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# How Pretreatment Affect the Dormancy, Germination and Seedling Growth of Caper (*Capparis Spinosa* L.) Populations

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## ABSTRACT

In order to investigate the effects of pretreatments on dormancy and seedling germination of two caper populations, a laboratory experiment was conducted (2019-2020) in Islamic Azad University (Isfahan Branch). The study was performed as a split factorial experiment in a completely randomized design. Main plot consisted of different leaching levels (soaking in distilled water for 0, 6, 12, 18 and 24 hours) and subplots included factorial of two seed populationes (Najafabad, Izeh) and nine levels of hormone or chemical treatments (gibberellic acid (100 mg/l), benzyl adenine 6 ($10^{-5}$ M), kinetin ($10^{-5}$ M), gibberellic acid+ benzyl adenine, Gibberellic acid+ kinetin, benzyl adenine+ kinetin, gibberellic acid+ benzyl adenine+ kinetin, potassium nitrate 0.4%, distilled water as control). According to the results, 18 hours leaching of Najafabad population plus gibberellic acid +kinetin and 24 hours leaching of Najafabad population with benzyl adenine showed the highest germination uniformity. The highest germination percentage was observed in 24 hour leaching of Izeh population plus gibberellic acid+ benzyl adenine+ kinetin and also 24-hour leaching of Izeh population plus gibberellic acid+ benzyl adenine. 12 hours leaching of Izeh population plus gibberellic acid+ benzyl adenine+ kinetin showed the highest germination time and seed vigor index. The highest germination rate was belonging to Najafabad population without leaching plus kinetin and also 6 hours plus kinetin. On the whole, 18 and 24 hours of leaching plus gibberellic acid and kinetin alone or in combination seem to be suitable to break the seed dormancy.

***Keywords**:* Leaching, Benzyl adenine, Gibberellin, Kinetin, Seed vigor index

## INTRODUCTION

The Caper (Capparis spinosa L.) is an annual creeper herbaceous shrub from Cleome genus of Capparidaceae family, with two cotyledons and detached petals. The plant has simple leaves with spinous auricles, white, large and fragrant flowers with unequal convex sepals, and 8 to 15 flags (Gianguzzi *et al*., 2019; Ashraf *et al*., 2018). This plant is the largest genus

of the family. Species of this family have various names in Iran (Rashedi *et al*., 2015). This genus has many tropical and subtropical species which are distributed in hot and dry regions of Iran. This plant plays an important role in the dynamics of ecosystems such as the Mediterranean ecosystem during the period of limited resources, such as dry summers. In addition to the mentioned characteristics such as resistance to high and low temperatures and drought resistance, Caper plant is also resistant to saline and poor soils and has shown good growth in these soils (Anwar *et al*., 2016). Due to the very low water requirement of this species, Caper is one of the best options to replace the current cover plants in landscaping. It also has high water retention due to its fleshy leaves. Dense plant assemblage as well as rich root system has given this plant a high ability to stabilize sandy soils (Sheikhi Hamuleh *et al*., 2019). Rehabilitation of degraded pastures requires compatible species which can prevent quantitative and qualitative degradation of soil in addition to forage production. Salinity and alkalinity of soil are two main factors which inhibit plant activity. However, there are species that tolerate harsh soil conditions and despite the mentioned limitations are able to grow and establish in desert lands (Shamsi *et al*., 2015). Therefore, in order to improve and rehabilitate arid and semi-arid rangelands, there is a need to introduce, reproduce and establish native species with high adaptability and yield.

Plant propagation through seeds is simple and inexpensive, but seed dormancy, especially in arid and semi-arid regions, has made it very difficult (Aryaeefar *et al*., 2018). The seeds of many rangeland and medicinal plants in natural habitats ensure their survival for many years through various dormancy types. A significant percentage of the seeds of wild populations are dormant at harvest, but dormancy breaking plus uniform germination is necessary for the propagation and cultivation of these plants (Hauvermale *et al*., 2015). Seed dormancy can cause serious problems in reproduction and domestication of rangeland plants, so that the seeds of many of these plants are not able to germinate even under favorable humidity and temperature conditions (Abbasi Sourki *et al*., 2019). Seed treatment improves germination successfully, especially in small seed plants (Gianinetti *et al*., 2018). Various factors are the causes of seed dormancy such as thickness of seed coat, imbalance of seed hormones concentrations and undeveloped embryos (Nelson *et al*., 2017).

Numerous studies have been performed on the dormancy of plant seeds, including the use of various treatments such as plant hormones, sulfuric acid, methanol, potassium nitrate, boiling water, stratification and normal water. However, different plant species show different responses to these treatments (Ganjali *et al*., 2015). Sometimes, these treatments require special materials and tools or are very difficult and time consuming. Therefore, it is necessary to achieve quick and easy methods to break the dormancy of seeds of plants such as caper and produce healthy and strong seedlings (Abbasi Sourki *et al*., 2019). Gibberellins play a major role in stimulating seed dormancy. It can be also a replacement for light, temperature or cold for seed germination (Zhong *et al*., 2015). It is necessary to change the biosynthesis of hormones and reduce the ABA / GA ratio to break dormancy and germination of seeds, which occurs with decreased sensitivity to ABA and increased sensitivity to GA (Nee *et al*., 2017). Due to the role of gibberellic acid in stimulating seed germination, seed treatments have been used by several researchers to break the dormancy (Aghababanejad *et al*., 2016). There are various interpretations of the effect mechanism of gibberellin on seed germination. The study of the biochemical mechanism of gibberellins has shown that gibberellins increase RNA polymerase activity and thus increase the transcription rate of DNA fragments. Gibberellins cause quantitative and qualitative changes in the synthesis of some proteins by changing the transcription or translation stages of some genes and finally stimulate the synthesis of hydrolyzing enzymes for grain storage molecules such as alpha amylase. These enzymes

catalyze the necessary reactions for producing energy and essential compounds for fetal growth and development and thus induce the germination phenomenon (Zhu *et al*., 2019). Gibberellins have been shown to be the main stimulus for seed germination.

Although cytokinins do not directly affect germination, they are necessary to complete the induction of germination by gibberellin and reduce the effect of germination inhibitors (Dobisova *et al*., 2017). Cytokinin is an important plant hormone which is mostly found in developing embryos and fluid endosperm. The presence of high levels of abscisic acid in the fetus during germination and low levels of bioactive gibberellic acid indicate that hormones other than gibberellic acid, which are cytokinins, play an important role in controlling plant germination (Tuan *et al*., 2019). In the study of the effect of cytokinins on seed germination, increased activity of alpha-amylase enzyme has been emphasized which leads to the breakdown of starch molecules, increased membrane permeability and exchange of storage materials (Noriega and Perez, 2017). Cytokinins are able to reverse the inhibitory action of ABA. They facilitate cell growth and division in seed embryos and stimulate germination by stimulating DNA and RNA synthesis in seeds (Chitnis, 2014). Gibberellin, on the other hand, has a stimulating effect on seed germination by stimulating seed storage sources and weakening the mechanical resistance of endosperm cells around the primary root tip. Seed soaking is also a proposed method of increasing physiological efficiency that is especially suitable for coarse seed species. Soaking the seeds in water and drying them before the germination process is a simple way to hydrate the seeds (Torabi Chafgiri *et al*., 2017).

Nitrogen-containing compounds such as potassium nitrate have long been used as one of the most important dormant seed treatments in seed laboratories (Tang *et al*., 2019). Potassium nitrate is the most widely used chemical to increase seed germination and its solution is used in conventional general germination experiments. It is recommended by the Association of Formal Seed Specialists and the International Seed Testing Association to test the germination of many species. One of the reasons for the positive effect of potassium nitrate on seed germination is probably balanced hormonal ratio and reduction of growth inhibitors such as abscisic acid. These chemical stimuli disrupt the physiological dormancy of seeds (Mansouri and Omidi, 2018).

The results of a study showed that application of sulfuric acid for 15 minutes plus gibberellic acid at a concentration of 2000 ppm at 20 and 30 °C was the best way to break the dormancy of caper seeds (Labbafi *et al*., 2018). In a study entitled Caper Seed Germination Using Naphthalene and Gibberellic Acid Treatments, it was found that this plant had a significant role in reducing the erosion of arid and desert areas and the highest germination percentage was belonging to Gibberellic acid2000 ppm for 24 hours (Soyler and Khawar, 2007).

Due to the wide variety of plant species as well as the variety of type and depth of dormancy, various treatments have been proposed to break dormancy and stimulate seed germination of plants, the most important of which are gibberellic acid and other hormonal treatments.

It should be noted that germination ecology and appropriate treatments for dormancy breaking can be quite different for different plants in a family, species of the same genus, and different ecotypes of a species (Sharifi *et al*. , 2015). For this purpose, this study was conducted to investigate different pre- treatments on seed germination of two caper populations.

**Materials and methods**

The research was conducted in the seed laboratory of the Faculty of Agriculture, Islamic Azad University, Isfahan Branch (2019-2020). The study was performed as a split factorial

experiment in a completely randomized design with three replications. Each sterile petri dish with 25 seeds was an experimental unit. Main plot consisted of different leaching levels (soaking in distilled water for 0, 6, 12, 18 and 24 hours) and subplots included factorial of two seed populationes (Najafabad, Izeh) and nine levels of hormone or chemical treatments (gibberellic acid (100 mg/l using Merck ethanol96% as solvent of GA3 salt), benzyl adenine 6 ($10^{-5}$ M using NAOH 1N), kinetin ($10^{-5}$ M using NAOH 1N), gibberellic acid+ benzyl adenine, Gibberellic acid+ kinetin, benzyl adenine+ kinetin, gibberellic acid+ benzyl adenine+ kinetin, potassium nitrate 0.4%, distilled water as control). To perform the experiment, seeds were collected and then populations were randomly selected. First the seeds that were visually healthy and uniform were separated by a loop. Then, seeds were disinfected using sodium hypochlorite0.5% for two minutes to prevent any kind of contamination and were immediately washed several times with distilled water. All instruments were sterilized using autoclave or oven and all steps of the experiment were performed in a sterile environment.

The germinator used for germination in this research was the Light Contorol model made by GROUC Company (Iran). After disinfection, the device was adjusted for 16 hours of light (600 micromoles of photons per square meter per second) and 8 hours of darkness, 70% humidity and a temperature of 25.15 ° C. For each treatment, 25 seeds were placed on a 4 cm Whatman filter paper in an 8 cm petri dish, moistened by 10 ml of the treatment solution and then placed in the germinator. The duration of the experiment was 30 days. Germinated seeds were counted every 24 hours to determine the percentage and rate of germination. The basis of germination was 2 to 3 mm growth of root. When the number of germinated seeds was the same for two consecutive counts, the germination evaluation was completed and this time was considered as the end of the germination period. At the end of this period, the desired traits were evaluated. Germination uniformity was calculated according to Equation (1) (Zafari *et al*., 2017):

Germination uniformity=

D = the number of days from the beginning of germination, D = average number of days from the beginning of germination, N = number of germinated seeds per day, Σn = total number of germinated seeds

Germination percentage was calculated using Equation (2) (Zafari et al., 2017):

Germination percentage = the number of germinated seeds / the number of all seeds

Mean germination time was calculated through Equation (3) (Rahimi et al., 2019):

MTG=□□□□nd)/ □N

MTG = average germination time, D = the number of days from the beginning of germination, n = the number of germinated seeds and N = total number of germinated seeds

Germination rate was calculated as the inverse of mean germination time (Rahimi et al., 2019).

RG= 1/MTG

RG = germination rate and MTG = mean germination time

Having the germination percentage and mean seedling length, the seed vigor index was calculated using Equation (5) (Rashidi et al., 2017):

Seed vigor index = average seedling length in millimeters (total stem and root length) × germination percentage / 100

Data were analyzed using MSTAT-C 4.1. Means were compared based on Duncan's multiple range test at 5% probability level. Graphs were drawn using Excel 2013.

## RESULTS AND DISCUSSION

*Germination uniformity*

The effect of leaching and the interaction of leaching and population on germination uniformity were not significant at 5% probability level. While, the effects of population, hormonal-chemical substances, interaction of population× substances, leaching× substances and their three-way effect were significant at 1% probability level (Table 1). Thus, 18 hours leaching of Najafabad population plus gibberellic acid and kinetin hormone and 24 hours leaching of Najafabad population plus benzyl adenine showed the highest germination uniformity (0.38 and 0.36, respectively). The lowest germination uniformity was observed in Najafabad population without leaching and gibberellic hormone. There was no statistically significant difference between some treatments at the 5% probability level of Duncan test (Table 2).

Table 1. Analysis of variance results of the effect of different treatments on some of the characteristics of seed germination of *Capparis spinosa* L.

| | | Average of Squares | | | | |
|---|---|---|---|---|---|---|
| (Source of Variations) | (Degree of Freedom) | (Germination Uniformity) | (Germination Percentage) | (Average Germination Time) | (Germination Rate) | (Index of Seed Vigor) |
| (Leaching) | 4 | 0.001 $^{ns}$ | 185.689 $^{ns}$ | 1.730 $^{ns}$ | 0.000 $^{ns}$ | 0.005 $^{ns}$ |
| (Error a) | 10 | 0.0001 | 288.652 | 2.737 | 0.0001 | 0.025 |
| (Cultivar) | 4 | 0.045$^{**}$ | 6931.200$^{**}$ | 837.902$^{**}$ | 0.022$^{**}$ | 0.337$^{**}$ |
| (Leaching × Cultivar) | 4 | 0.000 $^{ns}$ | 92.237$^{**}$ | 5.692$^{**}$ | 0.0001$^{**}$ | 0.009$^{**}$ |
| (Chemical hormonal substances) | 8 | 0.003$^{**}$ | 480.237$^{**}$ | 20.985$^{**}$ | 0.001$^{**}$ | 0.115$^{**}$ |
| (Cultivar × Chemical hormonal substances) | 32 | 0.001$^{**}$ | $^{**}$139.756 | 8.712$^{**}$ | 0.000$^{**}$ | 0.012$^{**}$ |
| Leaching × Chemical hormonal (substances) | 8 | 0.001$^{**}$ | 158.933$^{**}$ | 11.741$^{**}$ | 0.0001$^{**}$ | 0.074$^{**}$ |
| (Leaching × Cultivar × Chemical hormonal substances) | 32 | 0.001$^{**}$ | 59.637$^{**}$ | 7.187$^{**}$ | 0.0001$^{**}$ | 0.023$^{**}$ |
| Error b | 170 | 0.0001 | 7.115 | 1.044 | 0.0001 | 0.001 |

$^{ns}$ not significance, * and ** are significance at 5 and 1% probability levels, respectively.

Table 2. Results of the comparison of the effect of different treatments on some of the characteristics of seed germination of *Capparis spinosa* L.

| (Leaching) | (Cultivar) | *(Treatment)* | (Germination Uniformity) | (Germination Percentage) | (Average Germination Time) | (Germination Rate) | (Index of Seed Vigor) |
|---|---|---|---|---|---|---|---|
| 0 | *(Najafabad)* | *(Gibberellic acid)* | $0.09^{i-v}$ | $25.33^{l-p}$ | $14.63^{m-x}$ | $0.07^{cd}$ | $0.30^{z-c'}$ |
| | | *(Benzyl adenine)* | $0.26^{b-e}$ | $18.67^{q-t}$ | $13.82^{p-]}$ | $0.07^{cd}$ | $0.87^{h-p}$ |
| | | *(Kinetin)* | $0.22^{d-g}$ | $17.33^{r-u}$ | $9.52^{gh}$ | $0.11^{a}$ | $0.19^{c}$ |
| | | *(Gibberellic acid and Benzyl adenine)* | $0.36^{ab}$ | $14.67^{tu}$ | $10.28^{fgh}$ | $0.10^{ab}$ | $0.40^{u-a}$ |
| | | *(Gibberellic acid and Kinetin)* | $0.15^{e-o}$ | $24.00^{m-q}$ | $13.98^{o}$ | $0.07^{cd}$ | $0.46^{t}$ |
| | | *(Benzyl adenine and Kinetin)* | $0.23^{c-f}$ | $21.33^{o-r}$ | $13.74^{q-^}$ | $0.07^{cd}$ | $0.26^{c}$ |
| | | *(Gibberellic acid and Benzyl adenine and Kinetin)* | $0.20^{d-j}$ | $21.33^{o-r}$ | $11.69^{^-f}$ | $0.09^{bc}$ | $0.85^{h-q}$ |
| | | *(Potassium Nitrate)* | $0.07^{l-v}$ | $22.67^{n-r}$ | $14.80^{l-w}$ | $0.07^{cd}$ | $1.09^{f-g}$ |
| | | *(Distilled water)* | $0.10^{h-v}$ | $24.00^{m-q}$ | $12.42^{y-e}$ | $0.08^{c}$ | $0.22^{c}$ |
| | *(Izeh)* | *(Gibberellic acid)* | $0^{v}$ | $36.00^{fgh}$ | $14.47^{m-y}$ | $0.07^{cd}$ | $0.63^{o-x}$ |
| | | *(Benzyl adenine)* | $0.04^{r-v}$ | $41.33^{cde}$ | $17.74^{a-g}$ | $0.06^{cde}$ | $0.52^{r}$ |
| | | *(Kinetin)* | $0.17^{d-l}$ | $21.33^{o-r}$ | $12.85^{w-c}$ | $0.08^{c}$ | $2.29^{cd}$ |
| | | *(Gibberellic acid and Benzyl adenine)* | $0.06^{m-v}$ | $24.00^{m-q}$ | $14.77^{l-w}$ | $0.07^{cd}$ | $0.73^{k-t}$ |
| | | *(Gibberellic acid and Kinetin)* | $0.21^{d-i}$ | $13.33^{u}$ | $10.39^{e-h}$ | $0.10^{ab}$ | $0.08^{c}$ |
| | | *(Benzyl adenine and Kinetin)* | $0.06^{m-v}$ | $30.67^{i-l}$ | $17.74^{a-g}$ | $0.06^{cde}$ | $1.83^{de}$ |
| | | *(Gibberellic acid and Benzyl adenine* | $0.04^{s-v}$ | $37.33^{efg}$ | $18.70^{abc}$ | $0.05^{de}$ | $1.17^{fgh}$ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | *and Kinetin)* | | | | | |
| | | *(Potassium Nitrate)* | 0.03$^{tuv}$ | 33.33$^{g-j}$ | 18.02$^{a-f}$ | 0.06$^{cde}$ | 0.56$^{p-[}$ |
| | | *(Distilled water)* | 0.07$^{l-v}$ | 25.33$^{l-p}$ | 13.57$^{s-^}$ | 0.07$^{cd}$ | 0.28$^{z-c'}$ |
| | | *(Gibberellic acid)* | 0.10$^{i-v}$ | 26.67$^{k-o}$ | 11.49$^{–-g'}$ | 0.09$^{bc}$ | 0.31$^{y-c'}$ |
| | | *(Benzyl adenine)* | 0.13$^{f-t}$ | 22.67$^{n-r}$ | 11.83$^{]-f'}$ | 0.08$^{c}$ | 0.64$^{m-w}$ |
| *6* | *(Najafabad)* | *(Kinetin)* | 0.21$^{d-h}$ | 14.67$^{tu}$ | 9.00$^{h}$ | 0.11$^{a}$ | 0.14$^{a'b'c'}$ |
| | | *(Gibberellic acid and Benzyl adenine)* | 0.18$^{d-k}$ | 18.67$^{q-t}$ | 11.30$^{a'-g'}$ | 0.09$^{bc}$ | 1.05$^{g-k}$ |
| | | *(Gibberellic acid and Kinetin)* | 0.17$^{e-m}$ | 25.33$^{l-p}$ | 13.36$^{u-`}$ | 0.08$^{c}$ | 0.23$^{–-c'}$ |
| | | *(Benzyl adenine and Kinetin)* | 0.15$^{e-q}$ | 24.00$^{m-q}$ | 13.13$^{v-b'}$ | 0.08$^{c}$ | 1.14$^{f-i}$ |
| | | *(Gibberellic acid and Benzyl adenine and Kinetin)* | 0.05$^{p-v}$ | 36.00$^{fgh}$ | 14.21$^{n-[}$ | 0.07$^{cd}$ | 0.91$^{h-o}$ |
| | | *(Potassium Nitrate)* | 0.07$^{l-v}$ | 28.00$^{k-n}$ | 16.10$^{f-n}$ | 0.06$^{cde}$ | 0.68$^{l-u}$ |
| | | *(Distilled water)* | 0.11$^{g-u}$ | 18.67$^{q-t}$ | 12.05$^{\-f'}$ | 0.08$^{c}$ | 0.26$^{\-c'}$ |
| | | *(Gibberellic acid)* | 0.03$^{tuv}$ | 37.33$^{efg}$ | 18.16$^{a-e}$ | 0.06$^{cde}$ | 0.56$^{p-[}$ |
| | | *(Benzyl adenine)* | 0.07$^{l-v}$ | 32.00$^{h-k}$ | 15.84$^{g-p}$ | 0.06$^{cde}$ | 0.92$^{h-o}$ |
| | | *(Kinetin)* | 0.11$^{g-u}$ | 20.00$^{p-s}$ | 14.64$^{m-x}$ | 0.07$^{cd}$ | 1.07$^{g-k}$ |
| | *(Izeh)* | *(Gibberellic acid and Benzyl adenine)* | 0.04$^{p-v}$ | 29.33$^{j-m}$ | 17.17$^{b-j}$ | 0.06$^{cde}$ | 0.55$^{q-[}$ |
| | | *(Gibberellic acid and Kinetin)* | 0.07$^{k-v}$ | 14.67$^{tu}$ | 12.31$^{z-f'}$ | 0.08$^{c}$ | 0.11$^{b'c'}$ |
| | | *(Benzyl adenine and Kinetin)* | 0.07$^{k-v}$ | 30.67$^{i-l}$ | 16.75$^{c-l}$ | 0.06$^{cde}$ | 0.48$^{s-–}$ |
| | | *(Gibberellic acid and Benzyl adenine and Kinetin)* | 0.03$^{tuv}$ | 37.33$^{efg}$ | 17.31$^{a-i}$ | 0.06$^{cde}$ | 2.12$^{cd}$ |
| | | *(Potassium Nitrate)* | 0.04$^{r-v}$ | 44.00$^{bcd}$ | 18.36$^{a-d}$ | 0.05$^{de}$ | 2.31$^{c}$ |
| | | *(Distilled water)* | 0.06$^{l-v}$ | 29.33$^{j-m}$ | 19.03$^{ab}$ | 0.05$^{de}$ | 0.35$^{w-b'}$ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | *(Gibberellic acid)* | 0.10$^{h-v}$ | 25.33$^{l-p}$ | 15.37$^{i-u}$ | 0.07$^{cd}$ | 0.31$^{y-c'}$ |
| *12* | *(Najafabad)* | *(Benzyl adenine)* | 0.16$^{e-m}$ | 21.33$^{o-r}$ | 11.26$^{b-g}$ | 0.09$^{bc}$ | 0.22$^{-c'}$ |
| | | *(Kinetin)* | 0.07$^{k-v}$ | 24.00$^{m-q}$ | 12.00$^{\-f'}$ | 0.08$^{c}$ | 0.56$^{p-z}$ |
| | | *(Gibberellic acid and Benzyl adenine)* | 0.18$^{d-k}$ | 22.67$^{n-r}$ | 12.70$^{x-c'}$ | 0.08$^{c}$ | 0.99$^{h-l}$ |
| | | *(Gibberellic acid and Kinetin)* | 0.16$^{e-n}$ | 26.67$^{k-o}$ | 12.27$^{z-f'}$ | 0.08$^{c}$ | 0.62$^{n-x}$ |
| | | *(Benzyl adenine and Kinetin)* | 0.11$^{g-u}$ | 25.33$^{l-p}$ | 12.24$^{[-f'}$ | 0.08$^{c}$ | 0.59$^{o-y}$ |
| | | *(Gibberellic acid and Benzyl adenine and Kinetin)* | 0.16$^{e-o}$ | 22.67$^{n-r}$ | 10.32$^{f'g'h'}$ | 0.10$^{ab}$ | 0.50$^{r-\wedge}$ |
| | | *(Potassium Nitrate)* | 0.32$^{abc}$ | 14.67$^{tu}$ | 11.5$^{-g'}$ | 0.09$^{bc}$ | 0.66$^{l-v}$ |
| | | *(Distilled water)* | 0.13$^{f-u}$ | 20.00$^{p-s}$ | 14.58$^{m-x}$ | 0.07$^{cd}$ | 0.52$^{r-]}$ |
| | | *(Gibberellic acid)* | 0.04$^{q-v}$ | 37.33$^{efg}$ | 15.66$^{h-q}$ | 0.06$^{cde}$ | 0.34$^{x-b'}$ |
| | *(Izeh)* | *(Benzyl adenine)* | 0.09$^{j-v}$ | 28.00$^{k-n}$ | 16.15$^{e-n}$ | 0.06$^{cde}$ | 1.16$^{f-i}$ |
| | | *(Kinetin)* | 0.05$^{n-v}$ | 34.67$^{ghi}$ | 15.11$^{k-v}$ | 0.07$^{cd}$ | 0.12$^{f-i}$ |
| | | *(Gibberellic acid and Benzyl adenine)* | 0.04$^{q-v}$ | 40$^{def}$ | 15.22$^{j-u}$ | 0.07$^{cd}$ | 0.64$^{n-x}$ |
| | | *(Gibberellic acid and Kinetin)* | 0.03$^{tuv}$ | 24.00$^{m-q}$ | 17.15$^{b-k}$ | 0.06$^{cde}$ | 0.21$^{`-c'}$ |
| | | *(Benzyl adenine and Kinetin)* | 0.04$^{p-v}$ | 37.33$^{efg}$ | 17.29$^{a-i}$ | 0.06$^{cde}$ | 0.55$^{p-[}$ |
| | | *(Gibberellic acid and Benzyl adenine and Kinetin)* | 0.03$^{tuv}$ | 45.33$^{bc}$ | 19.23$^{a}$ | 0.05$^{de}$ | 4.66$^{a}$ |
| | | *(Potassium Nitrate)* | 0.08$^{k-v}$ | 24.00$^{m-q}$ | 13.85$^{p-]}$ | 0.07$^{cd}$ | 0.98$^{h-m}$ |
| | | *(Distilled water)* | 0.04$^{q-v}$ | 30.67$^{i-l}$ | 16.98$^{c-k}$ | 0.06$^{cde}$ | 0.38$^{u-a'}$ |
| | | *(Gibberellic acid)* | 0.10$^{h-v}$ | 22.67$^{n-r}$ | 14.20$^{n-[}$ | 0.07$^{cd}$ | 0.23^-c' |
| *18* | *(Najafabad)* | *(Benzyl adenine)* | 0.24$^{c-f}$ | 18.67$^{q-t}$ | 10.57$^{d'-h'}$ | 0.09$^{bc}$ | 0.17a'b' c' |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | *(Kinetin)* | 0.14$^{f-s}$ | 18.67$^{q-t}$ | 11.23$^{b'-g'}$ | 0.09$^{bc}$ | 0.40u-a’ |
| | | *(Gibberellic acid and Benzyl adenine)* | 0.08$^{k-v}$ | 28.00$^{k-n}$ | 12.53$^{y-d'}$ | 0.08$^{c}$ | 0.66l-v |
| | | *(Gibberellic acid and Kinetin)* | 0.38$^{a}$ | 17.33$^{r-u}$ | 12.23$^{[-f'}$ | 0.08$^{c}$ | 0.72k-t |
| | | *(Benzyl adenine and Kinetin)* | 0.12$^{g-u}$ | 25.33$^{l-p}$ | 12.04$^{\-f'}$ | 0.08$^{c}$ | 0.65l-v |
| | | *(Gibberellic acid and Benzyl adenine and Kinetin)* | 0.11$^{g-u}$ | 28.00$^{k-n}$ | 11.06$^{c'-g'}$ | 0.09$^{bc}$ | 0.9.h-o |
| | | *(Potassium Nitrate)* | 0.15$^{e-r}$ | 18.67$^{q-t}$ | 13.53$^{t-–}$ | 0.07$^{cd}$ | 0.22_-c’ |
| | | *(Distilled water)* | 0.15$^{e-q}$ | 21.33$^{p-r}$ | 14.05$^{o-\}$ | 0.07$^{cd}$ | 0.30z-c’ |
| | *(Izeh)* | *(Gibberellic acid)* | 0.04$^{r-v}$ | 32.00$^{h-k}$ | 16.90$^{c-k}$ | 0.06$^{cde}$ | 0.25]-c’ |
| | | *(Benzyl adenine)* | 0.19$^{d-j}$ | 24.00$^{m-q}$ | 13.58$^{r-^}$ | 0.07$^{cd}$ | 1.42fg |
| | | *(Kinetin)* | 0.06$^{m-v}$ | 29.33$^{j-m}$ | 15.94$^{g-o}$ | 0.06$^{cde}$ | 2.20cd |
| | | *(Gibberellic acid and Benzyl adenine)* | 0.04$^{p-v}$ | 44.00$^{bcd}$ | 16.98$^{c-k}$ | 0.06$^{cde}$ | 0.86h-q |
| | | *(Gibberellic acid and Kinetin)* | 0.06$^{m-v}$ | 30.67$^{i-l}$ | 15.46$^{i-t}$ | 0.06$^{cde}$ | 0.37v-a’ |
| | | *(Benzyl adenine and Kinetin)* | 0.03$^{tuv}$ | 36.00$^{fgh}$ | 16.85$^{c-k}$ | 0.06$^{cde}$ | 0.74j-t |
| | | *(Gibberellic acid and Benzyl adenine and Kinetin)* | 0.03$^{tuv}$ | 48.00$^{ab}$ | 17.52$^{a-h}$ | 0.06$^{cde}$ | 1.49ef |
| | | *(Potassium Nitrate)* | 0.03$^{tuv}$ | 34.67$^{ghi}$ | 17.73$^{a-g}$ | 0.06$^{cde}$ | 2.35c |
| | | *(Distilled water)* | 0.04$^{p-v}$ | 32.00$^{h-k}$ | 16.21$^{e-n}$ | 0.06$^{cde}$ | 0.72k-t |
| *24* | *(Najafabad)* | *(Gibberellic acid)* | 0.12$^{g-u}$ | 20.00$^{p-s}$ | 12.09$^{\-f'}$ | 0.08$^{c}$ | 0.65l-v |
| | | *(Benzyl adenine)* | 0.36$^{ab}$ | 16.00$^{stu}$ | 11.17$^{b'-g'}$ | 0.09$^{bc}$ | 0.74j-t |
| | | *(Kinetin)* | 0.13$^{f-t}$ | 25.33$^{l-p}$ | 13.09$^{v-c'}$ | 0.08$^{c}$ | 0.88h-p |
| | | *(Gibberellic acid and Benzyl adenine)* | 0.13$^{f-u}$ | 28.00$^{k-n}$ | 13.32$^{u-a'}$ | 0.08$^{c}$ | 0.62$^{n-x}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *(Gibberellic acid and Kinetin)* | $0.28^{abcd}$ | $21.33^{o-r}$ | $11.10^{b'-g'}$ | $0.09^{bc}$ | $0.90^{h-o}$ |
| | *(Benzyl adenine and Kinetin)* | $0.12^{g-u}$ | $28.00^{k-n}$ | $14.31^{n-z}$ | $0.07^{cd}$ | $0.80^{i-s}$ |
| | *(Gibberellic acid and Benzyl adenine and Kinetin)* | $0.08^{k-v}$ | $29.33^{j-m}$ | $11.37^{`-g'}$ | $0.09^{bc}$ | $0.74^{j-t}$ |
| | *(Potassium Nitrate)* | $0.08^{k-v}$ | $22.67^{n-r}$ | $14.79^{l-w}$ | $0.07^{cd}$ | $0.28^{[-c'}$ |
| | *(Distilled water)* | $0.06^{m-v}$ | $25.33^{l-p}$ | $13.68^{q-\wedge}$ | $0.07^{cd}$ | $0.24^{]-c'}$ |
| *(Izeh)* | *(Gibberellic acid)* | $0.04^{p-v}$ | $26.67^{l-o}$ | $13.58^{r-\wedge}$ | $0.07^{cd}$ | $0.16^{a'b'c'}$ |
| | *(Benzyl adenine)* | $0.14^{e-r}$ | $20.00^{p-s}$ | $15.62^{h-s}$ | $0.06^{cde}$ | $0.25^{]-c'}$ |
| | *(Kinetin)* | $0.07^{l-v}$ | $28.00^{k-n}$ | $15.64^{h-r}$ | $0.06^{cde}$ | $3.02^{b}$ |
| | *(Gibberellic acid and Benzyl adenine)* | $0.02^{uv}$ | $48.00^{ab}$ | $16.38^{d-m}$ | $0.06^{cde}$ | $1.10^{f-j}$ |
| | *(Gibberellic acid and Kinetin)* | $0.05^{o-v}$ | $37.33^{efg}$ | $17.37^{a-i}$ | $0.06^{cde}$ | $0.53^{q-\backslash}$ |
| | *(Benzyl adenine and Kinetin)* | $0.04^{r-v}$ | $44.00^{bcd}$ | $15.67^{h-q}$ | $0.06^{cde}$ | $0.66^{-v}$ |
| | *(Gibberellic acid and Benzyl adenine and Kinetin)* | $0.03^{tuv}$ | $52.00^{a}$ | $16.32^{e-m}$ | $0.06^{cde}$ | $1.83^{de}$ |
| | *(Potassium Nitrate)* | $0.03^{tuv}$ | $37.33^{efg}$ | $17.65^{a-h}$ | $0.06^{cde}$ | $0.97^{h-n}$ |
| | *(Distilled water)* | $0.06^{m-v}$ | $34.67^{ghi}$ | $14.60^{m-x}$ | $0.07^{cd}$ | $0.82^{h-r}$ |

In each column, the means with different letters have a significant difference at the level of 5% of the Duncan test.

Priming increases the percentage, rate and uniformity of seed germination and emerging. Germination is defined as the emergence of embryo from the seed by initiating a variety of synthesis and decomposition activities, including respiration, protein synthesis, and motility of food reserves after water uptake. Seed germination time is not exactly fixed under natural conditions and environmental changes can cause the seeds to start germinating. Ambient water potential also has a direct effect on the rate of water uptake and consequent plant germination. Water is the most important factor for activating seed germination and the low available water leads to impaired seed germination (Ahmadpour *et al*., 2016). Positive effects were also reported by moisture pretreatment on acceleration and uniformity of germination to repair damage caused by seed ripening stages of rapeseed populations (Najafi *et al*., 2015).

The advantages of moisture pretreatment are reduction of time, increased germination uniformity, synchronization in flowering, faster ripening and higher yield (Vaseii Kashani *et al*., 2015).

***Germination percentage***

The effect of leaching on germination percentage was not significant ($p>0.05$). The effects of population, hormonal-chemical substances, interactions of leaching× population, population× substances, leaching× substances and leaching× population× substances on germination percentage were very significant ($p<0.01$). According to the results, 24 hours leaching of Izeh population plus gibberellic acid, benzyl adenine and kinetin; 24 hours leaching of Izeh population plus gibberellic acid and benzyl adenine and 18 hours leaching of Izeh population plus gibberellic Acid, benzyl adenine and kinetin had the highest germination rates (52, 48 and 48, respectively). The lowest germination percentage was belonging to Izeh population without leaching plus gibberellic acid and kinetin, Najafabad population without leaching plus gibberellic acid and benzyl adenine and 6 hours leaching of Najafabad population plus kinetin (13.33, 14.67 and 14.67%, respectively) (Table 2).

Seed germination is a critical stage of plant life and strategies that plant adopts at this stage can significantly increase the survival of it (Shamsi *et al*., 2015). The mechanism of seed dormancy is different in various plants and different methods have been used to break dormancy and induce seed germination, which are different depending on the type of plants' dormancy (Aghababanejad *et al*., 2016). Seed germination begins with water uptake. Following water uptake, phytohormones are released, leading to the synthesis of hydrolytic enzyme and other enzymes by the endosperm. Cytokinins and gibberellins are from germination regulators that regulate reactions in plants via various mechanisms such as controlling transcription and synthesis of specific enzymes (Ahmad and Hussain , 2014). Gibberellins, cytokinins and inhibitors are essential growth regulators for dormancy or germination of seeds and the presence or absence of active concentrations of these three classes determines germination. The action site of gibberellin is the endosperm, cotyledons and embryo (Farhoodi and Makizadeh, 2015). Gibberellins are among the phytohormones that are directly involved in controlling and accelerating germination. Gibberellins promote cell growth by increasing the cell wall elasticity. Gibberellin subsequently hydrolyzes starch to sugar. This reduces cell water potential and thus facilitates the entry of water into the cell. Following this process, cell elongation occurs (Zhu *et al*., 2019). In the present study, gibberellin had accelerating effects on dormancy breaking and seed germination. In addition, gibberellic acid initiates germination by inducing biosynthesis of alpha-amylase, resulting in dormancy braking. In the germinating seed, gibberellic acid is made by the embryo and travels from the embryo to the scutellum and then diffuses into the endosperm to reach the aleurone layer. There, gibberellic acid releases hydrolytic enzymes such as alpha-amylase, which in turn break down starch into oligosaccharides (Zhang *et al*., 2019). The oligosaccharides are then broken down into glucose in steps. Molecular studies have shown that gibberellic acid is perceived by receptors on the cytoplasmic membrane and interacts with G-protein in the membrane, followed by GTP binding to G-protein (Zhu *et al*. 2019). In the presence of gibberellic acid, GTP-protein acts as a primary component of gibberellic acid messaging in aleuronic cells. GTP-protein produces cGMP. This intermediate molecule stimulates other processes such as activation of protein kinases, increase of Ca2 +, opening of ion channels, etc. All of these components are involved in some way in the transmission of gibberellic acid to the nucleus (Parvin *et al*., 2015). Once the hormone is detected in the

nucleus, the GA-MYB gene is transcribed to make its protein, a transcription factor that activates the alpha-amylase gene. Ultimately, this reduces the water potential of the cell and thus facilitates the entry of water into the cell. Following this process, cell elongation occurs (Choi *et al*., 2016). Also, the stimulating effect of gibberellin treatments is often related to the mobilization of storage sources and the weakening of the mechanical resistance of endosperm cells around the apex of the primary root. Gibberellins have been shown to be the main stimulants of seed germination and inhibitors and cytokinins have secondary roles which are inhibitory and encouraging, respectively (Wang *et al*., 2020).

Although cytokinins do not directly affect germination, they seem to be necessary to complete the induction of germination by gibberellin and reduce the effects of germination inhibitors. Cytokinins may affect membrane permeability and thus allow endogenous gibberellins to reach their sites of activity and initiate biochemical processes necessary for germination (Zurcher and Muller, 2016). The results of the present study showed that kinetin also increased the germination percentage of seeds.

Kinetin increases alpha-amylase activity, resulting in hydrolysis of starch, a process required for germination. Alpha-amylase can bind to glycoside bonds of amylose (stored polysaccharide in plant seeds) to play a role in the decomposition of starch and provide the energy needed for germination processes (Ahmadpour *et al*., 2016). In addition, cytokinins may affect cytoplasmic membrane permeability and membrane transport (Hu *et al*., 2018). Therefore, it seems that breaking the seed dormancy by kinetin is probably related to increased membrane permeability and exchange of stored materials. Cytokinins also stimulate DNA and RNA synthesis to increase the cell division process in the embryo, thereby facilitating seed germination. Thus, cytokinins are required to complement GA induction of germination and indirectly reduce the effects of growth inhibitors such as ABA (Tang *et al*., 2019). Hormones of the cytokinin group also increase the stimulating effect of the gibberellins. Numerous reports have been published so far on the effect of cytokinins on the induction of seed germination in a number of plants (Dobisova *et al*., 2017). Increased alpha-amylase activity and breakdown of starch molecules or membrane permeability and transferrin matters through membrane are possible reasons for the effect of cytokinins on germination. Gibberellic acid (500 ppm) has been reported to have the greatest positive effect on dormancy and seed germination of thyme species (Ghasemi-pierbalooti *et al*., 2005), two medicinal species of Galbanum (Nadjafi *et al*. ., 2006), teucrium and milk thistle (Nabaee *et al*., 2013). These results are consistent with the results of the present study. On the other hand, the positive effects of leaching on softening the seed coat, reducing germination inhibitors and increasing seed metabolic activity have been reported (Yan, 2016). Inhibitors are a variety of compounds that may delay germination in various ways, such as inhibiting the production or activity of gibberellin or invertase enzyme which plays main role in glucose metabolism (Su *et al*., 2016). The effect of soaking can be expressed in that if dormant seeds are soaked, water-soluble inhibitors are released from the shell or embryo. According to some reports, the most important seed inhibitor is abscisic acid, which is somewhat reduced by soaking or washing. In a study on bugloss plant, the results showed that treatment with running water had a significant effect on seed germination of this plant (Nowrouzian *et al*., 2016) which is in agreement with the results of this study. In addition, the increase in germination percentage as a result of hydropriming may be due to the fact that seed pretreatment induces biochemical changes such as hydrolysis, enzyme activation, DNA replication, increased RNA synthesis and protein synthesis. This increases the growth of the embryo and reduces the leakage of metabolites and ultimately improves the strength of seeds and germination. On the other hand, water pretreatment improves germination, emergence and rapid and optimal establishment of

seedlings in a wide range of environmental conditions by reducing the time required for water absorption (Zhu *et al*., 2019).

### *Mean germination time*

Variance analysis results showed that the effect of leaching treatment on germination time was not significant ($p>0.05$). But the effect of population, hormonal-chemical substances and interactions of leaching× population, population× substances, leaching × substances and leaching × population× substances were highly significant($p<0.01$) (Table 1). The highest germination time (19.23) was observed in 12 hours leaching of Izeh population plus gibberellic acid, benzyl adenine and kinetin (Table 2). The shortest germination time was obtained from 6 hours leaching of Najafabad population plus kinetin and Najafabad population without leaching plus kinetin (9 and 9.52, respectively). After absorbing water, gibberellin is released from the fetus and transferred to the aleurone layer. This layer acts both as a storage tissue and as a secretor of hydrolytic enzymes. Eventually, these enzymes are transferred to the cotyledons, hydrolyze the cotyledon storage compounds, including proteins, and produce amino acids during germination (Yusefi Tanha and Fallah, 2016). Since that gibberellin is effective in the synthesis of seed hydrolyzing enzymes and thus the breakdown of starch and other nutrients and ultimately the transfer of these substances to the growing embryo, it seems that as the level of gibberellin in the seed increases, the balance between inhibitors and stimulants increases toward stimulants and thus the average germination time will be increased (Coons *et al*. , 2014). In a study, it was observed that soaking pumpkin seeds increased seed germination percentage and decreased the average germination time (Rehman *et al*., 2015). Also, the results of a study on columbine seeds showed that the best germination characteristics belonged to 12 and 24 hours of moisture pretreatment (Nabi *et al*., 2014).

### *Germination rate*

The results of analysis of variance showed that the effect of leaching treatment on germination rate was not significant at 5% probability level. While the effect of population, hormonal-chemical substances and interactions of leaching× population, population× substances, leaching × substances and leaching × population× substances were highly significant($p<0.01$) (Table 1). The highest germination rate (0.11) was observed in Najafabad population without leaching plus kinetin and 6 hours leaching of Najafabad population plus kinetin. The lowest germination rate (0.05) was obtained from Izeh population without leaching plus gibberellic acid, benzyl adenine and kinetin and 6 hours leaching of Izeh population plus potassium nitrate and distilled water (Table 2). According to the results, kinetin hormone with or without leaching showed the highest germination rate in Najafabad population.

Germination rate is known as a suitable indicator of seedling establishment success. Increased germination rate in pretreated seeds can be attributed to hydrolyzing biochemical changes and increased activity of degrading enzymes that improve seed germination (Mahmoudi *et al*., 2018). Cytokinins are directly related to higher cell division and alpha-amylase enzyme activity, which can lead to increased germination rate and percentage. Accelerated germination in pre-treated seeds may be due to following reasons: water uptake shortens the germination time by improving the cytoplasmic membrane and DNA, reducing the leakage of metabolites and increasing metabolic activity including higher activity of

degrading enzymes such as alpha-amylase, increasing ATP, increasing RNA and DNA synthesis, increased number of mitochondrial function enhancement (Dobisova *et al*., 2017).

Therefore, increased germination rate due to seed pretreatment indicates an increase in the strength of these seeds, which can improve the growth rate of plants and increase the quality and quantity of yield (Sivasubramaniam *et al*., 2011). Increasing the germination rate of plant seeds by water can be due to the fact that there are germination accelerators in the seeds, which accelerates the germination process by absorbing water. It is stated that pretreated seeds absorb water quickly and seed metabolism is activated faster. As a result, seed pretreatment increases the germination rate and reduces the inherent physiological heterogeneity in the seed (Sumlu *et al*., 2010).

Increased germination rate due to hydropriming in wheat seeds (Sivasubramaniam *et al*., 2011) and mountain rye (Ansari and Sharifzadeh, 2012) have also been reported, which is consistent with the results of this study.

### *Seed vigor index*

The effect of leaching treatment on seed vigor index was not significant ($p>0.05$). While, the effects of population, hormonal-chemical substances, and the interactions of leaching × population, population× substances, leaching × substances and leaching × population×substances on seed vigor index were highly significant (Table 1 ). The highest seed vigor index (4.66) was observed in 12 hours leaching of Izeh population plus gibberellic acid, benzyl adenine and kinetin hormones. The lowest seed vigor index (0.08) was obtained from Izeh population without leaching plus gibberellic acid and kinetin (Table 2). Vigor index is a function of germination percentage and seedling length and is directly related to these two traits. Increasing the index can be considered as a good sign of quality improving of aged seeds. The beneficial effects of priming by gibberellin and cytokinin may be due to accelerating and improving germination as well as increased cell elongation and division in seedlings (Ansari *et al*., 2012).

Pretreatment of seeds with distilled water had a positive effect on seed vigor and caused an increasing trend compared to control and other treatments. Since seed vigor is directly related to germination percentage as well as seedling length, increasing the above indices increased seed vigor.

Many researchers have reported that increasing the duration of hydropriming in seeds increased germination index, final germination percentage and seed vigor index (Abdolrahmani *et al*., 2011).

## CONCLUSION

Increased germination, growth rate and seedling establishment in rangelands are among the factors that increase yield, protect soil and water and prevent wind erosion, especially in arid areas where suitable conditions for plant growth are not well prepared. Therefore, it is important to know the effect of different treatments on plant germination.

Treatments to break the seed dormancy are from the important principles for economic production of valuable caper plant, because without treatments, the plant cultivation will fail. The results of this study showed that different combinations of used plant hormones were highly effective. This not only shows that appropriate range of hormonal compounds were used, but also indicates that Cytokinins alone are less effective than when used in combination with gibberellins.

Among the ecotypes used in this study, Izeh ecotype showed the best responses, which can be due to the sensitivity and very high reaction of seeds of this ecotype to hormonal treatments. This reaction can be due to the different hormonal balance of the ecotypes and the adaptation of this ecotype to the specific climatic conditions of the respective habitat.

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