Selection and regeneration of purple sweet potato calli against drought stress simulated by polyethylene glycol [version 1; referees: awaiting peer review]

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Abstract

Background: Water shortage due to natural and/or technical drought stress, widespread throughout Sumatra, Java, Sulawesi and Kalimantan islands, significantly reduces crop production. The development of varieties tolerant to drought stress is important since it is more effective rather than improving irrigation infrastructure to increase the sweet potato productivity.

Methods: Selection and regeneration experiments assessing purple sweet potato callus tolerance of drought stress, simulated by polyethylene glycol (PEG), were conducted to generate new variant plants tolerant of drought stress. Sterile explants (leaf and petiole) generated from previous in vitro culture were inoculated to the Murishage and Skoog (MS) medium containing plant growth regulator combination as treatments to induce calli. The calli were then transferred to half-MS medium containing 0, 5, 10, 15 and 20% PEG as selection agent for drought tolerance. The surviving calli were regenerated in the MS medium containing 0, 0.5, 1 or 1.5 mg l⁻¹ 6-benzylaminopurine (BAP). The callus formation, growth and survivability during in vitro culture were measured.

Results: Calli were successfully formed in almost all media containing 2,4-Dichlorophenoxyacetic acid (2,4-D) with the concentration of 1, 2, 3 and 4 mg l⁻¹ and BAP (concentration: 0.5 and 1 mg l⁻¹), but the medium of MS + 2 mg l⁻¹ 2,4-D + 0.5 mg l⁻¹ BAP resulted in the highest number of induced calli per treatment (mean=11.36), with the percentage of responsive explants standing at around 96%. The higher the concentration of PEG, the lower the number of surviving calli. At 20% PEG, only 54.42% calli survived. There were five plants successfully regenerated from the survived calli at 20% PEG, using MS medium containing 1.5 mg l⁻¹ BAP.

Conclusions: The experiment has successfully produced putative drought-tolerant plants by callus screening using PEG as drought-tolerance-selecting agent in purple sweet potato.

Keywords
Callus formation, purple sweet potato, drought tolerance, Polyethylene Glycol, in vitro selection
**Introduction**

Drought stress is a major limiting factor in increasing the production of several important crops in a large number of regions in Indonesia. The effect of drought stress on plant growth is largely determined by the amount of stress the plant is exposed to and the growth phase the plant was in during drought stress. In addition, drought stress causes inhibition of plant growth and plant roots and as a consequence, plants will grow slowly and their productivity is severely reduced. Development of cultivated plants tolerant to drought stress, including sweet potato, is an important approach to solving water shortage issues.

In vitro selection is a breeding strategy widely used to produce new variant plants that are resistant or tolerant to disease, herbicides or extreme environmental stresses, including drought stress. This method selects genetic variation arising from tissue culture processes especially produced from natural or artificial mutation. The rapid multiplication of undifferentiated cells during callus formation increases the possibility of natural mutation due to rapid cell division. Such genetic changes are subsequently selected as useful traits in breeding programs.

Polyethylene glycol (PEG) solution can be used as drought-tolerant selecting agent in soybean and other plants. PEG is able to control the decrease of water potential homogeneously, therefore it can mimic the potential of groundwater. The long-term use of PEG will not cause cell damage, because PEG has molecular weight of more than 6000 g/mol that cannot be absorbed into plant tissues. This research attempted to produce new variant of purple sweet potato tolerant to drought stress via in vitro selection using PEG as a selection agent.

**Methods**

**Callus growth**

Young leaves and petioles from previous in vitro-generated plants grown using standard Murishage and Skoog (MS) medium were used as explants. The intact leaf were sliced into 1 cm² of lamina and 1 cm length of petiole. The explants were then inoculated to the MS containing plant growth regulator of 2,4-dichlorophenoxyacetic acid (2,4-D) (Merck, Cat. No.31518, Germany) combined with 6-benzylaminopurine (BAP, Merck, Cat. No. B3408, Germany) to induce calli. The explants were grown and placed at a sterilized 600 ml bottle containing around 20 ml solidified medium for a month at the culture room at a temperature of 25±2°C. The composition of the treatments were Z₁ (MS + 0 mg L⁻¹ 2,4-D + 0 mg L⁻¹ BAP), Z₂ (MS + 1 mg L⁻¹ 2,4-D⁻¹ + 0.5 mg L⁻¹ BAP), Z₃ (MS + 2 mg L⁻¹ 2,4-D⁻¹ + 0.5 mg L⁻¹ BAP), Z₄ (MS + 2 mg L⁻¹ 2,4-D⁻¹ + 1 mg L⁻¹ BAP), Z₅ (MS + 3 mg L⁻¹ 2,4-D⁻¹ + 0.5 mg L⁻¹ BAP), Z₆ (MS + 3 mg L⁻¹ 2,4-D⁻¹ + 1 mg L⁻¹ BAP), Z₇ (MS + 4 mg L⁻¹ 2,4-D⁻¹ + 0.5 mg L⁻¹ BAP), and Z₈ (MS + 4 mg L⁻¹ 2,4-D⁻¹ + 1 mg L⁻¹ BAP). All treatments were replicated five times. In total there were two types of explants x eight treatments x five replication x five explants per replication/bottle = 400 experiment units.

**Assessment of callus growth**

The green, fresh and compact calli produced at the first experiment were selected and transferred to MS medium with half the usual level of nutrients containing 0, 5, 10, 15 and 20% PEG as selection agent medium of drought tolerance. All treatments were replicated three times. In total there were five treatments x three replications x four explants per replication/bottle = 60 experiment units. The surviving calli in the PEG selection-agent medium were regenerated in MS medium containing 0, 0.5, 1 or 1.5 mg L⁻¹ BAP to induce shoots and roots. The growth variables during in vitro culture, such as the number of calli induced per treatment, percentage of responsive explants to induce calli, percentage of surviving calli under drought stress simulated by polyethylene glycol, and number of regenerated plants, were observed.

**Results and discussion**

**Callus production**

All media applied in the experiment could successfully induce callus formation except the basal medium containing no growth regulator (control). The existence of 2,4-D and BAP in various concentration combination was able to induce callus formation but the number of callus per explant and the percentage of responsive explant were varied (Table 1, Figure 1A, B). The highest number of induced calli per treatment was observed using the Z₁ treatment medium. These results were observed using both leaf and petiole (Figure 1A, B). Callus formation was successfully induced in sweet potato using both explants. The use of 2,4-D to induce callus formation is common. Callus formation can be induced by other plant growth regulators such as zeatin, or 1-naphthaleneacetic acid combined with gibberellin (GA₃). These calli emerged and was developed from the mesophyll cells in the leaf and protoplast from the stem or petiole.

**Drought-stressed callus growth**

The fresh, green and compact calli produced in the first experiment was transferred to the drought stressed medium simulated by MS medium containing different concentration of PEG to discover the putative drought tolerant calli of purple sweet potato. At least one callus survived in all PEG-containing medium, but the survival rate was varied (Table 2, Figure 1C). The higher the concentration of the PEG in the medium the lower survival rate of the calli in the selection medium. This indicates that drought stress caused by PEG simulation affects callus survivability and can be used as drought-tolerant selection medium, as also reported in other crop plants such as soybean, tobacco, grass, rice and chili. The putative drought-tolerant plants are screened from regenerated plants derived from the survived calli in the highest concentration of PEG. There was no significant different response between calli derived from the leaf and petiole (Table 2).

The surviving calli grown in medium containing 40% PEG were incubated in the regeneration medium to initiate shoot and/or root. The only medium MS containing 1.5 mg L⁻¹ BA can
Table 1. Callus induction using two different explants of purple sweet potato. Values shown are mean ±
standard deviation unless indicated.

<table>
<thead>
<tr>
<th>Callus induction medium</th>
<th>Leaf</th>
<th></th>
<th></th>
<th>Petiole</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calli induced per treatment</td>
<td>Responsive (%)</td>
<td>Calli induced per treatment</td>
<td>Responsive (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z₀ (MS + 0 mg L⁻¹ 2,4-D⁻¹ + 0 mg L⁻¹ BAP)</td>
<td>0.00 (± 0.00)</td>
<td>0</td>
<td>0.00 (± 0.00)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z₁ (MS + 1 mg L⁻¹ 2,4-D⁻¹ + 0.5 mg L⁻¹ BAP)</td>
<td>8.80 (± 1.94)</td>
<td>68</td>
<td>8.76 (± 2.09)</td>
<td>76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z₂ (MS + 1 mg L⁻¹ 2,4-D⁻¹ + 1 mg L⁻¹ BAP)</td>
<td>10.44 (± 2.01)</td>
<td>92</td>
<td>10.44 (± 1.41)</td>
<td>88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z₃ (MS + 2 mg L⁻¹ 2,4-D⁻¹ + 0.5 mg L⁻¹ BAP)</td>
<td>11.36 (± 2.25)</td>
<td>96</td>
<td>11.32 (± 2.62)</td>
<td>64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z₄ (MS + 2 mg L⁻¹ 2,4-D⁻¹ + 1 mg L⁻¹ BAP)</td>
<td>5.12 (± 2.00)</td>
<td>92</td>
<td>5.12 (± 3.04)</td>
<td>76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z₅ (MS + 3 mg L⁻¹ 2,4-D⁻¹ + 0.5 mg L⁻¹ BAP)</td>
<td>5.28 (± 0.83)</td>
<td>88</td>
<td>5.28 (± 1.04)</td>
<td>44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z₆ (MS + 3 mg L⁻¹ 2,4-D⁻¹ + 1 mg L⁻¹ BAP)</td>
<td>7.40 (± 1.98)</td>
<td>92</td>
<td>7.40 (± 1.36)</td>
<td>88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z₇ (MS + 0 mg L⁻¹ 2,4-D⁻¹ + 0 mg L⁻¹ BAP)</td>
<td>2.32 (± 1.75)</td>
<td>72</td>
<td>2.32 (± 2.20)</td>
<td>88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z₈ (MS + 0 mg L⁻¹ 2,4-D⁻¹ + 0 mg L⁻¹ BAP)</td>
<td>8.60 (± 0.80)</td>
<td>84</td>
<td>8.60 (± 1.86)</td>
<td>92</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MS, Murishage and Skoog medium; BAP, 6-benzylaminopurine.

Table 2. Percentage of surviving purple sweet potato calli under drought stress simulated by different concentration of polyethylene glycol (PEG).

<table>
<thead>
<tr>
<th>Explant</th>
<th>PEG Concentration (%)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>90.11</td>
<td>89.85</td>
<td>88.37</td>
<td>76.32</td>
<td>55.42</td>
<td></td>
</tr>
<tr>
<td>Petiole</td>
<td>96.88</td>
<td>86.88</td>
<td>81.67</td>
<td>76.98</td>
<td>57.92</td>
<td></td>
</tr>
</tbody>
</table>

successfully induce shoot and root growth (Table 3, Figure 1D). This indicates that the calli exposed with high concentration of PEG are still viable to regenerate into whole plant. The results increase the possibility to get a new purple sweet potato variant tolerant to drought stress as also reported in soybean ¹⁹,²⁰.

The raw data for calli and explant growth are available on OSF.²⁵

Conclusions and outlook
Initial production of putative drought tolerant plants by callus screening using PEG as drought tolerant-selecting agent in purple
sweet potato was successfully done in this experiment. Callus was successfully induced in MS medium containing many different combinations of 2,4-D and BAP. Drought-tolerant screening using PEG is generally effective since there was a strong indication that the increase of PEG concentration caused the reduction of the callus survivability. However, the high percentage of surviving calluses at the highest concentration of PEG (40%) may indicate that the drought stress applied in the experiment was not apparently sufficient to induce cell mutation. Therefore, a future experiment using higher concentrations of PEG could provide more tolerant calli and increase genetic mutations, especially with regards to drought tolerance. Genetic evaluation and field experiments using water shortage treatment experiments will be the next investigation to clarify the genetic mutations involved and the stability of the drought-tolerance characteristics.

Data availability
The raw data on the number of calli formed per bottle for different treatment conditions and subsequent explant growth are available on OSF. DOI: https://doi.org/10.17605/OSF.IO/DEGVY.

Grant information
The author(s) declared that no grants were involved in funding this work.

References


Table 3. Initiation of shoot and root derived from calli that survived 40% polyethylene glycol growth.

<table>
<thead>
<tr>
<th>Callus induction medium</th>
<th>Number of regenerated plants</th>
<th>Leaf</th>
<th>Petiole</th>
</tr>
</thead>
<tbody>
<tr>
<td>K₀ (MS + 0 mg L⁻¹ BAP)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K₁ (MS + 0.5 mg L⁻¹ BAP)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K₂ (MS + 1.0 mg L⁻¹ BAP)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K₃ (MS + 1.5 mg L⁻¹ BAP)</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>
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