EFFECT OF DIFFERENT N FORMS AND CONCENTRATIONS ON OLIVE SEEDLINGS GROWTH

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Abstract

The effect of different N forms on the growth of olive seedlings in the greenhouse was studied. KNO3, NH4NO3 and (NH4)2SO4 at different concentrations, as well as two types of substrates, sterile sand and a sand-peat mixture (1:1), were tested. In sterile sand, with N concentrations higher than 5 mM, serious damage was observed in the plants, so concentrations of 3, 4 and 5 mM of N were used for each N salt. N-NH4 treatments resulted in a significantly higher growth compared to KNO3 treatment. Using the peat-sand substrate and the N concentrations described above, seedling growth was higher than in sterile sand, but no differences among treatments were observed. However, the peat:sand substrate allowed application of higher N concentrations and using NH4NO3 differences in growth were obtained, reaching the best results with 14-28 mM.

1. Introduction

One of the main problems for the olive tree genetic improvement programs is the long juvenile period of the olive seedlings, during which the tree does not bloom, and hence is non productive (Hackett, 1985).

Some authors (García-Berenguer, 1991) pointed out the need of a minimum vegetative development for young olive plants to reach the mature stage. Lavee (1990) found ways to shorten olive seedling juvenile period, by training single shoot upright plants together with controlled thinning and shortening lateral juvenile branches under intensive growing conditions.

On the other hand, nutritional factors, and especially nitrogen, have considerable influence on olive seedling growth in greenhouse conditions (Bartolini et al., 1984; Troncoso et al., 1986) and it seems appropriate to consider that adequate nitrogen nutrition would be of great importance in obtaining a rapid vegetative growth which would shorten the juvenile phase of olive tree. Previous studies in plants indicated that mixed nitrate-ammonium nutrition offers better results than with either nitrogen source alone (Hageman, 1984; Olsen, 1986).

In this work, the influence of the N form and concentration on the olive seedling growth in greenhouse conditions as well as the influence of two different substrates have been studied.

2. Materials and methods

Olive seedlings, cv. Manzanillo, obtained from isolated embryos germinated in vitro were used as plant material. After germination, the plantlets were transferred to 250 ml pots with a substrate composed of perlite:sand (1:1) which were then covered with translucent plastic bags. Every 5-6 days a cut was made to the bags until the seedlings were able to survive in the external environment. Plantlets thus obtained were used for the following experiments:
- **Exp. 1:** Seedlings were transferred to 8 l. containers with inert sterile sand and treated (using a dip irrigation system) with Hoaglands, without N, 20% solution (basal solution) for 9 months and respectively the addition of the following N concentrations and forms: Control (3 mM of N as Ca(NO₃)₂) and 3, 4 or 5 mM of N as KNO₃, NH₄NO₃ and (NH₄)₂SO₄ salts.

- **Exp. 2:** The same as Exp. 1, but using a substrate composed of sand:peat (1:1).

- **Exp. 3:** For this experiment, the seedlings were previously grown in a sand:peat (1:1) substrate for 8 months, irrigated with Hoagland 20% solution. Afterwards, the following treatments were carried out for 11 months: Control (Hoagland 20%) and increasing concentrations of NH₄NO₃ (3.5, 7, 14, 21, 28, 40 and 46 mM of N).

For all the experiments, 3 repetitions of 10 plants per treatment were performed. Plant growth was measured, as the sum of all shoot lengths, and leaf mineral composition determined according to Pinta *et al.* (1973).

### 3. Results and discussion

In previous studies, Sarmiento *et al.*, 1994 had found that olive seedlings cultured in greenhouse conditions on inert sand suffered serious damage with concentrations of N higher than 5 mM. Lower N levels did not give differences in growth irrespective of the N form supplied after 6 months of culture. Beside this, a nitrification process affecting the N-NH₄ nutritive solutions was detected, which decreased NH₄ concentrations while NO₃ levels increased, thus changing the NH₄NO₃ balance. This fact could be responsible for the lack of differences observed in growth.

In the present work the nutritive solutions were frequently renewed to avoid the negative effect of NH₄ oxidation. With this modification, the results from experiment 1 (figure 1) regarding growth showed a clear difference among treatments. Thus, KNO₃ applications did not improve the growth obtained with the control treatment at any of the concentrations tested, while N-NH₄ treatments resulted in significantly higher growth than that obtained with both the control and KNO₃ applications, although no differences could be observed between NH₄NO₃ and (NH₄)₂SO₄ applications. Therefore, the ammonium application favoured olive seedling growth which is consistent with other results (Therios and Sakellariadis, 1988).

Despite the differences in growth, leaf mineral composition did not show clear variations among treatments (table 1). Nevertheless, it is worth mentioning that the highest P levels were found in leaves of plants treated with the N-NH₄ solutions. A similar observation was reported by Therios and Sakellariadis (1988) for the cv. Chondrolia Chalkidikis, where ammonium nutrition favoured P assimilation. On the other hand, leaves of plants treated with control and KNO₃ presented higher K content. This fact had already been found in our previous works with *in vitro* cultured embryos (Sarmiento *et al.*, 1994) and, as reported elsewhere (Kirkby and Mengel, 1967; Horst *et al.*, 1985), may be related to competence between NH₄⁺ and K⁺ ions to enter the cells. The (NH₄)₂SO₄ applications gave slightly higher S contents in leaves.

The use of sterile sand offered the advantage of a high sensitivity to small variations in supplied N, especially when the effect of ammonium oxidation to nitrate was avoided. On the contrary, it did not allow the use of high N concentrations, which established a narrow range between deficiency and toxicity of N. The results obtained from exp. 2 (same as exp. 1 but changing the substrate) (figure 2), showed a higher mean growth compared to exp. 1. There were no differences in growth regardless the N concentration and form applied, probably because of the presence of peat in the substrate which improved the growth of seedlings treated with KNO₃.

The results of Exp. 3 are shown in figure 3. As recorded in exp. 2, the lowest concentrations of N (3.5 and 7) did not improve growth compared to control but increasing the concentration of N as NH₄NO₃, produced a significantly higher growth.
Therefore, the presence of peat allowed higher applications of N \((\text{NH}_4\text{NO}_3)\) and thus the best results in growth were achieved at 14-28 mM. Higher levels than these showed a toxic effect on seedlings, which grew less than with the control treatment.

**Acknowledgement**

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**References**


Figure 1. Olive seedlings growth on sterile sand cultured for 9 months, after the addition of different concentrations and forms of N (Exp. 1). The asterisk indicates statistical significance with reference to the control.

Figure 2. Olive seedlings growth on sand:peat substrate (1:1) cultured for 9 months, after the addition of different concentrations and forms of N (Exp. 2).
Figure 3. Olive seedlings growth on sand:peat substrate (1:1) cultured for 11 months, after the addition of different concentrations of NH₄NO₃ (Exp. 3). The asterisk indicates statistical significance with reference to the control.

Table 1. Mineral composition of olive seedlings leaves cultured for 9 months on sterile sand substrate, after the addition of different concentrations and forms of N.

<table>
<thead>
<tr>
<th>mM of N</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>S</th>
<th>Fe</th>
<th>Mn</th>
<th>Zn</th>
<th>Cu</th>
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<tr>
<td>Control</td>
<td>3</td>
<td>2.11</td>
<td>0.10</td>
<td>2.15</td>
<td>0.90</td>
<td>0.22</td>
<td>1.16</td>
<td>0.28</td>
<td>50</td>
<td>11</td>
<td>42</td>
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<td>KNO₃</td>
<td>3</td>
<td>2.39</td>
<td>0.09</td>
<td>1.85</td>
<td>0.81</td>
<td>0.18</td>
<td>0.38</td>
<td>0.17</td>
<td>114</td>
<td>12</td>
<td>13</td>
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<tr>
<td>4</td>
<td>2.21</td>
<td>0.09</td>
<td>2.06</td>
<td>0.71</td>
<td>0.12</td>
<td>0.22</td>
<td>0.19</td>
<td>116</td>
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<td>7</td>
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<td>5</td>
<td>2.11</td>
<td>0.08</td>
<td>2.26</td>
<td>1.10</td>
<td>0.21</td>
<td>0.70</td>
<td>0.15</td>
<td>111</td>
<td>33</td>
<td>44</td>
<td>8</td>
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<tr>
<td>NH₄NO₃</td>
<td>3</td>
<td>2.19</td>
<td>0.16</td>
<td>1.36</td>
<td>0.82</td>
<td>0.21</td>
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<td>1.28</td>
<td>1.00</td>
<td>0.29</td>
<td>0.22</td>
<td>0.16</td>
<td>114</td>
<td>48</td>
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<tr>
<td>(NH₄)₂SO₄</td>
<td>3</td>
<td>2.16</td>
<td>0.23</td>
<td>1.07</td>
<td>1.16</td>
<td>0.31</td>
<td>0.26</td>
<td>0.28</td>
<td>104</td>
<td>22</td>
<td>21</td>
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