Effects of KNO$_3$ and CaCl$_2$ on Seed Germination of *Rheum khorasanicum* B. Baradaran & A. Jafari.

Reza Darrudi, Mohammad Reza Hassandokht and Vahideh Nazeri

Department of Horticultural Science, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

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ABSTRACT

For improving seed germination of *Rheum khorasanicum* B. Baradaran & A. Jafari, different treatments including KNO$_3$ and CaCl$_2$ were conducted. Treatments applied in this work arranged in two experiments was conducted a completely randomized design. The factors examined include seven levels of KNO$_3$ and CaCl$_2$ comprise (0, 5, 10, 15, 20, 25 and 30 mM). The results showed that a positive relationship between germination traits and concentrations KNO$_3$ and CaCl$_2$ was obtained. The higher germination percentage 80% and 75% occurred in seeds that treated with 30 mM CaCl$_2$ and KNO$_3$ respectively. Lower germination percentage (57.3%) observed in control seeds.

Keywords: *Rheum khorasanicum*, Seed germination, KNO$_3$, CaCl$_2$.

INTRODUCTION

Seed dormancy is one of important limiting factor in exploitation economically product of valuable specious [15]. Seed of endemic plants haven't uniform germination in natural ecosystems because they must to survive by seed dormancy. Seed dormancy and germination is depends on genetic factors and environmental conditions that effected on mother plants and harvesting conditions [6].

Rutheford and Ali [25] reported that cold storage is able to break dormancy in *Rheum palmatum* seeds. Farzami Sepeher and Ghorbanli [11] indicated that dormancy in *R. ribes* is imposed by the embryo and some inhibitors in embryo may be responsible for the dormancy and exhibited that KNO$_3$ and CaCl$_2$ broke dormancy and promoted germination in this specious. Nabaei et al. [22] reported that the highest germination percentage (96%) in Rheum ribes was obtained by using the combined treatment of GA$_3$ (500 ppm) and pre-chilling (for 25 days) in 2°C. External nitrogenous compounds represent effective substitutes for plant growth substance. Seeds of many crops and tree species exhibit elevated levels of seed viability and germination when treated with nitrogenous compounds [4,5,14]. Gupta et al. [15] reported a peak germination rate of 96% in Hippophae salicifolia when seeds are subjected to a 0.1% KNO3 pretreatment. Also 0.1% KNO3 solution increases the germination percentage of *Oriental lily* seeds [13]. Seeds of both marigold species primed with 50 mM CaCl$_2$ for 24 h significantly reduced mean emergence time and days to 50% emergence, increased seedling emergence uniformity, final seedling emergence percentage and seedling growth [2]. The genus *Rheum* comprises about 103 species and is distributed in the temperate and sub-tropical regions [23]. This genus is documented in Iranian flora by four species including *R. ribes*, *R. turkestanicum*, *R. persicum* and *R. Khorasanicum*, in which the last two species are endemic [18]. *Rheum khorasanicum* B. Baradaran & A. Jafari is a hardy perennial that was first reported by Jafari *et al.*, [18] and accepted as a new species. It’s morphological very close to but differs in some characters such as presence of bracts, different surface of inflorescence, the pedicle joint position and the epidermal cell shape and Its Persian name is "Rivas". *Rheum* species are medicinally important plants due to the presence of anthraquinone derivatives occurring in the subterranean parts of the plant [19]. Rhubarb roots are used as laxative medicine and an antipsoriatic drug in Iran [26]. Young shoots and petioles are used against diarrhea as well as stomachic and antiemetic, while juice of some parts of the plant is used against hemorrhoids, measles, smallpox and cholagogue [3]. Fresh stems and petioles are consumed as vegetable as well as digestive and appetizer [1]. Because of over harvesting due to increasing market request and very limited distribution, the wild resources of *R. khorasanicum* is decreasing rapidly and no attempt has been performed for its domestication. Therefore,
the aim of this work was to evaluate the effects of KNO\textsubscript{3} and CaCl\textsubscript{2} on seed germination of *Rheum khorasanicum* B. Baradaran & A. Jafari.

**Materials and Methods**

The seeds of *Rheum khorasanicum* were collected from a natural habitat from Neyshabour Mountains (Khorasan Razavi Province, Iran) in July 2012 and then immature seeds and those damaged by insects were removed. The seeds were surface sterilized by soaking in 1% sodium hypochlorite (NaOCl) for 10 min and subsequently rinsed thoroughly with sterilized water prior to applying any treatment. Control and treated seeds were placed in plastic pots filled with perlite. Each germination treatment was performed with three replications. In each replication, 30 seeds were used, divided equally among three pots, giving a total of 90 seeds for each treatment. Experiments conducted in order to completely randomized design at Horticultural Department, University college of Agriculture and Natural Resources, University of Tehran, Karaj, Iran in 2012-2013. *R. khorasanicum* seed treatments were carried out in two experiments including:

Experiment 1) Seeds were soaked in 15, 20, 25 and 30 mM CaCl\textsubscript{2} and stratified at 2\textdegree C for 30 days then were transferred to germinators with 5\textdegree C.

Experiment 2) Seeds were soaked in 15, 20, 25 and 30 mM KNO\textsubscript{3} and stratified at 2\textdegree C for 30 days then were transferred to germinators with 5\textdegree C. Germinated seeds were counted and removed every 24 h for 30 days. A seed was considered germinated when the tip of the radicle appeared [28]. The indices used to evaluate germination were the following:

- Percentage of germination = \((\text{n}/\text{N})\times 100\)
  
  \(\text{n} = \text{the total number of germinated seeds, } \text{N} = \text{the number of seeds used at the beginning of the experiment.}\)

- The germination rate (GR) was calculated using the following formula [28]:
  
  \[ \text{GR} = \sum (\text{number of germinated seeds since } n-1)/n, \]  
  
  with \(n\) being the days of incubation.

- Germination uniformity (GU) calculated using Germin Software [27].

- The time to 50% final germination (T\textsubscript{50}) of each treatment was determined as the day that 50% of final germination had taken place.

- Mean germination time (MGT) was calculated using the following equation [10]:
  
  \[ \text{MGT} = \sum (\text{n.d})/\text{N} \]

  \(\text{n} = \text{the number of germinated seeds between scoring intervals, } \text{d} = \text{number of days from the beginning of the test, } \text{N} = \text{the total number of germinated seeds in the treatment at the end of experiment.}\)

**Statistical Analysis:**

The data were subjected to analysis of variance using SAS software and significant differences were determined using Duncan’s Multiple Range Test (DMRT) at 0.05 probability.

**Results and Discussion**

The results indicated that CaCl\textsubscript{2} and KNO\textsubscript{3} concentration had significant effects on germination traits at 1% probability level (Table 1 and 2).

**Table 1: Effects of CaCl\textsubscript{2} on germination characteristics of *Rheum khorasanicum* B. Baradaran & A. Jafari.**

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>Degree of freedom</th>
<th>Germination Percentage</th>
<th>Germination Rate</th>
<th>T\textsubscript{50}</th>
<th>MGT</th>
<th>GU</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl\textsubscript{2}</td>
<td>6</td>
<td>235.94\textsuperscript{a}</td>
<td>0.00042\textsuperscript{a}</td>
<td>13.73\textsuperscript{a}</td>
<td>9.86\textsuperscript{a}</td>
<td>18.407\textsuperscript{a}</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td>8.71</td>
<td>0.000009</td>
<td>0.52</td>
<td>0.3</td>
<td>1.158</td>
</tr>
<tr>
<td>%cv</td>
<td></td>
<td>4.87</td>
<td>3.67</td>
<td>7.19</td>
<td>4.53</td>
<td>5.51</td>
</tr>
</tbody>
</table>

Notes: **, *, and ns: significantly different at 1%, 5% and not significantly difference, respectively.

**Table 2: Effects of KNO\textsubscript{3} on germination characteristics of *Rheum khorasanicum* B. Baradaran & A. Jafari.**

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>Degree of freedom</th>
<th>Germination Percentage</th>
<th>Germination Rate</th>
<th>T\textsubscript{50}</th>
<th>MGT</th>
<th>GU</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO\textsubscript{3}</td>
<td>6</td>
<td>156.98</td>
<td>0.00044</td>
<td>17.7</td>
<td>11.366</td>
<td>13.174</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td>7.95</td>
<td>0.000006</td>
<td>0.0543</td>
<td>0.1937</td>
<td>0.8954</td>
</tr>
<tr>
<td>%cv</td>
<td></td>
<td>4.32</td>
<td>3.1</td>
<td>7.55</td>
<td>3.2</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Notes: **, *, and ns: significantly different at 1%, 5% and not significantly difference, respectively.

**Germination percentage:**

The germination percentage of *R. khorasanicum* seeds that was soaked for 0 as control were below to compare of other treatments (Fig 1). With increasing concentration of CaCl\textsubscript{2} and KNO\textsubscript{3} from 0 to 30 mM improved germination percentage but at CaCl\textsubscript{2} (X=0.81) increased rapidly than KNO\textsubscript{3} (X=0.66).

**Germination rate:**

As seen in Fig 2, germination rate at both CaCl\textsubscript{2} and KNO\textsubscript{3} were raised. CaCl\textsubscript{2} (X=0.0012) increased rapidly than the KNO\textsubscript{3} (X=0.0011). Germination rate of 0 as control were lower than the other treatments.

\[ \text{GU} = \sum (n.d)/\text{N} \]  

\[ \text{MGT} = \sum (n.d)/\text{N} \]
Fig. 1: Germination percentage of *R. khorasanicum* seeds subjected to different concentrations of CaCl$_2$ and KNO$_3$.

**Time to 50 % final germination:**

With increasing concentration of CaCl$_2$ and KNO$_3$ from 0 to 30 mM decreased T$_{50}$ but at CaCl$_2$ (X= -0.1948) decreased rapidly than KNO$_3$ (X= -0.1329) (Fig 3).

**Mean germination time:**

Mean germination time of untreated seeds was higher than the treated seed with CaCl$_2$ and KNO$_3$ and in both treatments of CaCl$_2$ and KNO$_3$ MGT decreased from 15 to 10 days (Fig 4).

**Germination uniformity:**

With increasing the concentration of CaCl$_2$ and KNO$_3$, germination uniformity decreased (Lower GU is associated with higher uniformity). The rate of uniformity decreasing in CaCl$_2$ (-0.217) treatment was lower than KNO$_3$ (-0.9153).

Fig. 3: Time to 50 % final germination of *R. khorasanicum* seeds subjected to different concentrations of CaCl$_2$ and KNO$_3$. 
Calcium chloride had major effects on germination and increased germination characteristic in *rheum khorasanicum* seeds. The effect of calcium is in loosening of cell wall. Calcium is readily bound with free carboxy groups linking with pectin chains to expand highly hydrated gel networks [20]. Today is accepted the statement that Ca\(^2+\) is a central regulator of plant development and growth [17]. Ca\(^2+\) plays an important role in controlling membrane structure and function by binding to phospholipids and thus, stabilizing lipid bilayers and providing structural integrity to cellular membranes [8], which is particularly important in the germinating seed. Calcium modulates the activity of certain phosphatases and kinase enzymes that participate in the signal transduction during the germination process [9,16]. This study showed that Potassium nitrate also promoted germination traits in this specious. This agrees with a number of studies carried out earlier [21,7,24]. Several studies have examined the role NO plays in breaking dormancy and regulating germination [5,14]. NO may be a product of nitrite and nitrate decomposition. Moreover Nitrates could remove dormancy through pentose phosphate pathway and using oxygen produced during various oxidation processes [12].

**References**


