Effects of Kinetin, Salt Concentration, and Temperature on Germination and Early Seedling Growth of Lactuca sativa L.¹

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Abstract. The effect of salinity (0 to 0.12 M NaCl) and temperature (20-30°C) on germination of ‘Phoenix’ lettuce seed was studied. Increasing levels of both factors inhibited germination and reduced fresh weight of young seedlings. The salinity by temperature interaction was found to be statistically significant in the inhibition of germination. Although the mechanism of this interaction is not known, it was found that osmotic inhibition was reduced at lower temperatures. Kinetin largely overcomes both temperature and osmotic inhibition of germination, but its effect on early seedling development varies with temperature. At 15°C no significant effect was observed, but at higher temperatures (23°C and 25°C) it increased fresh weight at higher salinity levels but had no effect at lower salinity levels.

Introduction

MOST studies of the effect of salt concentration on plant growth have been with plants subsequent to early seedling stage. However, it is also important to know the effect of salt concentration on germination and growth of young seedlings. Commercial lettuce production, particularly in the irrigated valleys of the Southwest, is often located on soils which are somewhat saline or at least subject to salinization (1). Lerner et al. (14) and Mayer and Poljakoff-Mayber (16) reported osmotic inhibition of lettuce seed germination by NaCl. Frankland and Smith (5) osmotically inhibited the germination of half seeds and intact excised embryos of ‘Attracite’ lettuce seed with 0.15 M NaCl to demonstrate a stimulatory effect of chloramphenicol on lettuce seed germination. Uhvits (23) using alfalfa seeds showed that mannitol gives results somewhat different from those obtained with NaCl, and he ascribed the difference to ionic toxicity of the salt. Kahn (12) found that the red-light-reversible “dark-osmotic inhibition” of ‘Grand Rapids’ lettuce seed functions in 2 steps: the first osmotic and the second non-osmotic. He suggested that the non-osmotic step may be similar or identical to that induced by high temperature. Workman and West (24) studied the germination of Eruota lanata in relation to temperature and salinity. They found no significant interaction between temperature and salinity when the seeds were germinated at 6 levels of NaCl under 2 temperature regimes.

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Several workers have reported on the effect of temperature on the germination of photosensitive lettuce seeds (3, 4, 5, 7, 10, 22), however, there are limited reports on the commercially important but nonphotoblastic varieties. There is a paucity of information on the effects of a combination of salt and temperature on germination of lettuce seeds.

Both kinetin and thiourea have been shown to help overcome the germination inhibition of lettuce imposed by high temperature (6, 18, 21) but we are aware of very little information on the effect of growth regulators in overcoming germination inhibition due to salt.

The present study reports on some effects of variations in salt concentration and temperature on the germination of lettuce seeds. The effects of kinetin treatment on these interactions were also investigated.

Materials and Methods

The data reported in this paper were secured with one lot of Great Lakes 'Phoenix' seed. Its maximum germination was 98% at 25°C on water-saturated filter paper. No significant change in germination response was observed during 6 months' storage at room temperature and humidity.

Each replicate consisted of three 9-cm petri dishes of 50 seeds each placed on double sheets of filter paper (Whatman No. 1) wetted with 5 ml of experimental salt solution or distilled water. The salt solutions from 0.02-0.10 M were prepared by dissolving reagent grade NaCl in distilled water. The data presented are averages of 6 replicates representing a total of 900 seeds per treatment. A square root transformation (\( \sqrt{X} + 1 \)) was made for analysis of variance (19).

Seeds were treated by soaking 3 min in 10 mg/liter kinetin solution. After air-drying, the seeds were sprinkled on wet filter paper. This simple 3-min dip treatment has been shown to be as effective in promoting lettuce seed germination as adding the kinetin solution to the dishes (18). Soaking the seeds in distilled water for 3 min in the same fashion did not give significantly different results from planting dry seeds on wet filter paper without pre-soaking. Therefore, dry seeds were used as controls.

Germination was carried out in the dark in incubators at 20°, 23°, and 30° ± 1°C. For the seedling development studies 250 ml of fine washed silica sand No. 30 were placed in transparent plastic boxes each 12 \( \times \) 10 \( \times \) 3.5 cm, moistened with \( \frac{1}{4} \) strength Hoagland's solution containing dissolved sodium chloride. Twenty-five seeds were selected and planted in each box. The boxes were placed in incubators at 15, 23, and 25° with a photoperiod of 16 hr maintained by white fluorescent lights 10 inches above the covered dishes. After 3 weeks 10 seedlings were carefully removed from each box, washed to remove any adhering sand particles, and blotted dry before determining fresh weight.

Results

Interaction of salt and temperature. Since the germination responses at 23 and 27°C were intermediate to those at the other temperatures used, only the results obtained at 20, 25, and 30° are presented here (Fig. 1). At these temperatures the higher the salt concentration the slower the rate of germination. Germination in the presence of the lowest salt levels (.02 and .04) did not differ from water controls at 20°, but at 30° it was considerably reduced. At 30° 0.04 M NaCl completely inhibited germination while at 25° there was still 20% germination after 96 hr with as high as 0.08 M NaCl. At 20° inhibition was not complete even at 0.10 M salt level. After 72 hr the dishes incubated at 30° were transferred to 25° (Fig. 1E) where the seeds subsequently germinated at rates similar to those placed initially at 25°.

Analysis of variance for the germination data from untreated seeds showed that temperature, salinity level and the temperature-salinity level interaction were all significant at the 0.01 level. Salt and temperature combined to inhibit germination significantly.

Effect of kinetin on salt and high temperature inhibition. Kinetin increased germination at most temperatures and at most salt concentrations used in these experiments. There was no apparent increase in germination in the presence of 0.02 or 0.04 M NaCl at 20°C as maximum germination was attained in 24 hr. At the higher salt concentrations tested there was an initial lag in the germination rate.
during the first 24 hr. This lag nearly disappeared with kinetin treatment at the 0.08 M salt level but was still evident at 0.10 M salt level at both 20° and 25°. At 30° kinetin did not overcome the initial lag in germination rate at 0.04 M to 0.10 M salt levels (Fig. 1).

The analysis of variance of the germination data from kinetin-treated seeds showed that temperature, salinity level, and the temperature-salinity level interaction were all significant at the 0.01 level. Although kinetin treatment increases germination under both high salt and high temperature conditions there is still a highly significant interaction of salt and temperature. This was also indicated in Fig. 1 where kinetin was less effective when both high temperature and high salinity conditions were imposed upon the seeds.

The effects of salt concentration on early germination (24 hr) of both kinetin-treated and control seeds at 30° were examined in the present study and Table 1 shows the reversibility of the salt effect at 25°. Transferring the ungerminated seeds from the salt medium after 144 hr to distilled water restored the full capacity of the seeds to germinate. Incubating the seeds for as long as 12 days in the salt solution at 25° did not impair the capacity of the ungerminated seeds to germinate after transferring to pure water.

Temperature and salinity effect on initial seedling growth. Table 2 shows the effect of salinity on early seedling growth at 15°, 23°, and 25°C. There was a decrease in fresh weight per plant with increasing temperature within the narrow range of temperatures observed. This decrease occurred at all salinity levels, but it was more pronounced at the higher levels (0.06 to 0.10 M).

The effect of kinetin on early seedling growth seems to vary with salt concentration and temperature. At 15° there was very little difference between kinetin-treated and non-treated seeds at all salt levels. At 23° and 25° there was also very little difference at the lower salinity levels (0.06 M NaCl) but at higher levels (0.06-1.0 M) kinetin treatment increased seedling fresh weight over non-treated seeds.

**Table 1. Recovery of lettuce seed germination from salt inhibition. Ungerminated seeds incubated for 144 hr at 25°C in different NaCl concentrations were transferred to water.**

<table>
<thead>
<tr>
<th>NaCl Molar Concentration</th>
<th>0.04 M</th>
<th>0.06 M</th>
<th>0.08 M</th>
<th>0.10 M</th>
<th>0.12 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per cent germination after 144 hr</td>
<td>90</td>
<td>47</td>
<td>22</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Per cent germination 24 hr after transfer to water</td>
<td>100</td>
<td>94</td>
<td>92</td>
<td>90</td>
<td>89</td>
</tr>
<tr>
<td>Per cent germination 48 hr after transfer to water</td>
<td>100</td>
<td>97</td>
<td>98</td>
<td>96</td>
<td>96</td>
</tr>
</tbody>
</table>

**Table 2. Effect of salinity and kinetin on early seedling growth at 15°, 23°, and 25°C.**

Average fresh weight (mg) per seedling after 3 weeks. Each figure is average of 2 replicates.

<table>
<thead>
<tr>
<th>Salt molar concentration</th>
<th>15°</th>
<th>23°</th>
<th>25°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control seeds</td>
<td>Kinetin-treated seeds</td>
<td>Control seeds</td>
<td>Kinetin-treated seeds</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>0.00</td>
<td>66.1</td>
<td>65.2</td>
<td>58.0</td>
</tr>
<tr>
<td>0.02</td>
<td>53.3</td>
<td>50.9</td>
<td>50.0</td>
</tr>
<tr>
<td>0.04</td>
<td>52.0</td>
<td>50.1</td>
<td>50.5</td>
</tr>
<tr>
<td>0.06</td>
<td>48.6</td>
<td>42.1</td>
<td>38.6</td>
</tr>
<tr>
<td>0.08</td>
<td>31.0</td>
<td>26.0</td>
<td>21.8</td>
</tr>
<tr>
<td>0.10</td>
<td>29.2</td>
<td>27.7</td>
<td>20.6</td>
</tr>
</tbody>
</table>

**Discussion**

We have found that the salinity by temperature interaction was statistically highly significant in the inhibition of lettuce seed germination. Although interactions between variables are quite well understood statistically, the physical mechanisms involved are not always obvious. Lockhart (15) using a growth system where one factor promoted and another inhibited, developed a technique for testing for interaction between these factors. Although it is difficult to apply all of the criteria of his analysis to seed germination, it is interesting that when the curves of Fig. 2 are inverted they resemble the model curves presented by Lockhart (15) for variables which are interacting rather than seeming those of variables which are acting independently.

Although the nature of the interaction is not known, the results shown in Fig. 1 and 2 indicate that inhibition due to high salinity levels was reduced at the lower temperatures (20 to 23°C). This effect could be similar to the negation of dark-osmotic inhibition of photosensitive lettuce seed by cold treatment (12).

Complete recovery from inhibition is obtained within the salt concentration range used and recovery was still complete after incubating the seeds in the salt solution for as long as 12 days. The results agree with those of Kahn (12) that the effect of NaCl at this concentration range is principally osmotic and nontoxic. A similar reversible response of lettuce seeds to high temperature was shown by Smith et al. (18).

There are numerous examples of kinetin overcoming the heat dormancy of lettuce seed. Kinetin has been found in the present study also to overcome osmotic inhibition due to NaCl. The extent to which kinetin is effective is related to the temperature at which germination is attempted. Kahn (13) showed that gibberellic acid (GA₃) was able to overcome the dark-osmotic inhibition of lettuce seed germination. According to Ikuma and Thimman (8) action of kinetin is presumably in the cotyledons, whereas GA₃ action is in the embryo axis. GA₃ contributes the primary growth stimulus (hydrolytic activity) while kinetin stimulates cotyledon expansion (cell division) resulting in seed coat rup-
ture. The mechanism they proposed for these actions (9) is that kinetin stimulates proteinase, pectinase, and cellulase activity in the cotyledons which in turn reduces the endosperm-integument restrictions. On the other hand, GA₃ promotes amylase activity which supplies the monosaccharides for embryo respiration. It has not been possible to demonstrate a stimulatory effect of GA₃ on the germination of the 'Phoenix' variety used in the present study (18). However, in contrast Ikuma and Thimman (8) reported that although kinetin enhances germination of 'Grand Rapids' lettuce, it is not sufficient in itself; but GA₃, alone induces the germination process. If the effect of kinetin is to reduce the endosperm-integument restrictions, then it will bring the embryo in contact with the salt solution, thereby increasing chances of osmotic injury. Yet, as shown in Table 2, kinetin improves seedling growth at the higher salt concentrations. Therefore, the proposed role for kinetin in photosensitive lettuce seeds (8, 9) does not adequately explain its effectiveness in overcoming osmotic inhibition and an alternative explanation needs to be sought.

The primary action of osmotic inhibition is retardation of water uptake by a tissue which is crucial for the germination process (12). Perhaps it could be postulated that kinetin reduces the moisture requirement of this tissue by some unknown mechanism so that germination and growth can still take place under inadequate moisture supply to the tissue. This is, of course, complicated by the findings of Shull (17) that moisture intake of Xanthium seeds increases with increasing temperature. On the other hand, kinetin may be acting to increase protein synthesis during germination. Ben-Zioni, Itai, and Vaadia (2) have shown that kinetin helps overcome decreased protein synthesis induced by osmotic and salt stress of tobacco plants.

The increased seedling growth due to kinetin pretreatment may perhaps be related to the findings of Itai and Vaadia (11) who showed that cytokinin activity decreased in root exudates of sunflower plants which had been subjected to water deficits or osmotic and salinity stresses. However, one would expect that lettuce seeds would take up a very small amount of kinetin during the 3-min treatments and would therefore be supplying very little cytokinin to the growing seedling. After soaking pea seeds in concentrations of benzyladенine from 5-40 mg/liter for 6 hr, Sprent (20) found that the seedlings, grown in soil, showed reduced root and shoot growth and increased branching.

The effects of kinetin in increasing germination of lettuce under high temperatures and saline conditions is potentially a useful tool to facilitate precision planting of lettuce in desert areas, particularly if increased seedling growth due to kinetin seed treatment can be reproduced under field conditions.

**Literature Cited**


