Conference presentation

The effects of ultra-high diluted giberellic acid on in vitro lettuce seed germination.

Carolina Santos Barreto¹, Fortune Homsani², Carla Holandino², Nina C. B Silva¹

1- Laboratory of Applied Botany- Pharmacy Faculty, Federal University of Rio de Janeiro, 21941-902, Rio de Janeiro, RJ, Brazil.

2- Multidisciplinary Laboratory of Pharmaceutical Sciences and Laboratory of Research and Development of Integrative and Complementary Medicine, Department of Drugs and Medicines, Pharmacy Faculty, Federal University of Rio de Janeiro, 21941-902, Rio de Janeiro, RJ, Brazil.

INTRODUCTION

Laboratory bioassays have been allowed researchers to eliminate possible interferences through perfectly controlled experimental designs and manipulation of nearly all parameters. So, investigators can manipulate complex conditions one at a time to search for answers to a while kind of scientific questions (Mancías et al., 2000).

Lettuce seeds bioassays have been used for testing many different things like allelopathic responses, discovering of new drugs and toxicity tests, for instance (Mancías et al., 2000). This bioassay has been recommended by some Governmental Agencies as U.S. Environmental Protection Agency, the Food and Drug Administration, and the Organization for Economic Cooperation and Development, because it has proven to be an easy and inexpensive mean of testing the toxicity of different types of contaminants of concern in water and sediments, including heavy metals and some pesticides and other organic toxicants.

In most cases, lettuce (Lactuca sativa L., Asteraceae) has been used because of its sensitivity, simultaneous and fast germination, reliability of germination percentage and homogeneity of seeds. Others advantages are they are less expensive and easy to culture, when compared with other organism usually used, and require no upkeep between experiments. It's used extensively and allows comparison of bioassays from many different compounds.

Considering homeopathy research, according to Majewsky et al., (2009) and Jäger et al., (2015), the main plant used in plant models in general is wheat followed by Dwarf peas and duckweed. Lettuce had been used in very few experiments regarding impaired adult plants and seedlings (Bonfim et al., 2011) and, specifically to experiments using healthy plants, almost none papers reporting the use of lettuce seeds were found (Hopkins, 1998), although seedling model is the most frequently experimental plant model used on basic research in homeopathy (Majewsky et al., 2009).

AIMS

The main goal of this research was to evaluate the effects of ultra-high diluted giberellic acid (GA₃) on lettuce seeds germination and seedling growth.
METHODS

Whole experiment was performed using seeds of *Lactuca sativa* L. cv Regina 500. During all test the seeds were kept at 25°C in controlled environment growth chamber under 16 day light hours provided by fluorescent tubes.

The experiment was conducted in two different laboratories, one of them was in account of preparing test substances and the other responsible for conducing the seed bioassay. The experiment was blinded all the time.

Preparing test substances

The experiment was conducted using Gibberellic acid 3CH (10-6) and 12CH (10-24), water 12CH, a 3µmol GA$_3$ solution as positive control and no potentized water as negative control.

All test solutions were prepared freshly on the day of the experiment from the same batch of distilled water.

GA$_3$ (SIGMA -Aldrich) was dissolved in distilled water in order to obtain the 3µmol stock solution that was used for further potentization process.

According to Brazilian Homeopathic Pharmacopoeia (Brazil, 2011), the whole process consists in successive centesimal (1:99) dilutions steps followed by succussion that, in this case, was carried out through a "mechanic-arm" device model known as "DENISE". By the same method, 12CH water was prepared. All test solutions were submitted to sterile filtration using membranes with 0,24 µ size pores.

After preparation, all solutions were randomized and coded by a person not involved in the next experimental step.

Lettuce seed bioassay

10 Lettuce seeds were placed in Petri dishes containing one sheet of Whatman nº01 filter paper as support watered with 1ml of common distilled water. In each Petri dish 2ml of the different treatment solutions were added. At this step, treatments were coded again. Every 2 days, one millilitre of each solution was added.

The experiment was carried out over 7 days and seed germination was evaluated every 2 days. The root and shoot length were manually measured in the end of the 7th day. Seedlings were separated in groups of 10 individuals, frozen and stored using liquid nitrogen until chlorophyll and carotenoids analyses.

Chlorophyll and Carotenoids content analysis

To determine total chlorophylls (Chl a, Chl b and a/ b ratio) and carotenoids content, seedlings were lyophilized and then were weighed followed by macerated in dimethyl sulfoxide (DMSO) at 50°C for 12 h. After this, the material was filtered through paper filter (Whatman No. 01). The spectrophotometrically quantification was determined through absorbance measurement according to Wellburn (1994).
Statistical analysis

After 30 days, plantlets were kept out from vessels and evaluated for number of shoots, shoot length, rooted plants (%), callus development (%) and fresh biomass.

The experiment was repeated twice and each one consisted in 5 Petri dishes per treatment with 10 seeds per treatment. The final sample size was 100 seeds per treatment.

The data were analysed by one-way analysis of variance (ANOVA) following by Duncan's and t-test (p<0.05)

RESULTS AND DISCUSSION

All seeds germinated at same time in the second day from the experiment start. At the end of the 7th day it was observed a hundred percent germination in almost all treatments. Only seeds treated with GA$_3$ 3CH showed slight lower germination, nevertheless without statistical difference to others (Table 1). This fact improves assay reproducibility and increases the statistical level of acceptability (Mancías et al., 2000).

Table 1: Lettuce seed germination and seedling development after 7 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination Rate (%)</th>
<th>Radicle length (cm)</th>
<th>Shoot length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (no dilution no succussion)</td>
<td>100$^a$</td>
<td>3.92$^{bc}$</td>
<td>0.49$^b$</td>
</tr>
<tr>
<td>GA$_3$ 3µmol</td>
<td>100$^a$</td>
<td>3.68$^c$</td>
<td>2.05$^a$</td>
</tr>
<tr>
<td>GA$_3$ 3 CH</td>
<td>98$^a$</td>
<td>4.10$^b$</td>
<td>0.43$^b$</td>
</tr>
<tr>
<td>GA$_3$ 12 CH</td>
<td>100$^a$</td>
<td>3.83$^c$</td>
<td>0.48$^b$</td>
</tr>
<tr>
<td>Water 12 CH</td>
<td>100$^a$</td>
<td>4.59$^a$</td>
<td>0.47$^b$</td>
</tr>
</tbody>
</table>

Mean values followed for same letter, in a column, do not differ significantly in Duncan's test or Student-test (for percentages analyses) at 5% level of statistical significance

Concerning the seedling development, radicle length was affected just by Water 12 CH, in which the longest length (4.59 cm) was observed when compared with other treatments (Table 1). This result is in accordance with others in which potentized and non potentized water produced different responses (Brizzi et al.,2000; Brizzi et al., 2009) nevertheless different others in which no statistically significant difference was found (Baumgartner et al., 2008; Jäger et al., 2010; Jäger et al., 2015).

Despite having no significantly difference from water, the radicle length was reduced in GA$_3$ at molar concentration which is in accordance with the statement that a strong gibberellin stimulus inhibit root growth. On the other hand, the potentized substances didn't promote shoot elongation and the longest shoots were developed when gibberellin was added in micro molar concentration (Table 1).

It has been demonstrated that potentized gibberellic acid significantly affect development of different healthy plant test species (Hamman et al., 2003; Majewsky et al., 2009; Scherr et al., 2009, Endler et al., 2015). Hamman et al (2003) studying barley seedlings have demonstrated that roots of medium-vigor seed lots treated in GA$_3$ 15CH were longer than others treatments. The content of photosynthetic pigments was significantly higher in seedlings developed under Water 12CH treatment where Chlorophyll a and b as well as total carotenoids were significantly different from both controls (Table 2).

Table 2: Photosynthetic pigments content extracted from 7 days old lettuce seedlings.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chl a (µg/ mg dry weight)</th>
<th>Chl b (µg/ mg dry weight)</th>
<th>Total Carotenoids (µg/ mg dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (no dilution no succussion)</td>
<td>1.79$^b$</td>
<td>0.72$^b$</td>
<td>0.24$^b$</td>
</tr>
<tr>
<td>GA$_3$ 3µmol</td>
<td>0.99$^c$</td>
<td>0.36$^c$</td>
<td>0.16$^c$</td>
</tr>
<tr>
<td>GA$_3$ 3CH</td>
<td>1.84$^b$</td>
<td>0.79$^a$</td>
<td>0.27$^a$</td>
</tr>
<tr>
<td>GA$_3$ 12 CH</td>
<td>1.80$^b$</td>
<td>0.73$^b$</td>
<td>0.25$^b$</td>
</tr>
<tr>
<td>Water 12 CH</td>
<td>1.95$^a$</td>
<td>0.81$^a$</td>
<td>0.27$^a$</td>
</tr>
</tbody>
</table>

Mean values followed for same letter, in a column, do not differ significantly in Duncan's test at 5% level of statistical significance.

CONCLUSION

These preliminary results suggest that potentized water altered growth in the lettuce seedlings, promoting root growth by an unknown mechanism and improving photosynthetic pigments content. Studies regarding histological issues and other biochemical parameters may contribute to a better understanding of this response. Lettuce seed bioassay could be as useful for basic homeopathy studies as it has been extensively used for other investigations. Compared with other test organisms used, lettuce brings the opportunity to test homeopathy in a dicotyledonous group specie. Obviously, it must take in account that the effects on germination of dicotyledonous species are generally lower than effects expressed in monocotyledons. It is of paramount importance that more experiments be undertaken in order to verify if the test system is stable and reliable.

References


https://doi.org/10.51910/ijhdr.v15i4.864
5. Endler et al. Wheat and ultra high diluted gibberellic acid e further experiments and re-analysis of data. 2015