The Effect of Different Explant Sources on Adventitious Shoot Regeneration in Flax (Linum usitatissimum L.)

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Abstract: Hypocotyl and stem explants excised from in vitro and greenhouse-grown seedlings of Madaras, 1886 Sel. and Omega flax cultivars were compared in terms of percentage of explants producing shoots, shoot number per explant and total shoot number per petri dish. Hypocotyl explants resulted in better shoot regeneration. In vitro-grown seedlings were found to be more suitable than greenhouse-grown seedlings as an explant source.

Key Words: Explant sources, adventitious shoot regeneration, flax

Introduction

The aim of genetic transformation experiments is to regenerate whole fertile transformed plants. The adventitious shoot regeneration capacity of cells or tissues to be used in transformation studies affects the success of gene transformation significantly (1-3).

Some dicotyledonous plants from the families Solanaceae, Begoniaceae, Crassulaceae, Gesneriaceae and Cruciferae regenerate more easily in vitro. Flax has a good regeneration system (4-6). However, the production of transformed flax plants from transformed callus is very low. In particular, after inoculation with Agrobacterium, the regeneration capacity of explants is somehow decreased dramatically. The possible explanations for this phenomenon are: (1) Plant cells may perceive Agrobacterium infection as an attack, and (2) inoculation process may influence plant regeneration negatively.

In vitro shoot regeneration is greatly affected by genotype, physical situation of explant, basal medium content, and culture conditions such as light and temperature. Adventitious shoot regeneration subjected to gene transfer should be increased as much as possible in order to obtain high frequency transgenic plants. Therefore, an efficient adventitious regeneration system should be established before gene transfer to flax.

Flax is an important crop, which is widely used all over the world as a source of natural fibres and industrial oil, and has the potential of meeting edible oil and protein deficiencies (7). Moreover, it has been used as a model system for genetic manipulation studies due to its small nuclear genome.

In the present study, the effects of different explant sources on the regeneration capacity of hypocotyl and stem explants of flax were investigated. Knowledge on the effect of explant source on regeneration is very important not only for flax but also for other crop plants. In addition, it will be useful in solving the problem of regeneration, especially after Agrobacterium inoculation.
Materials and Methods

Madaras, 1886 Sel. and Omega flax cultivars obtained from Northern Crop Science Laboratories, in North Dakota, USA, were used in the study.

Hypocotyl and stem explants were excised from two different sources: *in vitro* and greenhouse-grown seedlings. In order to obtain *in vitro*-grown seedlings, seeds were surface-sterilized with 40% commercial bleach containing 6% sodium hypochloride for 20 min with continuous stirring and then were washed three times with sterile water. After that, sterilized seeds were germinated on a basal medium of Murashige and Skoog’s (MS) mineral salts and vitamins (8), 3% sucrose and 0.7% agar.

Greenhouse-grown seedlings were obtained in pots 12 days after sowing. Seedlings were surface-sterilized before culture as described above for seed sterilization.

For regeneration, 5 mm sections of hypocotyl and stem explants from both *in vitro* and greenhouse-grown seedlings were cultured on MS medium supplemented with 1 mg l$^{-1}$ BAP+0.2 mg l$^{-1}$ NAA and 1 mg l$^{-1}$ BAP+0.02 mg l$^{-1}$ IBA, respectively. All cultures were incubated at 25±1°C under cool white fluorescent light with a 16 h light/8 h dark photoperiod.

The pH of MS medium was adjusted to 5.8 prior to autoclaving. Four replicates were designed, and there were 10 explants per replication. Data from different explant sources were statistically analyzed by Independent-Samples *t*-test in the SPSS for Windows program. Data presented in percentages were transformed by arcsines ($\sqrt{X}$) before statistical analysis (9).

Results and Discussion

In the present study, hypocotyl and stem explants excised from *in vitro* and greenhouse-grown seedlings were compared with regard to percentage of explants producing shoots, shoot number per explant and total shoot number per petri dish. Total shoot number per petri dish is a good indicator for estimating the success of gene transfer. The more shoots per petri dish the more success in gene transfer.

In hypocotyl explants, the best shoot regeneration was obtained when *in vitro*-grown seedlings were used as explant source in all cultivars tested. On the contrary, explants from greenhouse-grown seedlings caused dramatic decreases in shoot regeneration (Table, Figure). For example, in 1886 Sel. cultivar, percentage of explants producing shoots, shoot number per explant and total shoot number per petri dish decreased from 87.50%,

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Shoot regeneration (%)</th>
<th>Shoot number per explant</th>
<th>Total shoot number per petri dish</th>
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<tbody>
<tr>
<td></td>
<td>Hypocotyl</td>
<td>Stem</td>
<td>Hypocotyl</td>
</tr>
<tr>
<td>In vitro</td>
<td>Green House</td>
<td>In vitro</td>
<td>Green House</td>
</tr>
<tr>
<td>Madaras</td>
<td>80.00</td>
<td>37.50</td>
<td>77.50</td>
</tr>
<tr>
<td>t value</td>
<td>4.281**</td>
<td>NS</td>
<td>5.177**</td>
</tr>
<tr>
<td>1886 Sel.</td>
<td>87.50</td>
<td>22.50</td>
<td>65.00</td>
</tr>
<tr>
<td>t value</td>
<td>4.339**</td>
<td>NS</td>
<td>9.309**</td>
</tr>
<tr>
<td>Omega</td>
<td>82.50</td>
<td>40.00</td>
<td>70.00</td>
</tr>
<tr>
<td>t value</td>
<td>2.705*</td>
<td>NS</td>
<td>8.137**</td>
</tr>
</tbody>
</table>

Every cultivar was analyzed separately according to explant sources
Each value is the mean of 4 replications each with 10 explants
** significantly different at the 0.01 level
* significantly different at the 0.05 level
NS not significant
14.43 and 126.26 to 22.50%, 1.31 and 2.94, respectively (Table and Figure).

In stem explants, the best results were also recorded from in vitro-grown seedlings regarding to shoot number per explant and total shoot number per petri dish in all cultivars. When greenhouse-grown seedlings were used, results decreased significantly (Table). Percentage of stem explants producing shoots was not influenced by the explant source significantly. In stem explants, decreases in shoot regeneration between in vitro and greenhouse-
grown seedlings were lower than those of hypocotyl explants. For example, in 1886 Sel. cultivar, shoot number per explant and total shoot number per petri dish decreased from 8.72 and 56.68 to 6.48 and 43.74, respectively (Figure).

In hypocotyl and stem explants, the best results were obtained from in vitro-grown seedlings. The results from hypocotyl explants were better than stem explants in every case. When greenhouse-grown seedlings were used as explant sources, shoot regeneration decreased dramatically. The degree of this reduction was higher in hypocotyl explants than in stem explants. It was obvious that hypocotyl explants were extremely sensitive and easily affected by preculture treatments such as surface sterilization, and culture conditions.

Total shoot number per petri dish is a good indicator of gene transformation. This should be as high as possible for efficient gene transformation. In this study, when hypocotyl explants were excised from greenhouse-grown seedlings, total shoot number per petri dish decreased at a rate of 85.00% in Madaras, 98.00% in 1886 Sel. and 93.00% in Omega cultivars.

It was previously reported that hypocotyl was the most suitable explant for gene transformation in flax (2,3,10-12). This was verified once more in the present study.

The application of biotechnology to crop improvement requires efficient adventitious plant regeneration protocols. The introduction of foreign genes coding for agronomically important traits into plant cells is useless unless transgenic plants are produced. The adventitious shoot regeneration capacity of cells or tissues could be the main factor affecting the success of all transformation techniques. However, shoot regeneration capacity is greatly affected by genotype, physical situation, age and size of explant, basal medium content, and culture conditions such as light and temperature.

In conclusion, the data presented in this study clearly indicated that seedlings used as explant sources should be grown in vitro for high frequency shoot regeneration.

References