Impacts of PEG-6000 pretreatment for barley (*Hordeum vulgare* L.) seeds on the effect of their mature embryo in vitro culture and primary investigation on its physiological mechanism

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Abstract

In this paper, we studied polyetheneglycol (PEG) pretreatment effect on the mature embryo culture in vitro by using barley (*Hordeum vulgare* L.) seeds. Meanwhile, we analyzed and assayed its mineral element and endogenous hormone level. The experimental results were as follows: (1) PEG-6000 imbibition could obviously slow down the water timecourse absorbed by barley seeds; (2) 10% PEG-6000 treatment of barley seeds for 3 h had a positive effect on germination in vitro and callus induction of the barley seed mature embryos; (3) 10% PEG-6000 treatment inhibited soluble leakage from the seeds; (4) N leakage was mainly from the endosperms, Mn2+ leakage from embryos; (5) PEG-6000 treatment changed greatly the hormone level (ABA, IAA, GAs), which influenced the percentage of plantlets from the mature embryo callus. The results can provide some clues to scientific sowing of crop seeds, pretreatment for the purpose of uniform seedlings, and the explant response quality in plant tissue culture.

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Keywords: Pretreatment; Barley seeds; Mature embryos; Mineral elements; Endogenous hormones

1. Introduction

Pretreatment and imbibition are common problems for farmers to encounter with in practice, which is disregarded very often [1,2,15,16] and understood little in relation to physiological and biochemical mechanisms [3,4,9,24]. Scientific soaking for crop seeds and pretreating with diverse regulatory substances on the basis of good realization of these mechanisms are of importance for obtaining high germination rate and uniform seedlings with good quality, which are the pre-conditions for higher production of crops [10,25].

Former research displayed that PEG-6000 treatment for seeds had a positive action on seed germination [5]. The stem from the mature embryo cultures of the seeds pretreated by PEG-6000 had an increasing callus rate [6,24]. McDonald et al. [13] reported that some anti-growing factors came from wheat seed coats during imbibition. Rice hulls had an anti-oxidative activity [8,21,22]. PEG-6000 addition to the nutrient medium of wheat seedlings led to a rapid cessation of leaf growth and shrinkage, followed by a partial restoration of extension in the subsequent 40 min. This osmotic shock resulted in IAA accumulation in the shoots [9]. These results further exhibited that PEG-6000 use in pretreating, soaking and osmotic regulating should be paid to much attention as a main experimental regulator because of

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its extensive biological functions, which remain unclear in physiology, biochemistry, and molecular biology. Our work showed that 10% PEG-6000 treatment could prompt the ex-plant quality of embryos, seedlings and other organs by regul-ulating imbibing course [10,11]. Here, we further reported our current study in this aspect, i.e. effects of PEG-6000 pretreatment for wheat seeds on their mature embryo in vitro culture and physiological mechanical investigation for the purpose of providing scientific basis for cultivation of seedlings in fields and tissue culture and enriching crop seed physiology.

2. Materials and methods

2.1. Plant material

Hundred Barley mature seeds (Hordeum vulgare L. cv. Zaozhu No. 3), which were healthy and uniform, were se-lected and rinsed five times by deionized water with a room temperature of 22 °C and humidity of 55%.

2.2. PEG-6000 pretreatment for the seeds

Treat barley seeds for 3 h by using 10% PEG, and then soak the seeds in deionized water for 21 h. The total time for imbibition was 24 h (PEG-pretreatment 3 h + soaking 21 h).

2.3. Separation for the mature embryos

Filter paper was used to dry the seeds by absorbing the water. Separate the embryos from the seeds by anatomical knives on superclean desk.

2.4. Medium and culture condition

See Refs. [10,11] and write down rates of callus induc-tion, bud germination, root germination, and corresponding plantlets. Culture temperature, 22 °C; humidity, 55%.

2.5. Soluble leakage detection

Put 100 seeds into the deionized water with a volume of 20 ml and soak them for 30 min. Then determine the leakage by applying DDS-11A Electronic Conductor (Shanghai Sec-ond Analytical Instrument Factory) and O.D. 268 value repre-senting macromolecules by using Shimazu UV-Spectrometer [11].

2.6. Mineral element content analysis

Take 100 embryos or 50 endosperms, and detect K+, Mg2+, Mn2+ as described in [12], and N as described in [10].

2.7. Endogenous hormone determination

Measure the hormones (IAA, GAs, ABA) of each sample of 100 embryos or 50 endosperms as described by Wu et al. [12] during different periods. The applied standard samples were bought from SERVA (ABA) and Sigma Chemical Co. (IAA, GAs >99.9%).

The experiments (measurements and culture) above were triplicated. The other chemicals not mentioned above were in the rank of Analytical Pure.

3. Results

3.1. PEG-6000 pretreatment on the leakage of soluble substances (macromolecules) in the barley seeds

Fig. 1 showed that 10% PEG-6000 pretreatment could effectively retard the leakage of solutes and macromolecules from the seeds by comparing the control ones, and the O.D. 268-like substances (nucleic acids and proteins) were more significant. The figure also implied that the leakage content decreased as the timecourse became longer. These phenom-enas suggested that proper PEG-6000 pretreatment could slow down the soluble leakage, which was beneficial to subsequent morphogenesis including seed germination and in vitro cul-ture response of the mature seeds.

3.2. Effect of PEG-6000 pretreatment on mineral element content in embryos, endosperms and seeds

Table 1 told us some interesting results as follows: (a) Im-bibition with different time course could led to net leakage of N, K+, Mg2+, and Mn2+, whose percentage was 36–40%, 17–60%, 10–34%, and 10%, respectively. (b) PEG-6000 pre-treatment generally reduced the leakage, especially N and this pretreatment had different actions on different mineral elements, such as K+ and Mg2+ with different net leakage content. (c) There was different transfer of mineral elements with various amounts between embryos and endosperms of the seeds. (d) N net leakage was mainly from endosperms and with progressive imbibition, N transferred to embryos from endosperms. (e) Different loss content in embryos and endosperms reflected different laws in leaking.

![Fig. 1. Changes of the solute-leakage intensity during imbibition.](image-url)
Table 1

Changes of mineral element content from PEG-6000 pretreatment and imbibition with different time (0 h, 3 h, and 24 h) in embryos, endosperms and seeds (unit: g/embryo or embryo/endosperm)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Kinds</th>
<th>N</th>
<th>K⁺</th>
<th>Mg²⁺</th>
<th>Mn²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Em</td>
<td>En</td>
<td>Em + En</td>
<td>Em</td>
<td>En</td>
</tr>
<tr>
<td>0</td>
<td>Con</td>
<td>3.7</td>
<td>536.2</td>
<td>539.9</td>
<td>22.3</td>
</tr>
<tr>
<td>3</td>
<td>Con</td>
<td>8.5</td>
<td>513.0</td>
<td>521.5</td>
<td>21.6</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>5.1</td>
<td>441.4</td>
<td>446.5</td>
<td>16.3</td>
</tr>
<tr>
<td>24</td>
<td>Con</td>
<td>21.3</td>
<td>322.0</td>
<td>343.3</td>
<td>19.2</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>13.5</td>
<td>312.7</td>
<td>326.2</td>
<td>8.5</td>
</tr>
</tbody>
</table>

Note: Em, embryo; En, endosperm; Con, control; Pre, pretreatment.

3.3. Influence of PEG-6000 pretreatment on endogenous hormone content in the seeds

From Fig. 2, the result showed that: (a) During the imbibition (0–24 h), IAA content had obvious change in the embryos and endosperms, GAs had change mainly in the embryos, and ABA basically in the internal endosperms. (b) PEG-6000 pretreatment and imbibition could make IAA content increase rapidly in the embryos and late in the endosperms. (c) Progressive imbibition led to a general decrease in ABA content in the endosperms. (d) Non-PEG-6000 pretreatment could produce an increase in IAA, ABA, and GAs in the endosperms, and a decrease in IAA, ABA and GAs in the embryos. (e) PEG-6000 Pretreatment could significantly retard endogenous hormone change in the seeds.

3.4. PEG-6000 pretreatment and in vitro culture response of the mature embryos

From Table 2, the result showed that: (a) PEG-6000 pretreatment could obviously raise in vitro culture effect whether seeds or mature embryos were used as explants. (b) PEG-6000 pretreatment and imbibition for 24 h got better effect in terms of root emergence, bud emergence, callus induction and regenerated plantlets. (c) Comparing the control, PEG-6000 pretreatment could increase the percentage of root and bud emergence by 40%, callus by 200%, plantlets by 400% in the seeds of imbibition for 3 h, respectively. Twenty-four-hour pretreatment and imbibition exhibited that root emergence percentage increased by about 1.5 times, bud emergence percentage by approximately 2 times, callus induction percentage by 6 times, plantlet percentage by about 7 times in the seeds, and by 10 times, 1 time, and 6.5 times, respectively, with an exception of root emergence in the embryos.

4. Discussion and conclusions

There have been some reports related to seed imbibition and its physiology, for instance, seed coat retardation for water absorption [13], solute leakage during imbibition [3,7,14], protein synthesis during early imbibition [2,7,8], new pattern change of isoperioxidases (POD) during imbibition [4,14], IAA increase in imbibition [9,14] and so on. Our study also reflected the above phenomena to larger extent under our experimental system. Fig. 1 showed that imbibition could in-

Fig. 2. Influence of PEG-pretreatment on the endogenous content in the barley seeds (embryos and endosperms).
Impact of PEG-6000 pretreatment on in vitro culture response of the mature embryos

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Treatments</th>
<th>Cultures</th>
<th>Root emergence (%)</th>
<th>Bud emergence (%)</th>
<th>Callus (%)</th>
<th>Plantlets (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Control</td>
<td>Seeds</td>
<td>15</td>
<td>20</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Embryos</td>
<td>0</td>
<td>31</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>Seeds</td>
<td>21</td>
<td>28</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Embryos</td>
<td>6</td>
<td>29</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>24</td>
<td>Control</td>
<td>Seeds</td>
<td>17</td>
<td>22</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Embryos</td>
<td>0</td>
<td>32</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td></td>
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<td>Seeds</td>
<td>45</td>
<td>61</td>
<td>42</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Embryos</td>
<td>22</td>
<td>5</td>
<td>63</td>
<td>29</td>
</tr>
</tbody>
</table>

PEG-6000 is a routine chemical reagent, which is extensively applied in biological treatment, in particular, in osmotic pressure treatment of plant materials [17–23,26]. But its complicated physiological and biochemistry functions are generally ignored. Our current study proves that PEG-6000 pretreatment can influence mineral element change and endogenous hormone content change greatly, further promoting in vitro culture effect, which can be explained easily on the basis of traditional biological theory. Veselov et al. [9] provided good evidence for this in the study of wheat seedlings by PEG-6000 pretreatment. Our research shows that endosperms of seeds function not only in storage of nutrients [4] but also in regulating germination and subsequent morphogenesis [21,24]. In a word, when utilizing PEG for treatments, its possible biological actions should be taken into consideration and related experimental results should be analyzed on the basis of this consideration. Purity of PEG is also paid attention to.

Plant tissue culture regeneration system is of importance to molecular biological study and biotechnological breeding, in which PEG treatment plays a certain role such as in protoplast fusion [25]. Our research suggests that 10% PEG-6000 and treatment for 3 h can have better in vitro culture effect. Higher concentration and longer time treatment may produce opposite effect [22,23]. Besides, this paper also provided new thinking for culturing seedlings in laboratories, regulating explant characters and quality, matter exchange between embryos and endosperms, which are lacking in seed physiology and very important to agricultural production.

In summary, pretreatment is a kind of stress, involved in physical and biochemical process, which is crossed with other stresses like drought, salt, low temperature [26,27]. PEG, as a common osmotic regulator, has multifunction in physics and biology, which are realized by regulating mineral element (ion balance), hormone change, protein metabolism, and further by impacting signal transduction [25,27]. So many questions in this field remain to be solved and are worth being noticed.

Acknowledgement

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References