Influence of different explants on micropropagation of gwarpatha [Aloe vera (L.) Burm.]

Divya Dixit, Mohan Lal Jakhar, Sarfraz Ahmad, Suman Chahar, Kassim Yahya Mitlimbanya and Hari Ram Jat

Abstract

The present investigation was carried out to study effect of different explants on micropropagation of Aloe vera. The experiment was conducted using completely randomized design with ten replications at Tissue Culture Laboratory of S.K.N. Agriculture University, Jobner, India during 2018-19. Auxiliary shoot and apical shoot were used as explants in MS media containing same concentrations of plant growth hormone. The cultures were incubated at 25 ± 2°C under 14:10 hour’s photoperiod with a light intensity of 3000 lux. Among these two explants auxiliary shoot showed significantly higher number of shoots (11.0) with longer shoots length (9.65 cm) compare to apical shoot explant. Also, auxiliary shoot induced higher number of roots (6.8) with longer root length (7.89 cm) than apical shoot explants. Thus for large scale micropropagation, auxiliary shoot explants were significantly better than apical shoot explant for both shoot proliferation and root induction of Aloe vera.

Keywords: Aloe vera, apical shoot, auxiliary shoot, explant, micropropagation, root induction, shoot proliferation

Introduction

Plant tissue cultures are initiated from tiny pieces, called explants, taken from any part of a plant. Practically all parts of a plant have been used successfully as a source of explants. In practice, the “explant” is removed surgically, surface sterilized and placed on a nutrient medium to initiate the mother culture, that is multiplied repeatedly by subculture. The type of explants, size, position, age, physiological state and the manner in which it is cultured can affect the initiation of the cultures and further morphogenetic response [4]. Micro propagation using stem and lateral shoot pieces of Aloe vera had already been proved successful [1, 6, 7]. However, source of explants, size, age, genotype, media composition, culture conditions, phenolic content of explants, exogenous supply of the plant growth regulators and media discoloration greatly affect shoot regeneration from different genotypes of the same species. Therefore, the present study aimed to study the effects of different explants on micropropagation as an alternative protocol for rapid and high frequency in vitro propagation of Aloe vera.

Materials and Methods

The present investigation was carried out at Tissue Culture Laboratory of Department of Plant Breeding and Genetics, S.K.N. Agriculture University, Jobner, Rajasthan, India during 2018-19. For micropropagation of Aloe vera, two types of explants viz. apical shoot and auxiliary shoot were used and cultured at most responsive levels of plant growth regulators for shoot proliferation and root induction. Apical shoots were used as explants after removing the surrounding leaves while auxiliary shoot explants were used after removing the apical shoot. For shoot proliferation, BAP at 4.0 mg/l used singly and BAP (4.5 mg/l) with NAA (0.6 mg/l) were used in combination. For root induction, IBA used alone at 1.5 mg/l and IBA (2.0 mg/l) with NAA (0.5 mg/l) was used in combination. All chemicals used in the present study were of analytical grade. Murashige and Skoog (1962) [5] medium was used throughout the course of investigation. All the cultures were maintained in an air conditioned culture room at a temperature of 25 ± 2 °C under fluorescent light in a 14:10 hours’ photoperiod.

Observations recorded

Standard procedures had been during observations on period of initiation of shoot, number of shoots per explant, morphogenetic response (Percent), shoots length (cm), number of days...
taken for root induction, number of roots, root length (cm) and mean days taken for callus initiation of the callus.

**Statistical analysis**
Each experiment was conducted in completely randomized design and data were analyzed for means and standard error accordingly as described by Snedecor and Cochran (1972) [8]. Standard error was calculated only after value transformation for the characters where response was less than 100 per cent. The value for each replication was transformed by square root transformation as follows

\[ \sqrt{Y} + \frac{1}{2} \]

Where, Y= original value

Tests of significance were done according to Duncan’s Multiple Range Test (DMRT) for different traits [3].

**Results and Discussions**
In the present investigation two kinds of explant viz. apical shoot and auxiliary shoot were inoculated on media supplemented with the most responsive levels of plant growth regulators. For shoot proliferation BAP singly used at the concentration of 4.0 mg/l and in combination BAP at 4.5 mg/l with 0.6 mg/l NAA was used. For root induction 1.5 mg/l IBA used alone and in combination IBA (2.0 mg/l) with NAA (0.5 mg/l) was used. Both the explants showed different results of shoot bud break and root induction.

**Effect of explants on shoot bud proliferation**
Significant differences were observed for shoot length and number of shoots between both the explants at same levels of plant growth regulators. Auxiliary shoot explants started proliferating at 15.5 days after inoculation while apical shoot explants takes 22.3 days (Table 1). After 8 weeks of culture the auxiliary shoot explants showed higher number of shoots (11.0) with longer shoots length (9.65 cm) compare to apical shoot (Fig. 1 and Fig. 2). Frequency of shoot proliferation was 100 per cent in both the explants. Further, MS medium supplemented with 4.5 mg/l BAP + 0.6 mg/l NAA, auxiliary shoot explants showed higher number of shoots (12.8) with larger shoot length (8.53 cm), while apical shoot explants resulted into lesser numbers of shoots proliferated (Fig. 3 and Fig. 4). Callusing was not reported in both the explants supplemented with these plant growth regulator levels. Among the two kinds of explants auxiliary shoot responded considerably better on the media for shoot proliferation which were similar with the observations of Abdi et al. (2013) [1] in Aloe vera for shoot proliferation in auxiliary shoots along with sheath. They obtained maximum shoot proliferation in the MS medium supplemented with 4.0 mg/l BAP.

**Effect of explants on root induction**
When proliferated shoots from both the explants were inoculated on media supplemented with most responsive levels of auxins, there was 100 per cent frequency of root induction for both the explants. Auxiliary shoot explants induced higher number of roots (6.8) with longer length (7.89 cm) than apical shoot explants (Table 2 and Fig. 5). In combination of plant growth regulators, auxiliary shoot explants also induces more number of roots (5.6) than apical shoot explants (3.7) with longer length of the roots (Fig. 6). Significant difference was found between the length and number of shoots at both the explants Similar pattern of results was found for root induction also where auxiliary shoot explants exhibited significantly better results as compared to apical shoot explants These results indicated that auxiliary shoot explants were significantly better than apical explants for both shoot proliferation and root induction.

**Acknowledgements**
The author acknowledges the support from all the staff of Tissue culture laboratory and department of Plant Breeding and Genetics, S. K. N. Agriculture University, Jobner, Rajasthan (India) for conducting the experiment.

**Table 1: Effect of different explants of Aloe vera at most responsive levels of plant growth regulators (4.0 mg/l BAP and 4.5 mg/l BAP + 0.6 mg/l NAA) on shoot proliferation**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Explant</th>
<th>Days taken in shoot initiation</th>
<th>Morphogenetic response (per cent)</th>
<th>Number of shoot buds/explant</th>
<th>Shoot length (cm)</th>
<th>Days to callus initiation</th>
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<tr>
<td>1.</td>
<td>Apical shoot</td>
<td>22.3</td>
<td>100</td>
<td>3.1 ± 0.23 b</td>
<td>5.3 ± 0.25 b</td>
<td>3.09 ± 0.19 b</td>
</tr>
<tr>
<td>2.</td>
<td>Auxiliary shoot</td>
<td>15.5</td>
<td>100</td>
<td>7.3 ± 0.260 a</td>
<td>11.0 ± 0.39 a</td>
<td>9.65 ± 0.51 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0 mg/l BAP</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1.</td>
<td>Apical shoot</td>
<td>20.5</td>
<td>100</td>
<td>3.7 ± 0.213 b</td>
<td>5.7a ± 0.213 b</td>
<td>4.3 ± 0.18 b</td>
</tr>
<tr>
<td>2.</td>
<td>Auxiliary shoot</td>
<td>12.2</td>
<td>100</td>
<td>8.2 ± 0.327 a</td>
<td>12.8a ± 0.327 a</td>
<td>8.53 ± 0.44 a</td>
</tr>
<tr>
<td></td>
<td>4.5 mg/l BAP+ 0.6 mg/l NAA</td>
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</table>

Values followed by same letters in each column are not significantly different (p<0.05) using DMRT.

**Table 2: Effect of different explants of Aloe vera at most responsive levels of plant growth regulators (1.5 mg/l IBA and 2.0 mg/l IBA + 0.5 mg/l NAA) on root induction**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Explant</th>
<th>Days taken in root initiation</th>
<th>Rooting response (%)</th>
<th>Number of roots/explant</th>
<th>Root length (cm)</th>
<th>Number of shoot buds/explant</th>
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<tr>
<td>1.</td>
<td>Apical shoot</td>
<td>29.8</td>
<td>100</td>
<td>2.9 ± 2.333 b</td>
<td>2.25 ± 0.150 b</td>
<td>1.3 ± 0.15 b</td>
</tr>
<tr>
<td>2.</td>
<td>Auxiliary shoot</td>
<td>22.3</td>
<td>100</td>
<td>6.8 ± 0.367 a</td>
<td>7.89 ± 0.224 a</td>
<td>1.9 ± 0.23 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5 mg/l IBA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Apical shoot</td>
<td>24.2</td>
<td>100</td>
<td>3.7 ± 0.197 b</td>
<td>3.17 ± 0.277 b</td>
<td>1.1 ± 0.1 b</td>
</tr>
<tr>
<td>2.</td>
<td>Auxiliary shoot</td>
<td>18.3</td>
<td>100</td>
<td>5.6 ± 0.327 a</td>
<td>8.02 ± 0.319 a</td>
<td>2.3 ± 0.26 a</td>
</tr>
<tr>
<td></td>
<td>2.0 mg/l IBA + 0.5 mg/l NAA</td>
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Values followed by same letters in each column are not significantly different (p<0.05) using.
Fig 1: Shoot proliferation in Apical shoot explants at medium supplemented with 4.0 mg/l BAP

Fig 2: Shoot proliferation in Auxiliary shoot explants at medium supplemented with 4.0 mg/l BAP

Fig 3: Shoot proliferation in apical shoot explants at medium supplemented with 4.5 mg/l BAP + 0.6 mg/l NAA

Fig 4: Shoot proliferation in auxiliary shoot explants at medium supplemented with 4.5 mg/l BAP + 0.6 mg/l NAA

Fig 5: Root induction from auxiliary (left) and apical (right) explants at medium supplemented with 1.5 mg/l IBA

Fig 6: Root induction from auxiliary (right) and apical (left) explants at medium supplemented with 0.5 mg/l IBA + 2.0 mg/l NAA

References