RESEARCH ARTICLE

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Determination of effective surface sterilization protocol in in vitro tissue culture for Giant Snowdrop (*Galanthus elwesii* Hook) bulbs

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Abstract

Giant Snowdrop (*Galanthus elwesii* Hook) is a species of snowdrop belonging to the Amaryllidaceae family. In this study, the deformation rates of the bulbs was calculated as % (percent). For sterilization G. elwesii bulbs were sterilized at different concentrations (1, 3, 5, 7, 9, 11 and 13 %) of sodium hypochlorite (NaOCl) for 5, 10, 15, 20, 25 minutes. The bulbs were rinsed with sterile distilled water 3 times for 5 minutes and then transferred to the MS medium. Contamination rates in MS nutrient medium were determined as percent (%) after 7 days. Deformation was not observed in the bulbs sterilized with 1 and 3% sodium hypochlorite solution however 100% contamination was detected. The most effective surface sterilization was obtained by soaking in 9-11% sodium hypochlorite solution for 5 and 10 minutes. As the sodium hypochlorite concentration and the application time increased, the surface sterilization of the bulbs increased, but the deformation rate of the scaly leaves of the bulbs increased due to this increase.

Keywords: Giant Snowdrop, Bulb, Sterilization, Contamination, Deformation

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INTRODUCTION

In terms of seed plant diversity and plant genetic resources, our country is one of the richest countries in the world and by means of biodiversity it is compared with the Europe. Biodiversity is the natural resources of the countries and it refers to the diversity of life at all organizational levels, from the genetic, population and species levels to communities and ecosystems, and a dynamic feature of the ecosystem. Protection, development and sustainable use of this resource should be among the main objectives (Leveque and Mounolou, 2003). Considering that the origin of the basic foodstuffs necessary for our life is formed by wild species in nature the importance of preserving biological equality will be better understood (Arpa, 2012).

Bulbous plants which are called as geophytes have underground organs such as bulbs, tubes, and rhizomes They contribute to biodiversity since most of the geophytes have a very long time to form new shallots in their natural environment and the bulb formation rate is very low (Baktır et al., 1997). Giant Snowdrop reproduces in the natural environment with the formation of seeds and new shallots. For the formation of a new bulb from the seed, a long period of 4-5 years is required. Snowdrop reproduces in the natural environment with the formation of seeds and new shallots. The removal of bulbs from nature and long life cycle of the plantlead to the decline of the species belonging to the genus *Galanthus*. Therefore, rapid propagation methods should be designed for generation of these plants (Tıpırdamaz et al., 1999).

An effective sterilization method should provide both effective sterilization on the surface of the plant material and against possible infection sources on the plant material. In *in-vitro* studies usually same type of surface sterilization methods are used however these methods might not be effective against field-borne in-seed and bulbus pathogen contamination.

On the other hand, Girmen and Zimmer (1988) found that efficiency of sterilization was different between explants and seeds in *Galanthus*, *Leucojum* and *Tulipa* plants.

In a sterilization study using NaOCl, they reported that they obtained *Aloe pretoriensis* (*Liliaceae*) seeds by soaking them in 5% NaOCl and 1% $HgCl_2$ for 30 minutes, then in Nalauryl sulfate for 10 minutes and rinsing them with distilled water four times (Groenewald et al., 1975).

According to Gochhayat et al. (2017), hybrid *Lilium* Cv. Explants of Tresor plants were subjected to surface sterilization with $HgCl_2$ at different times (Control, 3, 4, 5, 6, 7, 8, 9 minutes) and at least 8 minutes of fungal and bacterial contamination was observed 15, 30, 45 days after inoculation. In their study, they applied different doses of BAP (0.5, 1 mg/l) and 2,4-D (0.5, 1, 1.5, 2, 2.5 mg/l) hormones and the highest callus production was obtained from the application which they applied 1 mg/l BAP and 1.5 mg 2,4-D hormones together.

Farooq et al. (2022), applied different concentration of carbendazim, mercuric chloride and ethyl alcohol sterilants to bulb shells and young leaf segments of "Indian Summerset" and "Nashville" Lilium LA hybrids at different times. The maximum percentage of asepsis was found in the application of Carbendazim 0.02 % for 30 minutes, mercuric chloride 0.1% for 5 minutes, and ethyl alcohol 70% for 10 seconds in both varieties.

Kone et al. (2011), conducted a study on the effect of substrate type and bulb size on *in vivo* production of seedlings in three plantain cultivars. They determined that the application of furadan+mancozeb in all cultivars in their study was successful in reducing contamination than other applications (Javel 0.25%, water 50°C, Javel 0.25%+water 50°C). In that study, the average number of buds in the bulb obtained from the soil substrate application was higher than the other applications (sawdust, sand). In addition, the largest bulbs were obtained from the least amount of buds in all cultivars.

This study was carried out to determine an effective sterilization method against microbial pathogens found in the bulbs of *Galanthus elwesii* plant. For this purpose, *Galanthus elwesii* bulbs were kept in 1, 3, 5, 7, 9, 11, 13 and 15% sodium hypochlorite (NaOCl) solution for 5, 10, 15, 20 and 25 minutes.

MATERIALS AND METHODS

In this study, the bulbs of *Galanthus elwesii* plants were used as material. Bulbs were washed under tap water for 10 minutes, and the soil, mud and other foreign matters were removed. In order to achieve an effective surface sterilization; 1, 3, 5, 7, 9, 11, 13 and 15% sodium hypochlorite (NaOCl) solutions (Y1, Y3, Y5, Y7, Y9, Y11, Y13, Y15) were applied to the bulbs for 5, 10, 15, 20 and 25 minutes (S5, S10, S15, S20, S25). The rate of deformation that occurred in bulbs after NaOCl application was determined as percent (%) and is shown in (Table 1). The sterilized bulbs were rinsed by passing them through distilled water 3 times for 5 minutes. After rinsing, bulbs were cultured in MS (Murashige and Skoog, 1962) nutrient medium. The contamination rate that occurred 7 days after the bulbs were cultured was determined in percent % and shown in (Table 1). In the study, nutrient mediums, tools and equipment were sterilized by autoclave under 1.05 atmosphere pressure at 120°C for 21 minutes. The pH of the nutrient medium was adjusted to 6.1 using 1 N KOH and 1 N HCl. Bulbs taken into the culture medium were cultured at 25±0.5°C in a climate cabinet with 18 hours of light (350 µmol m^{-2} s^{-1}) and 6 hours of darkness. For each repetitions, 4 bulbs were used, the trials were planned as 3 repetitions and the average of the percent (%) values formed as a result of these repeated trials was calculated. Relationships between the investigated features were determined by correlation analysis (Table 1). Biplot analysis was used in the interpretation of the data and the data were evaluated on the graph (Figure 1-2).

RESULTS AND DISCUSSION

We found a significant negative correlation between the contamination and deformation rates in terms of solution concentration and treatment duration (Table 1).

Table 1. Correlation coefficients between contamination and deformation rates

	Contamination rate	Deformation rate
Contamination rate	1,000	-0,8278***
Deformation rate	-0,8278***	1,000

The resulting inverse ratio was also seen in the graph of the correlation analysis. It can be concluded that as the solution concentration and duration of the treatment were increased the contamination rate was decreased while the deformation rate was increased.

In this study, keeping the bulbs in 9-11% hydrochloric acid for 5-10 seconds both sterilized and did not degenerate. The samples in the subjects with high deformation rate were deformed which meant that their structures were deteriorated due to the effects of high hypochlorite concentration or treatment time. It was found that the samples with high contamination rate were infected by bacteria or fungi due to low hypochlorite concentration or treatment time. When the biplot graph is examined, it can be said that the subjects closer to the origin than other subjects such as Y11S15, Y11S10, Y7S25, Y7S20, Y11S5, Y9S10, Y9S5 comply with the described definition

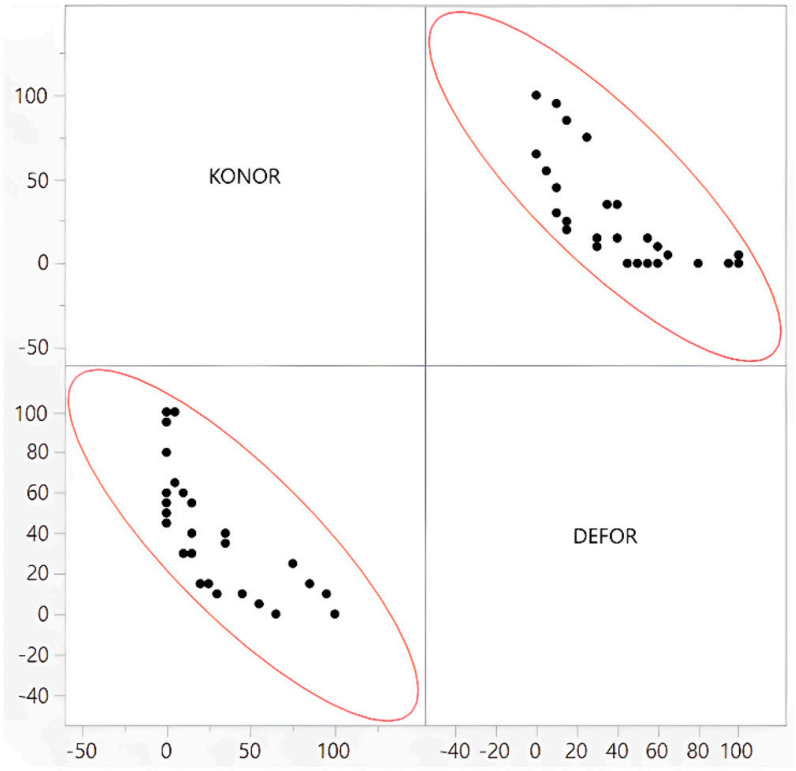

Figure 1. Correlation Analysis (Konor: Contamination rate, Defor: Deformation rate).

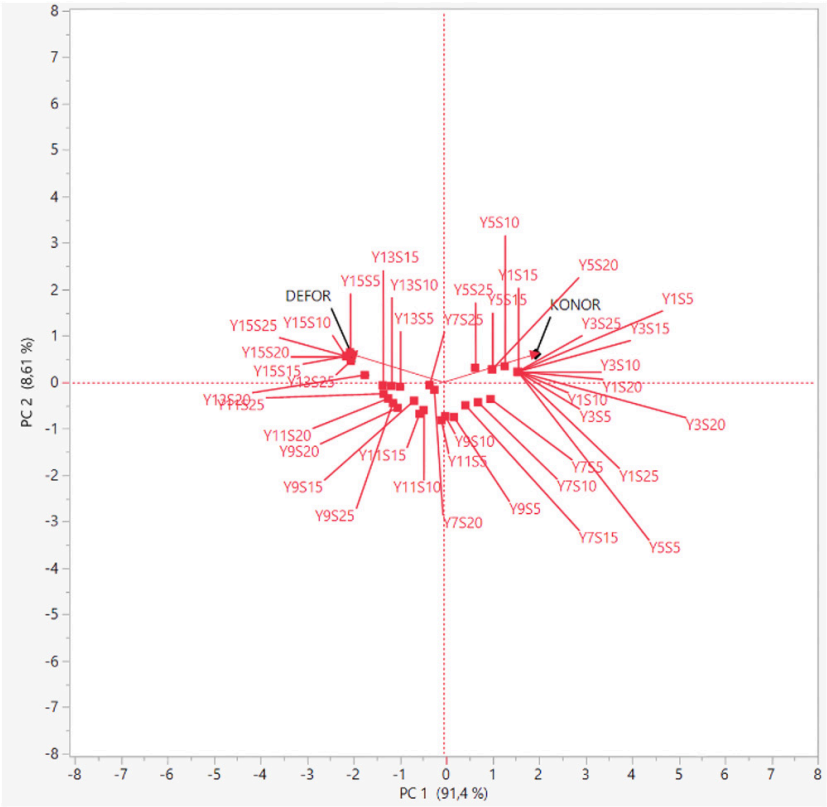

Figure 2. Graph of Biplot Analysis (Konor: Contamination rate, Defor: Deformation rate).

and undergo less contamination and deformation than other subjects, that is, they are more usable.

According to the results of the study, the average contamination rate was determined as 100% and the average deformation rate was 0% in all bulbs dipped in 1% NaOCl for 5, 10, 15, 20 and 25 minutes. The average contamination rate was determined as 100% and the average deformation rate was 0% in all bulbs dipped in 3% NaOCl for 5, 10, 15, 20 and 25 minutes.

The most effective surface sterilization was obtained by 9-11% sodium hypochlorite solution for 10 and 5 minutes. The deformation rates of scaly leaves of the bulbs was increased by high hypochlorite concentrations and treatment time.

In another study, the surface sterilization of the bulb explants of *G. nivalis* and *G. elwesii* was followed by sterilization of the whole bulb with 2 and 3% NaOCl solution for 20 minutes. It provided almost complete infection control in all of its batches, making the use of PPM or fungicides in the medium unnecessary. Therefore, they recommend the use of NaOCl in the surface sterilization of plant materials of the genus *Galanthus* (Staikidou et al., 2008).

CONCLUSION

Before starting the studies using plant tissue culture technique, the surface sterilization of the plant material to be used as material should be done effectively. If the surface sterilization of the plant material cannot be performed effectively, plant material, time and chemicals will be lost due to possible contamination problems. Therefore, it is very important to develop an effective surface sterilization protocol for the plant material used. Deterioration in tissue culture due to sterilization and hydrochloric acid (NaOCl) used can be a problem for researchers. With this study, keeping the bulbs in 9-11% hydrochloric acid for 5-10 seconds both sterilized and did not degenerate. Information has been obtained that can directly benefit those who will do tissue culture with Giant Snowdrop bulbs. Apart from these, many scientists around the world have tried sterilization in different organs and tissues of various plants by using a wide variety of substances and methods, and naturally they have reached very different results. The study provided convenience to those who do plant tissue culture studies. According to the results obtained, considering the applied concentration and time, it can be suggested that NaOCl can be used successfully in the sterilization of the bulbs of the Giant Snowdrop plant.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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