

The amount of water absorbed=W1 - WoW
Imbibition rate= $(W_1 - W_0) / W_0$
Here: W1 - Seed weight determined at the end of the experiment;
Wo - Seed weight determined at the beginning of the experiment.
Seed germination rate is determined using the Timson germination index '.
Seed germination rate index = ΣG/t
Here: G - the percentage of germinated seeds, %
t - growth period, days
The mean germination time (MGT) for measure the germination speed calculated using the formula:
MGT=Σ (n×g) /N,
Where: n - is number of seeds germinated on day g and N is the total number of germinated seeds (Muhammad Fareed Hafz et al.2021)
Final germination percentage (FGP)
FGP = Nt/N× 100
Where Nt is the number of germinated seeds and N is the number of tested seeds (Nassima Baha 2021).

A morphometric study conducted to study the dynamics of seed growth. Seeds measured once a day for the length of the initial root and initial stem during the study period. In order to study the effect of phytohormones on the formation of autotrophic function in seeds, the amount of chlorophyll at the stage of photomorphogenic development of seeds was determined. The samples were ground and frozen in 96% ethyl alcohol at - 18° C for 1 day. The content of chlorophyll a, chlorophyll b and total chlorophylls in the alcohol extract was determined spectrophotometrically according to Arnon method (Amit Kumar et al.2020) on an SP - UV1100 spectrophotometer.

3. Result and Discussion

The study of seed germination stages of medicinal plants is important for determining the quality indicators of medicinal plants. This initiates several biochemical events necessary for seedling development. Activation of water hydrolase enzymes in the early stages of seed germination is important in formation of hydrostatic pressure for cracking of the seed coat and in the metabolic process. The first stage of fermentation absorbed by diffusion into a certain amount of water. Water absorbed into the sperm cells is a key factor in the activation of biochemical processes that take place in the cell of seeds

In the experiment, the amount of water absorbed into the seeds, soaked in a solution of gibberellins acid of various concentrations, was determined. The results shown in the first diagram.

It observed that water absorption into the seed accelerated during the 4 - 8 hours of seed germination. The highest rate (11.4 mg) observed in seeds treated with a 0.01 µmol solution of GA3. The following values observed in 0.1 and 1.0 µmol solutions of GK3. However, it found that the swelling of seeds moistened with a solution of GK3 at 0.001 µmol was slower than in the control variant.

The speed of water swelling of seed affects the germination energy. Because the cracking of the seed coat of the embryo depends on the rate at which it swollen by water. The diagram shows the speed at which seeds germinate in the experimental variants.

It also observed that the speed of water swelling of the seeds correlated with the swelling power of the seed. In the control variant, the rate of swelling of the seeds was 0.398 mg, 0.379 mg in 0.001 µmol GK3, 0.465 mg in seeds soaked of 0.01 µmol solution, 0.463 mg in 0.1 mol solution and 0.461 mg. The plant hormone GK3 used in the experiment also found to affect the germination index of seeds (The mean germination time, germination, germination rate indices). The plant hormone GA3 used in the experiment was also found to affect the seed germination quality indicators (The mean germination time, final germination percentage, germination rate index).

It has been observed that GK3 affects the quality of maize seed germination. The best results observed in seeds treated with a solution of GK3 with a concentration of 500 mg L - 1. Low values observed in seeds moistened with 10 and 100 mg of L - 1 GK3 solution compared to the control variant [10].

In our study, it observed that the germination energy of seeds varies in different concentrations of gibberallin solutions. Based on the results of our above experiments, it can be concluded that the swelling power of the seed affects the germination energy and Seed germination rate index of the seed. This is because the rate of seed water absorption accelerates the cracking of the seed coat. In seeds immersed in 0.01 µ molar gibberellin solution, their germination observed to be better than in the control variant. However, it was observed that in the variant using a 0.001 µ molar solution, the forgetfulness was lower than in the control variant. The germination rate index and germination energy also showed low results in seeds treated with this concentration.

In the experiment, along with the quality indicators of the seedling, the phytohormone influenced the growth of the primary root and primary stem of the seedling. The results given in the table below.

It observed that the initial root length of the plant was higher than the other variants (21.2 mm) in 0.01 and 0.1 µmol solution. In the variant using 1.0 µmol solution, the average length of the primary root was found to be 15.8 mm, while in plants using 0.001 µmol GK3 solution, the lowest root length was observed. It was observed that the initial stem of the plant was higher in the variants using a solution of GK 0.1 µmol. It found that the initial root length was longer in the control variant plants than in the seeds soaked in 0.0001 µmol solution. However, the opposite was true of the initial stem length.

The amount of chlorophyll in seedlings was determined to study the effect of GK3 on chlorophyll synthesis in the last stage of plant life, i. e. autotrophic feeding.

Experiments have shown that gibberilin influences plant photomorphogenesis. The highest value was found in 2 variants, in seeds treated with 0.001 µ molar solution. In the 1.0 µmolar solution variant, the second value was 5.49 mg/g. The lowest value is - 4.11 mg / g in 0.1 µ molar solution. Chlorophyll "a" and "b" were found to be proportional to this amount.

4. Conclusion

Seed germination from a physiological point of view, germination divided into 3 stages. The intensity of the germination stages affects the intensity of the germination process. In the process of germination, biochemical processes take place in the cells. The cell also stretches and divides. All the processes that take place during the germination of seeds controlled and regulated by plant hormones. Studies have shown that gibberellic acid affects the initial stage of seed germination. The experiment found that gibberellinic acid has different effects at different stages of germination. Seed germination rates observed to be high in a 0.001 µmol solution of gibberellic acid, and the medicinal plant could be used to improve germination rates.

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Declarations

Conflicts of interest The authors declare that there is no conflict of interest associated with this research.

Author contribution statement GMR and AAN conceived and designed the experiment. The experiment was performed by GMR and MBF. ZIA collected the experimental data. GMR and ZOT performed the analysis and wrote the manuscript. All authors read and approved the final manuscript.

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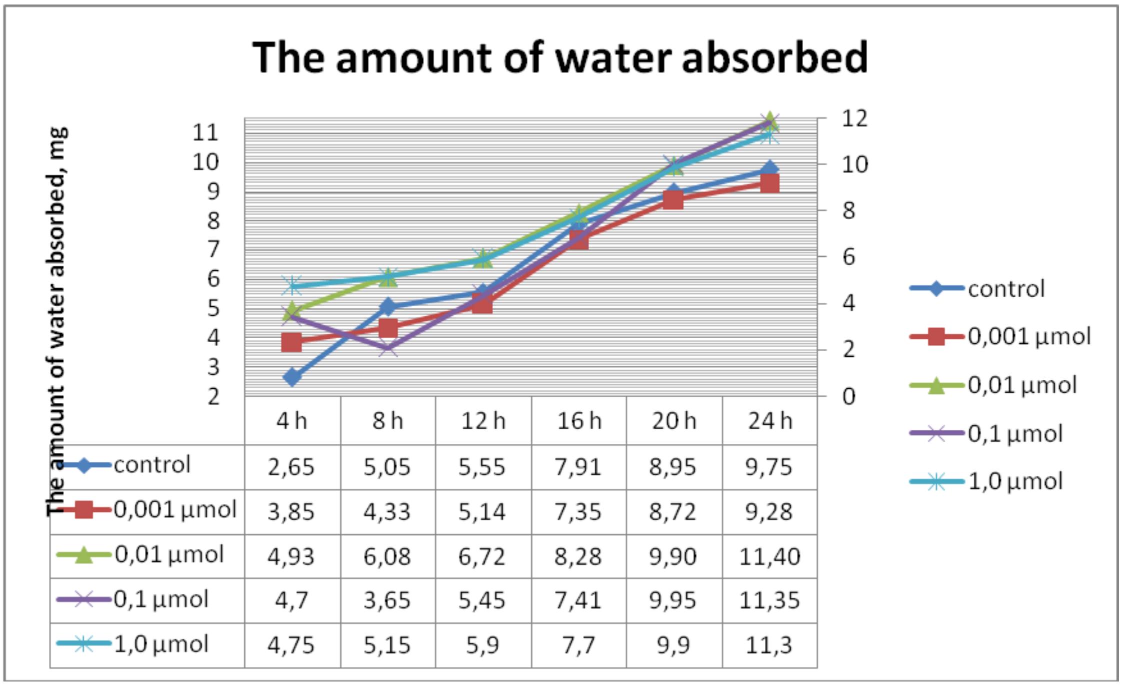

1 - diagramm. The amount of water soaked in the seeds from 0 to 24 hours was determined by weighing.
A - control option. B - Seeds soaked in 0.001 µmol solution. C - Seeds soaked in of 0.01 mol solution. D - Seeds soaked in 0.1 µmol solution. E - Seeds soaked in 1.0 µmol solution.
The amount of water soaked in the seeds on the vertical axis, mg.
Time to inflate the water on the horizontal axis, hours.

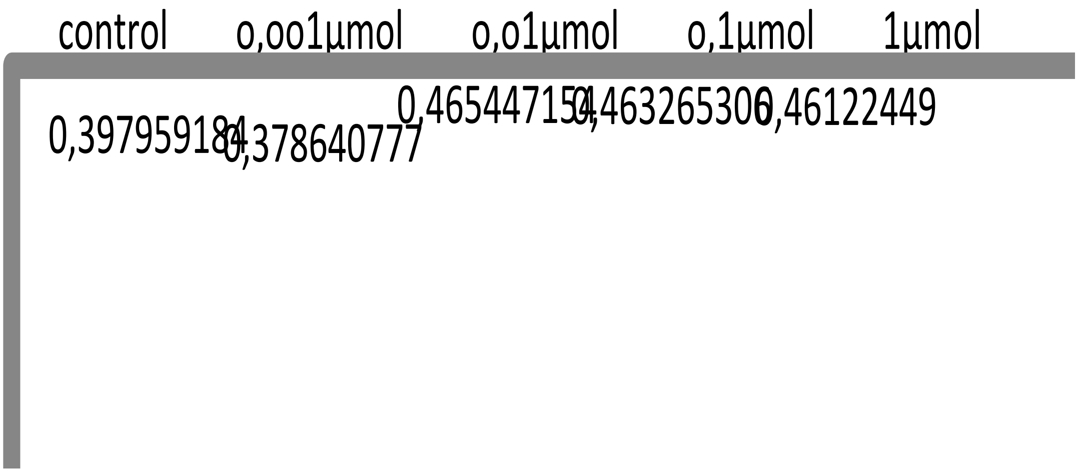

Figure 2: Imbibation rate of seeds and GK3, mg. A - control option. B - Seeds soaked in 0.001 µmol solution. C - Seeds soaked in of 0.01 mol solution. D - Seeds soaked in 0.1 µmol solution. E - Seeds soaked in 1.0 µmol solution

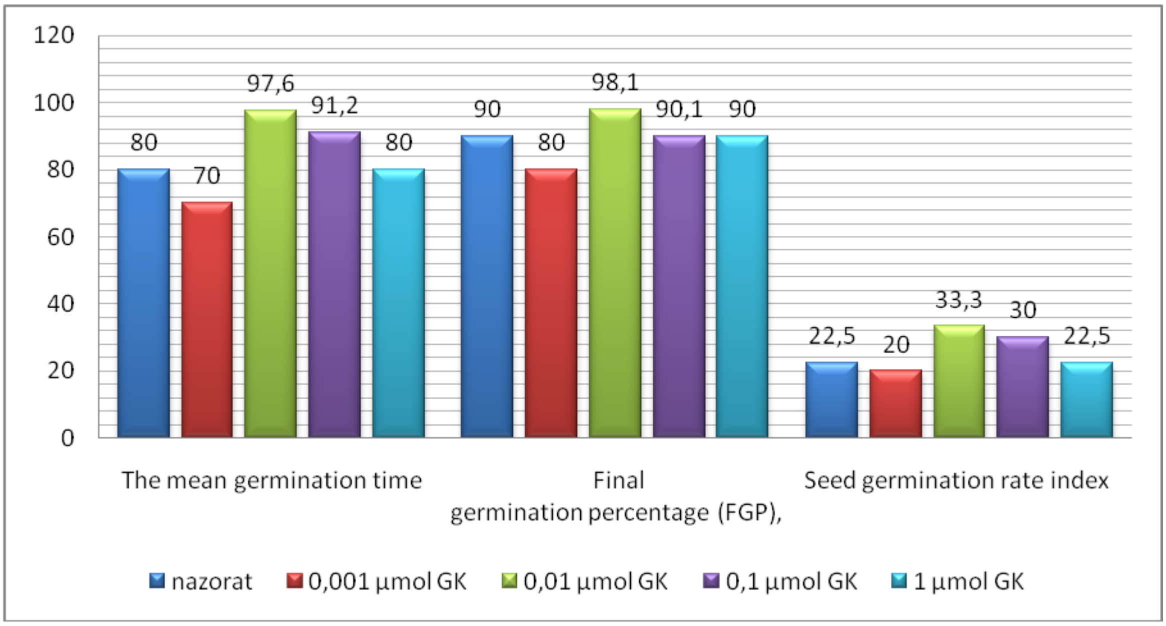

Figure 3: Effect of GA3 on the main germination time, final germination percentage and seed germination rate index. A - control option. B - Seeds soaked in 0.001 µmol solution. C - Seeds soaked in of 0.01 mol solution. D - Seeds soaked in 0.1 µmol solution. E - Seeds soaked in 1.0 µmol solution.
MGT - the main germination time, FGP - final germination percentage, GRI - seed germination rate index

The average length of the intial root and stem of the *Carduus marianus* L., mm

		control	0, 001 µmol	0, 01 µmol	0, 1 µmol	1, 0 µmol
1	The initial root lenght, mm	8, 1	9, 2	16, 6	16, 9	8, 9
2	the initial stem lenght, mm	14, 2	8, 8	21, 2	21, 2	15, 8

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