Selection of the optimal sterilization protocol of safflower seeds by the "Lysoformin-3000"

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Abstract. Safflower (Carthamus tinctorius L.) is an economically important oil seed crop. Contamination of its seed leads to a decrease in seed germination, crop yields and product quality. The research of determination of the safflower seeds optimal sterilization protocol by the "Lysoformin-3000" at the stage of introduction to the in vitro culture was carried out. The research consisted of several experiments. The first stage of the study included the seeds treatment by a sterilizing agent with a concentration of 0.5 % to 5 % and an exposure time of 3 and 7 minutes, followed by germination in Petri dishes according to the generally accepted method (GOST 12038-84). The second stage was the germination of safflower seeds directly in vitro culture using the best sterilization protocol from the first experiment. The absence of manifestations of contamination in the samples, as well as the germination of seeds was assessed. It was found that the highest yield of viable aseptic explants can be obtained by keeping the seeds in a 5 % aldehyde sterilizer solution for 3 minutes.

1 Introduction

Safflower (Carthamus tinctorius L.) is a plant that belongs to the Asteraceae family cultivated mainly because of orange-red dye (cartamine) found in its flowers and the oil in its seeds [1]. Safflower is resistant to cold, drought, soil salinity and stress caused by these conditions, and therefore it can be successfully grown in a rid climates with insufficient and unstable moisture. It is assumed that safflower seeds can adapt to adverse climatic conditions, especially to global warming, which provokes unstable environmental conditions. Monounsaturated fatty acids rich in oleic acid and polyunsaturated fatty acids rich in linoleic acid are obtained from safflower seeds. [2]. Recent studies of the health food market [1-4] demonstrate the active and successful use of safflower oil. In addition, after extracting the oil, safflower pulp (husk) is a suitable vegetable protein feed for animals. [5]. Safflower oil can also be considered as an alternative to oil [6]. It was shown [1] that safflower also has pharmacological properties, such as antifibrotic, antidiabetic, antitumor, anti-inflammatory, hepatoprotective, anticoagulant, antihyperlipidemic and antioxidant activity.

Safflower is a multi-purpose plant cultivated all over the world due to its various

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functions. Therefore, the selection of an effective sterilization regime for safflower seeds, and further development of a micropropagation protocol, can greatly contribute to the cultivation of this potential and adaptive oilseed crop.

Previous research in this area includes sterilization of safflower seeds by soaking for 30 minutes in a 30 % commercial bleach “Axion” containing 6 % sodium hypochlorite [7] or in a 0.1 % solution of mercury chloride with an exposure of 8-10 minutes [8]. The authors [9] used hydrogen peroxide and persulfuric acid in various concentrations as sterilizers.

The paper presents the results of a study of the concentration of “Lysoformin-3000” and exposure time influence on the viability of safflower seeds, and also shows the possibility of safflower seeds using as primary explants for introducing into in vitro culture in order to improve the quality of the culture and increase of production volumes.

2 Materials and methods

Safflower of Alexandrite variety was chosen as the object of study in this work. “Lysoformin-3000” was used as a sterilizing agent. The composition of this drug includes the following active ingredients: - glutaraldehyde (9.5 %), - glyoxal (7.5 %), - didecyldimethylammonium chloride (9.6 %), - as well as auxiliary components. The pH of “Lysoformin-3000” is 3.7 ± 0.3.

At the first stage of the study, the effectiveness of “Lysoformin-3000” was evaluated for the germination of safflower seeds according to the generally accepted methodology (GOST 12038-84). Safflower seeds were washed with water using laundry soap to remove foreign, visually visible particles from their surface. Then the seeds were washed for 1 hour in running tap water, dried with filter paper and subjected to chemical sterilization. Safflower seeds treated with Lysoformin-3000 were washed in 3-4 portions of distilled water and germinated according to the method described in GOST 12038-84. Safflower seeds, which were not subjected to sterilization, were used as a control. The concentration of “Lysoformin-3000” varied from 0.5 % to 5 %, the exposure time was 3 and 7 minutes. The experiment was carried out in triplicate. Germination was determined on the 10th day. The presence of infected, aseptically viable and aseptically nonviable explants was recorded daily.

The most appropriate seed sterilization regime was selected based on the data obtained from the experiment described above. Safflower seeds were washed with water using laundry soap and then washed for 1 hour in running tap water for introduction into in vitro culture. Further, under the conditions of the microbiological safety box “Laminar-S” NEOTERIC (class II, type A2), the seeds were subjected to chemical sterilization, after which they were washed in 3-4 portions of sterile distilled water. The treated explants were placed in test tubes, Petri dishes or jars on agar nutrient medium and covered with cling film. We used a nutrient medium prepared according to the Murashige and Skoog prescription [10], with the addition of growth regulators: 6-benzylaminopurine (BAP) – 1 mg ⸱ l⁻¹, 1H-indolyl-3-butyric acid (IBA) – 0.5 mg ⸱ l⁻¹ for cultivating safflower seeds. Containers with seeds were placed in a climatostat KS-200 (illuminance – 3000 lx, temperature – 25 °C, relative air humidity – 70 %). The number of aseptic viable, aseptic non-viable, infected plants was recorded daily for 10 days.

Work in a laminar flow box, as well as the preparation and autoclaving of nutrient media, treatment of the premises, sterilization of utensils and instruments were carried out in accordance with generally accepted standards and methods of work in aseptic conditions.
3 Results and discussion

Soil pathogens have a negative impact on agricultural crops, including safflower, which leads to a decrease in seed germination, their yield and quality. One of the ways to solve such problems is to obtain a healthy seed material by in vitro micropropagation. Sterilization of plant objects is the main condition for the successful introduction and cultivation of culture in vitro.

"Lysoformin-3000" at a concentration of 0.5 %, 1 %, 2 %, and 5 %, with exposure time of 3 and 7 minutes was used to obtain a sterile safflower culture (Alexandrite variety). The absence of heavy metals in the composition is an advantage of this sterilizing agent. The experiment was performed in triplicate under aseptic conditions. The concentrations of "Lysoformin-3000" were chosen based on the literature and experimental data. It was experimentally found that for the sterilization of safflower seeds it is necessary to use the concentration of "Lysoformin-3000" above 0.1 %, since when using the concentration of the sterilizing agent 0.1; 0.05; 0.025 % (exposure time 7 min), mold appeared on the 5th day. This indicates that the solution "Lysoformin-3000" with a concentration of less than 0.1 % does not have a sterilizing effect on safflower seeds. Sterilization of safflower seeds was carried out with constant stirring, then the seeds were washed with sterile water.

Scientists have shown that the use of the solution "Lysoformin-3000" increases the number of washings with sterile water from 2 to 3–4 times for better removal of the "Lysoformin-3000" from the surface of the explants. In this experiment, after washing, the seeds were placed in Petri dishes between layers of filter paper (2 layers on the bottom of the Petri dish, one layer covered the seeds on top), moistened with distilled sterile water. Safflower seeds that were not treated with Lysoformin-3000 were used as a control. Safflower seeds were germinated according to the method described in GOST 12038–84. Seeds were also laid out in Petri dishes between layers of moistened filter paper. The filter paper was soaked in sterile distilled water. Seeds were germinated at a temperature of 25 °C, without lighting in an electric dryer-air thermostat TS-1/80 SPU. The assessment of the state of the studied seeds was carried out within 10 days from the date of the start of the experiment through visual inspection. Seed germination was assessed on day 10.

Table 1. Germination of safflower seeds without their preliminary sterilization.

<table>
<thead>
<tr>
<th>Total number of seeds, pcs</th>
<th>Number of germinated seeds, pcs</th>
<th>The number of germinated seeds, %</th>
<th>Sprouts length, cm</th>
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<tbody>
<tr>
<td>50</td>
<td>17</td>
<td>34</td>
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“Lysoformin 3000” at a concentration of 0.5 %, 1 %, 2 %, and 5 %, the exposure time was...
Fig. 1. Dependence of the viability of safflower seeds on the sterilization protocol: 1–5 % solution of “Lysoformin-3000”, exposure time 3 min; 2–2 % solution of “Lysoformin-3000”, exposure time 3 min; 3–1 % solution of “Lysoformin-3000”, exposure time 3 minutes; 4–0.5 % solution of “Lysoformin-3000”, exposure time 3 minutes; 5–5 % solution of “Lysoformin-3000”, exposure time 7 min; 6–2 % solution of “Lysoformin-3000”, exposure time 7 min; 7–1 % solution of “Lysoformin-3000”, exposure time 7 min; 8–0.5 % solution of “Lysoformin-3000”, exposure time 7 min.

It was found that the number of germinated seeds decreases with increasing treatment time and the use of “Lysoformin-3000” at a concentration of 5 % with exposure time of 3 minutes leads to the maximum yield of aseptic viable explants – 53 %. This sterilization regime was used for further studies on the introduction of safflower into in vitro culture.

Seeds after sterilization were placed on the surface of an agar nutrient medium, covered with cling film and placed in a climatostat. Accounting of the number of aseptic viable, aseptic non-viable, infected plants was carried out daily for 10 days. The number of aseptic viable explants was 50 %.

The effectiveness of the chosen sterilization regime, as well as the possibility of using safflower seeds as primary explants is clearly demonstrated in Figure 2, which shows the germination of a safflower fetus on day 10.
4 Conclusions

The results of the conducted experiments showed that "Lysoformin-3000" can be used as a sterilizing agent for safflower introduction into in vitro culture. It was established that the sterilization regime with a concentration of "Lysoformin-3000" – 5 % and exposure time of 3 minutes leads to a higher yield of aseptic viable explants.

5 Acknowledgments

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