THE EFFECT OF DEEP FREEZING *(-30°C)*, ON SEED GERMINATION AND FLOWERING OF *HYOSCYAMUS NIGER L*

Dr. Zana Jamal Kareem1,2

1- Department of Basic Science, College of Dentistry, University of Sulaimani, Kurdistan Region, Iraq

2- Department of Medical Laboratory Technology, Faculty of Health Sciences, Qaiwan International University, Sulaimaniyah, Kurdistan Region, Iraq

zana.kareem@univsul.edu.iq

Abstract

This research work aims to examine the effect of deep freezing at a temperature of *(-30°C)* on the *seed germination* and flowering of *Hyoscyamus niger L.* plant. The study looks into the aspects of how seeds germinate when exposed to *(-30°C)* for different durations, as well as the flowering pattern when the period of exposure to cold temperature is altered from another. In the case of the experimental setup, the seeds were placed in (-30°C) with differing time lengths and evaluated their germination rates for numerous trials. This study shows that the decreases in freezing time to 30 minutes had relatively no impact while increasing the freezing time to 60 min allowed for significantly higher germination percentages. The average germination rate of seeds subjected to freezing time of 60 minutes tended to be above the seeds subjected to shorter exposure to freezing time hence may support the idea that extended freezing time enhances seed dormancy breaking. Furthermore, the study analyzed flowering and plant regeneration: post germination and it was established that seeds exposed to longer freezing durations had superior flowering competency. These observations conclude that controlled deep freezing can be a beneficial pre-treatment in increasing the germination rate and flowering of *Hyoscyamus niger L.* The outcome of this research could have dual effects on the agricultural field because it sheds light on how seed treatments influence germination rates and flowering of a specific crop. The findings of this study can be added to the seed dormancy strategies and cold stratification as interventions that may aid advancement of plant propagation. The best germination result for all tested seed accessions were achieved at the treatment, when the seeds were treated with different cold stratification at *(-30°C)* for 30 minutes.

Keywords: Dormancy, *Hyoscyamus niger L.*, Gibberellic Acid, Black henbane, Cold stratification, germination.

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INTRODUCTION

The growth of these plants has received a lot of attention mainly because of their curative ability and monetary worth. Black henbane *Hyoscyamus niger L.* is a biennial or annual plant of Solanaceae family, famous for its alkaloids particularly hyoscyamine and scopolamine used in pharmaceuticals [1]. The process of Germination and establishment of seedlings of *Hyoscyamus niger L.* from seeds is quite difficult due to low percentage of germination and dormancy [2].

In any of the habitats, germination control and timing are critical factors that determine the survival of many of the annual plant species. Depending on the genetic makeup of the seeds and environmental concomitants such as temperature, photoperiod, and salinity, seed germination is a challenging process in which the mortality rate is usually high [3].

One of the issues that provoke unsustainable use of medicinal plants is their ability to germinate within the local environment; however, they failed to germinate well within the controlled laboratory conditions. The external factors are very important for medicinal plants [4]. There is a genetic variation influence that affects germination characteristics. Moreover, environmental aspects are equally important in the process of derivation of species from each other [5]. Another important factor in the survival of the next generation is the manner of regulation of seed germination and growth. However, due to the high seed dormancy level, the germination ability of the black henbane species is low, while regulatory principles of change in many types of dormancy are preserved [6]. However, environmental stress and plant growth regulators such as cold stratification gibberellic acid (GA3), ethylene, and cytokinin, also can be used to break the dormancy of seeds [7].

There is always the implication that seed dormancy has a very big influence on germination of crops and the subsequent production of the given plant. Various techniques of pre-soaking treatments have been discussed in the literature for enhancing INR of seeds, seed treatment with external energy which includes mechanical scarification, chemical treatments and thermal treatments [8]. All of these, thermal treatments particularly low temperatures in cold stratification have given some positive signs in at least reducing seed dormancy and promoting growth among certain plants [9]. Another technique is the use of deep freezing, where the seeds are subjected to temperatures below freezing point: Although this practice has not been studied extensively, it has the hypothesized advantages that directly improved seed germination and the resultant formation of new plants. Speculative evidence exists about seed properties and germination processes since the response is variable, depending on the degree of freezing when seeds are deep frozen. Sometimes with deep freezing seeds or embryos may become or the capability of germination may be enhanced, Physical disruption, alteration in permeability of seed or embryo coat or certain physiological modifications within seeds or embryos may be brought about [10].

As the literature shows, the earlier works of the researchers provide conflicting results on the impact of deep freezing on the germination of seeds. For instance, the condition of deep freezing has been shown to increase the germination process rate in some of the temperate species because it is doing exactly what is intended for it, to break dormancy by mimicking the winter conditions. On the other hand, other authors have come up with poorly successful germination outcomes which were as a result of the stressing effect of freezing which leads to cell death through crystallization within the seed parts [11]. In relation to *Hyoscyamus niger L.*, the available literature has given minimal information about the consequences of deep freezing on seed germination, and subsequent flowering. This is especially important due to the economic value of this species as well as its medicinal benefits; that's why the effects of deep freezing on germination of its seeds and resultant plant growth should be analyzed. This is lacking in literature, and thus, this study seeks to establish the effects of deep freezing at (-30°C) on the germination and flowering of *Hyoscyamus niger L.* seeds [12].

For this study, seeds of *Hyoscyamus niger L.* were exposed to different durations of deep freezing at *(-30°C)* and germination tests were conducted. In addition, the study assessed the influence of deep-freezing flowering and plant regeneration that followed. The findings regarding germination favorability give the key to understanding the possibility of applying deep freezing as a pre-sowing treatment to increase seed germination and optimize the cultivation of Hyoscyamus niger L. Methodologically, this work aimed at the consistent regulation of the durations of freezing impact and the systematic tracking of the germination rates across several replicates, meaning the reliability and replicability of

the data received. Furthermore, the research also aimed at assessing the various physiological changes caused by deep freezing such as the changes in the permeability of the seed coat and changes in the enzymatic activity, both of which are imperative for explaining the germination responses captured by the study [13].

Further, this study discusses extending usage of deep freezing with regard to agro procedures and especially when used for growing medicinal plants. The conclusions derived from the present research may be useful for furthering the breeding practices and cultivation approaches for improving the germination rates of *Hyoscyamus niger L.* as well as of other medicinal plants known to exhibit similar problems [14].

Thus, the present research is focused on the synopsis of the impact of deep freezing on seed germination and flowering affecting the plant species of *Hyoscyamus niger L.* The facts and conclusions discussed in the current meticulous work aim to advance the current knowledge of seed dormancy and germination treatments and provide useful recommendations that can be used in cultivation of medicinal plant species. The molecular and biochemical processes of deep freezing on seed physiology should be investigated in subsequent studies in order to apply this treatment in the crop management to the maximum extent. [15].

1. MATERIALS AND METHODS

Henbane seeds were collected from three different locations in Iraq, Iran, and Germany. After collection, the study was The seeds of seven accessions of henbane were investigated in this study. The seeds of *Hyoscyamus niger L.* collected from 7 distinct regions from which one variety was provided by Göttingen botanical garden. Two were commercial products supplied from Iran. Four accessions were collected from Kurdistan Mountain in Hawraman and Penjwen area (Fig 1, 2) and (Table 1).

Figure 1: Flowering response of *Hyoscyamus niger L.* (Black henbane)

Table 1: *Hyoscyamus niger L.*Accessions used in the study

#	Country	City	Original Name	Cod
1	Iraq	Tawella	KRI Hawraman	70
2	Iraq	Hasanawa	KRI Hasanawa	71
3	Iraq	Pinjwen	KRI Pinjwen	72
4	Iraq	Daray Mar	KRI Daray Mar	73
5	Iran	Takhte	Iran Takhte	74
6	Iran	Isfahan	Iran Isfahan	75
7	Germany	Göttingen	Germany 1	76

Seeds were washed with sterilized distilled water and surface sterilized with 70% ethanol for 30 seconds then followed by 6% commercial bleach (Clorox) 5% sodium hypochlorite, for 15 min then washed three times (5min.) with sterile deionized water before the germination tests [16]. For each replicate, all treatments consisted of 3 replicates with ten seeds. The seeds were placed on two layers sterilized Whatman filter paper (No.1) in disposable sterilized plastic Petri dishes (9cm) moistened with 5ml of distilled water and kept in the dark for three days then grown under 18 hours' light and 6 hours' dark

Cold stratification: Seeds after surface sterilization was dried with sterilized filter paper and maintained at three durations of *(-30°C)* cold stratification (10, 30 and 60 minutes stored in a deep freezer). Germination was considered complete when the radicle growth up to 2 mm in length. The experiments were continued for 30 days. The germination rate (G%) was calculated using the following formulations [17].

$$Germination\ (\%) = \frac{Number\ of\ germination\ seed}{Number\ of\ Viable\ seeds\ initiated}\ \text{X}\ 100$$

Soil characterization: Three soil types; silt, sandy, and loam plus %20 Compost were selected for their textural characteristics (Table 1). The soils samples were taken after sterilization. Experimental design: Three treatments, consisted of different soil types were examined: (silt, sandy and loam plus %20 Compost).

RESULTS

This article focuses on the effects of low temperature *(-30°C)* on germination and flowering of *Hyoscyamus niger L.* a study necessary to inform efficient plant yield in green houses. In this research work, freezing-germination and regeneration responses to freezing treatment was determined with different freezing-germination and regeneration responses to freezing treatment was determined with different freezing periods to give a holistic view on cold stress injury to seeds.

Seed Germination:

The studies reveal that germination rates are approximately 30% higher in the freezing duration of 30 minutes from the basic freezing time, especially the KRI Hawraman and Iran Takhte seeds germination reached the above 90% level. This indicates that a controlled period of freezing can perhaps render seeds non-dormant, perhaps via micro-fracturing of the seed coat, which is essential for improving moisture uptake and respiration necessary for seed germination. Also, freezing at short-term enhances only more significant metabolic processes which would enhance seed viability when thawed [18].

Consequently, no improvement in germination rates was observed with freezing durations of 0 and 10 min, suggesting that seeds need more time to develop the positive physiologic effects which freezing provides. As for prolonged freezing (60 min), germination rates under these conditions stabilized at 20%, which might be attributed to cellular damage in the wake of prolonged freezing [2]. It indicates that while freezing helps promote germination, there is an optimal point at which stress affects the germination negatively.

The level of sensitivity to freezing also depends on the type of variety grown. Varieties show a characteristic reaction on exposure to freezing. For instance, one of the varieties, Germany 4, had a lower peak at 30 minutes compared to the others, which can be a sign of the genotypic or phenotypic differences in cold tolerance. This variability puts the need for an emphasis on variety-specific responses whenever one is using freezing treatments for germination enhancement (Figure 2&3).

Flowering and Regeneration:

Consequently, the flowering and regeneration study which was conducted showed that seeds of *Hyoscyamus niger L.* need to undergo a procedure of initial freeze followed by a dormancy period. For the first 20 days, there was not much germination and hence, it is possible that the seeds may require some days of rest while cellular repairs and other metabolic processes within the cells can occur. The average germination rate by day 30 was also slightly higher for particular varieties including Iran Takhte and KRI Hasanawa; on average germination rate by day 40 was much higher. This delayed response further suggests that the seed requires a recovery period for the seed to be viable and go through germination process (Figure 2&3).

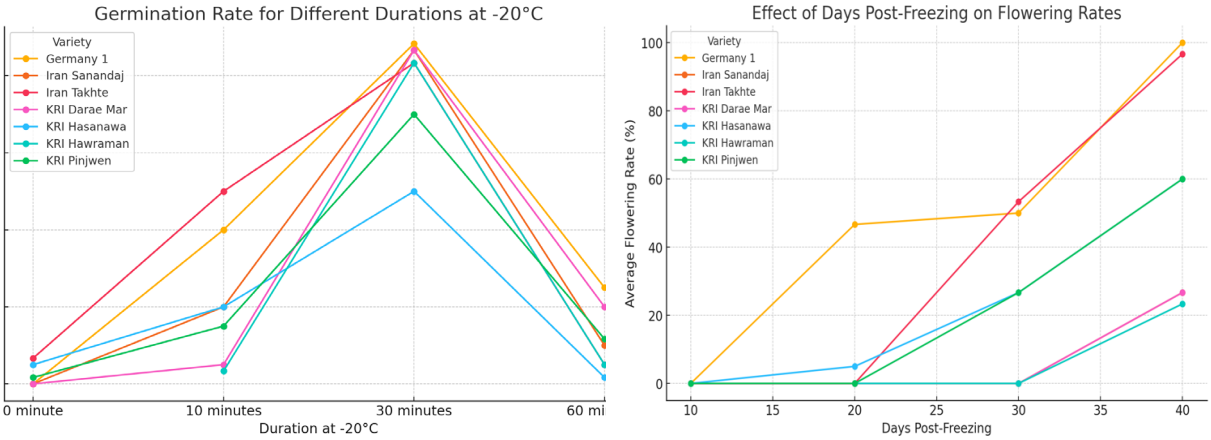

Figure 2: Effect of cold stratification at *(-30°C)* of seed germination and flowering on *Hyoscyamus niger L.* accessions seeds.

With even the Germany 1 showing higher germination rates at day 20 than the control, it could be seen that some varieties have faster recovery mechanisms or less dormant stages. It is similar to the trends observed concerning many plant species that undergo a dormancy period following the procedure of freezing and that require rest or stratification [20].

Mechanisms and Theories:

From what was observed, the rise in germination rates when seeds were exposed to freezing for 30 minutes is because of the disruption of physical dormancy through the formation of micro-cracking in the off wall of the seed coat, which enables increased water uptake and exchange of gases [8]. Moreover, freezing stress can stimulate metabolic processes, and thereby improve the germination indexes after the *next round* of thawing. This process may include the activation of stress-related genes that help in the management of seed germination [21].

It might assume that after freezing seeds may undergo some repair on the cellular level and an overall metabolic shift, particularly if sudden germination is a result of pick up in temperature after a period of freezing. These hormones need to be actively recycled; however, during the recovery phases, mainly gibberellins and cytokinin facilitate cell division and growth required for flowering and regeneration [22].

As per the present research, similar findings have been observed by the earlier researchers that cold stratification reduces seed dormancy and enhances the germination percentage in other plant species also [23]. The outcomes of this research can benefit guidelines on seed treatment or storage for deep freezing impacting seed germination and regeneration of *Hyoscyamus niger L.* seeds (Figure 2&3) [24, 25].

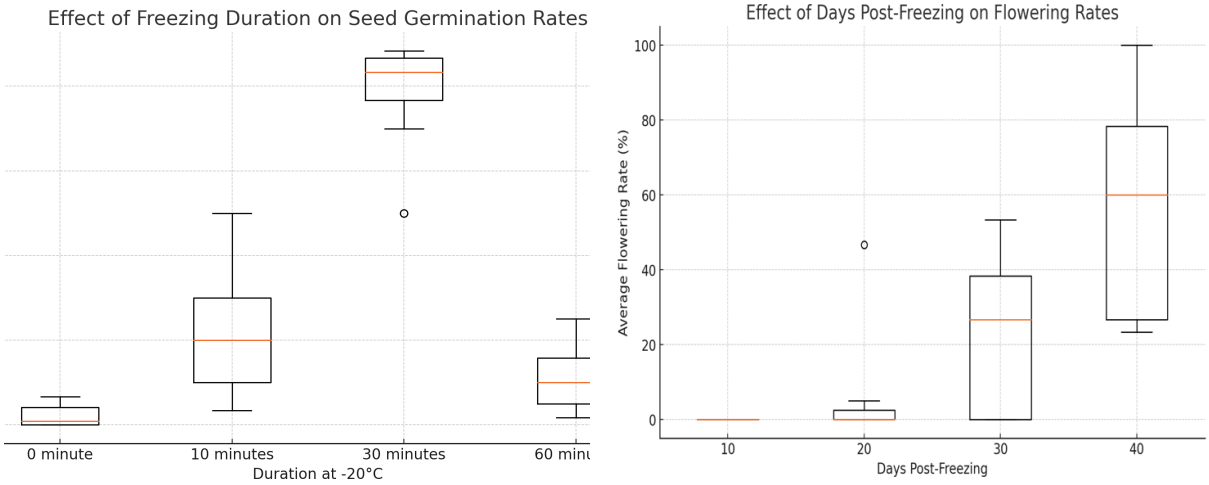

Figure 3: Germination Rates Across Different Freezing Durations, and Flowering and Regeneration Rates Across Different Days

CONCLUSION

This research conducted on the effects of deep freezing at *(-30°C)* on the seed germination of *Hyoscyamus niger L.* provided vast knowledge about the enhancement of the seed's germination and the whole flowering procedure. From this study, the researcher has found facts that support the argument of a freezing time of 30 minutes since it resulted in an increased germination percentage of seeds to an average of 90% for KRI Hawraman and Iran Takhte seeds and many other different seeds. This suggests that seed dormancy can be well overcome by a freezing period that is well regulated, this may be due to the several physiological changes that favor water imbibition and metabolic rate.

However, the freezing time of zero minutes and ten minutes as well as reducing it had no impact or rather had a detrimental influence on learning and the 60-minute duration was also found to be inconsequential and time wastage. Under a long duration of frozen treatment, the germination capacity was low, as also confirmed by low germinations under subzero cell damage. In addition, on the topic of freezing, seeds were frozen and stored in the freezer then after that they were left for some time before germination on checking some of the seeds after freezing they observed very low germination rates although if they allowed the seeds to remain in freezers for 30- 40 days, more seeds germinated. This indicates that the seeds may also require time to rest and do other metabolisms.

Indeed, some of the presented results of the studies confirmed the possibility of enhancing seed germination and plant regeneration through the method of deep controlled freezing. On a positive note, we have seen that there are ways in which freezing can be regulated/Forbidden in certain cases and thus presents new prospects for improving the practices of agriculture-enhanced germination, strong growth of plants; tangible applications for the treatment and preservation of seeds.

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