Effect of Genotype, Explant Type and Culture Medium on Shoot Regeneration in Tomato (Lycopersicon esculentum Mill.) in vitro

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Abstract


The regeneration capacity of three explants types (segments from hypocotyls, cotyledons and true leaves) in eight tomato lines (Lycopersicon esculentum Mill.) was studied. Explants were cultured on MS basal medium supplemented with different concentrations of plant growth regulators BA and IAA. In the studied treatments of these regulators highest regenerative activity was established in leave explants (44.0% and 36.4%, respectively), comparatively lower in the cotyledon (33.3% and 30.6%, respectively) and hypocotyl segments (21.7% and 19.3%, respectively). The highest regenerative frequency was observed in line XXIVA (65.3%) and the lowest one - in line №8 (3.6%). The best results were obtained in medium supplemented with 2.0 mg/l BA and 0.2 mg/l IAA that proves the strongest dependence of in vitro regeneration on genotype as well as less on type of explants and medium composition.

Keywords: Lycopersicon esculentum Mill., tomato, in vitro regeneration

Introduction

The initial genetic diversity is a necessary basis for successful development of the applied breeding. At present, the necessity of rich and various initial breeding material for creation of high qualitative and competitive varieties and F₁ hybrids requires parallel application of traditional and modern biotechnological methods.

In vitro tomato regeneration is achieved through direct and indirect organogenesis and embryogenesis. These problems are studied by many authors marking dependence of the process from genotype and explant type (Moghaieb et al., 1999; Gubis et al., 2003; Bhatia et al., 2004). It was established that on the regeneration activity there is an effect of medium content, explant size and age (Plastira and...
Perdikaries, 1997; Chandel and Katiyar, 2000; El-Bakry, 2002).

Study of the regeneration capacity and optimization of in vitro regeneration conditions both in all plants and tomato is a prerequisite for inducing of inherited mutations by: somaclonal variation, treatment of explants with different mutagenic factors in vitro, cultivation of immature embryos obtained after interspecific hybridization and genetic transformation (Crino et al., 1994; Sanchez-Donaire et al., 2000; Park et al., 2003).

The aim of this experimental work is to study the regeneration capacity of the cotyledon, hypocotyl and leave explants from different tomato genotypes (L. esculentum Mill.) which will be included in future in vitro experiments with mutagenic treatments.

Materials and Methods

Seeds of eight tomato lines (№ 8, 9, 10, 355, 373, 177, 163 and XXIV A) were sterilized in 5% calcium hypochlorite solution and sown on Murashige and Skoog (1962) (MS) medium without plant growth regulators. Explants of cotyledons and hypocotyls were excised from one week-old plants, but the segments of mature leaves were excised from three week-old plants all grown in vitro. The explants were cultivated in Petri dishes on basal medium contained major and minor salts by MS, vitamins as in B5 medium (Gamborg et al., 1968), 2% sucrose, 0.7% agar and plant growth regulators in two different concentrations:

I variant - 2.0 mg/l N6-Benzyladenine (BA) + 0.2 mg/l Indolil-3-acetic acid (IAA)

II variant - 2.2 mg/l BA + 1.6 mg/l IAA

and transfer after 20 days on medium contained - 4.4 mg/l BA + 1.6 mg/l IAA

The pH of the medium was adjusted to 5.8 before autoclaving. For the different variants 20 explants were cultivated in 3 replications of each of the genotype, at 25°C ±1°C, 4000 lux and 16/8 h day/night. The regeneration capacity (% explants with regeneration) was reading at the end of 75-days cultivation period. The obtained results were calculated by two-factor analysis of variance and Duncan's Multiple Range Test (1955).

Results and Discussion

The data presented in Figure 1 show that the average regeneration capacity of the studied tomato genotypes varies from 3.6% to 65.3% regardless of the used medium composition. Higher regeneration frequency was established in all tomato lines in the case of explant cultivation on variant I of the medium - from 4.4% to 71.0%, compared with variant II of the used medium - from 2.8% to 54.0%. These results proved that in our experiment the lower concentrations of the plant growth regulators (BA and IAA) induced better regeneration answer of the different explants.

The data presented in Table 1 prove that the higher average regeneration activity was registered in leave explants (44.0% and 36.4%, respectively on the two studied variants of the medium), compared with explants of cotyledons (33.3% and 30.6%, respectively) and explants of hypocotyls (21.7% and 19.3%, respectively). These results are in correspondence with the experimental data published by Duzyaman et al. (1994). The authors reported that the regeneration frequency depends on the explant type and increases to the direction of hypocotyls<
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Fig. 1. Tomato explants cultivation in vitro on MS basal medium with different concentrations of plant growth regulators (BA and IAA)

Table 1
Regeneration frequency of cotyledon, hypocotyl and leave explants from eight tomato genotypes

<table>
<thead>
<tr>
<th>Explant types</th>
<th>Medium I</th>
<th>Medium II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>Hypocotyl</td>
<td>Cotyledons</td>
</tr>
<tr>
<td>----------------</td>
<td>----------</td>
<td>-----------</td>
</tr>
<tr>
<td>8</td>
<td>0.0b 2.88</td>
<td>11.7a 2.88</td>
</tr>
<tr>
<td>9</td>
<td>11.7 ns 7.63</td>
<td>8.3 ns 2.88</td>
</tr>
<tr>
<td>10</td>
<td>11.7bc 5.77</td>
<td>13.3bc 5.77</td>
</tr>
<tr>
<td>355</td>
<td>16.7c 2.88</td>
<td>46.7ab 7.63</td>
</tr>
<tr>
<td>373</td>
<td>41.7b 5.77</td>
<td>58.3a 7.63</td>
</tr>
<tr>
<td>XXIVA</td>
<td>51.7c 2.88</td>
<td>70.0b 0.00</td>
</tr>
<tr>
<td>177</td>
<td>20.0a 5.00</td>
<td>6.7b 2.88</td>
</tr>
<tr>
<td>163</td>
<td>20.0c 5.00</td>
<td>55.0a 8.66</td>
</tr>
<tr>
<td>Average</td>
<td>21.7 c 33.3 ab</td>
<td>44.0 a 4.40</td>
</tr>
</tbody>
</table>

a,b,c,…P<0.05 Duncan’s Multiple Range Test
ns – not significant
The differences, reported by other authors are mainly due to the specificity of the genotypes and the conditions of cultivation (Moghaieb et al., 1999; Gubis et al., 2003).

On the base of the performed experiments the highest regeneration frequency was established in line XXIV A (65.3%) and the lowest one - in line № 8 (3.6%). Developing of plant-regenerates in the hypocotyl segments of this line was not observed or some explants regenerated in rare cases what is probably due to the specific influence of genotype or the experimental conditions.

The statistically analyzed data presented in Table 2 indicate that the differences between the studied genotypes and explant types in regeneration induction are highly significant while the differences between medium composition, explant type and genotype are not significant. These results are in agreement with those of Plastira et al. (1997), Moghaieb et al. (1999) and Gubus et al. (2003) who reported that in vitro plant regeneration in tomato is genotype specific process but in some level type of explants and conditions of cultivation also influence it.

Table 2
Analysis of the influence of nutrient medium, explant type and genotype on regeneration frequency in tomato

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Fcrit.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient medium - NM</td>
<td>1</td>
<td>127.3136</td>
<td>127.3136</td>
<td>0.27</td>
<td>3.09</td>
</tr>
<tr>
<td>Explants - E</td>
<td>2</td>
<td>6580.207</td>
<td>3290.104</td>
<td>7.04***</td>
<td>3.06</td>
</tr>
<tr>
<td>NM x E</td>
<td>2</td>
<td>61.01056</td>
<td>30.50528</td>
<td>0.07</td>
<td>3.06</td>
</tr>
<tr>
<td>Error</td>
<td>138</td>
<td>64484.6</td>
<td>467.2797</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>143</td>
<td>71253.13</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Fcrit.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype - G</td>
<td>7</td>
<td>53819.71</td>
<td>7688.53</td>
<td>233.76***</td>
<td>2.09</td>
</tr>
<tr>
<td>Explants - E</td>
<td>2</td>
<td>6652.831</td>
<td>3326.415</td>
<td>101.14***</td>
<td>3.07</td>
</tr>
<tr>
<td>G x E</td>
<td>14</td>
<td>6648.078</td>
<td>474.8627</td>
<td>14.43***</td>
<td>1.77</td>
</tr>
<tr>
<td>Error</td>
<td>120</td>
<td>3946.8</td>
<td>32.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>143</td>
<td>71067.42</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Fcrit.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient medium - NM</td>
<td>1</td>
<td>141.2136</td>
<td>141.2136</td>
<td>1.1</td>
<td>3.91</td>
</tr>
<tr>
<td>Genotype - G</td>
<td>7</td>
<td>553819.71</td>
<td>7688.53</td>
<td>59.75***</td>
<td>2.08</td>
</tr>
<tr>
<td>NM x G</td>
<td>7</td>
<td>636.5553</td>
<td>90.93647</td>
<td>0.71</td>
<td>2.08</td>
</tr>
<tr>
<td>Error</td>
<td>128</td>
<td>16469.94</td>
<td>128.6714</td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>143</td>
<td>71067.42</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; *** P<0.001
Conclusions

The obtaining results and their analysis allow formulating that:

• The regenerative activity in the studied eight lines depends on the highest level from the genotype nature and less from the type of explants and culture medium.

• The better regeneration activity is registered in variant I of the basal MS medium supplemented with 2.0 mg/l BA and 0.2 mg/l IAA in true leave explants.

References


