**Investigation of tTCLs Technique in Regeneration of African Violet (Saintpaulia ionantha)**

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**ABSTRACT**

tTCLs technique can justify, commercial and mass production of ornamental plants by decrease of explants size and increase of micropropagation yield. In present study, were investigated in tTCLs technique, effect of size and type of explants on regeneration of African violet influence of growth regulators. For regeneration were used, explants of leaf, petiole and pedicel in different size (leaf segments 3x3mm, 6x6mm, 10x10mm; segments of petiole and pedicel to thickness of 0.4mm, 2mm, 4mm). In multiplication stage were employed MS medium supplemented with B/A and NAA (1mg/l from each). In regenerated samples, were investigated, fresh weight, dry weight, size of callus and shoot number. High in vitro regeneration and shoot produced capacity were observed for calli formed from petiole explants with 4mm thickness and 10x10mm leaf explants with significant increase in other parameters. Explants of 0.4mm and 2mm thickness petioles, 0.4mm thickness pedicel and 3x3mm size leaf had lowest regeneration.

INTRODUCTION

*Saintpaulia ionantha* or African violet belongs to the Gesneriaceae family. This plant with different cultivars in various colors and shapes is one of popular commercial houseplants in world [8]. African violet due to having characteristics such as visual appeal, tolerance of low light room, ability to flower under artificial light and may of vegetative propagation in all of year, have been ever noteworthy for interested to production of plants. This plant is propagated commonly by vegetative leaf cutting. When, was produced one or several shoot from leaf cutting, being restriction in growing point, leads to production of asymmetric plants on form of leaf growth. For overcome these difficulties, in vitro micropropagation are employed to commercial and mass production of single crown and proper shape plantlets, similar to parent plant in short period of time [21]. Until now, regeneration of this plant has been made successfully initiated by different sources explants containing leaf [18], petiole, flower bud [11], stamen [24] and protoplast [7]. Culture of thin cell layer (TCLs) as one of new micropropagation techniques would be an adequate approach against requirements of farmers in gardening industries in future century. The thin cell layer system relies on small size explants prepared from various plant organs which they are excised either longitudinally (iTCL), or transversally (tTCL) [23]. This technique has benefits such as economical productivity of micropropagation, simple gene transfer and plants breeding, may of investigation of morphogenesis stages [23]. Thin cell layer culture influence of different PGRs was reported as an efficient approach for regeneration of *Lilium ledebourii* as ornamental plant [10]. Also, in a study on tTCL culture system for in vitro propagation of ginger were shown morphogenetic potential in tTCL ginger explants [20].The aim of this experiment was investigation of regeneration in different explants of leaf, petiole, pedicel and their size in tTCLs technique for *Saintpaulia ionantha*.

MATERIALS AND METHODS

African violet as mature plant, purchased from Miss Ashrafi’s greenhouse in Sad Abad of Tehran. The explants were taken from inflorescence (several days before opening of main flower), petiole and semi mature leaves. The explants after washing in running tap water were surface sterilized with 2% Etanol for 20 second and 1% of Sodium hypochlorite for 15 minutes under Laminar Air Flow. Followed, the explants were rinsed
three times with sterile distilled water. The explants sections of petiole and pedicel were prepared with thickness of 4mm, 2mm and 0.4mm. The leaf explants were prepared too, with size of 10×10mm, 6×6mm and 3×3mm. In multiplication stage for all explants were employed MS medium [12] with 200mg/L Casein hydrolysate, 3% Sucrose, 1mg/L Naphthalen acetic acid (NAA) and 1mg/L Benzyl adenine (BA) and 0.7% Agar. The pH of medium was adjusted to 5.7 before autoclaving. The sections of explants, germinated with consideration of polarity in 10cm diameter Petri dish with 30ml MS medium under Laminar flow. All Petri dishes were incubated at 25c in 3000 Lux light and 16h photoperiod. After 6 week of culture cultivation, were determined several parameters such as length, width, high, fresh wt, dry wt and shoot number in explants. In next stage, the explants were transferred to the MS basal medium which it has been semi solid with Agar. After 6 week and rooting, plantlets were hardened in 50% peat moss and 50% perlite. The effect of two explants treatment of petiole, pedicel and sections of its bottom (3 sections) were investigated with replication on different parameters as factorialities and in completely randomized designs. The effect of different sections of leaf, were investigated too, by 5 replication on different parameters in completely randomized designs. Statistics analyses were done using Excel and MSTAT-C soft wares.

Results:

Callus formation was started for leaf explants after a week and for explants of pedicel and petiole two week of culture cultivation. According to Table 1, the petiole explants with 4mm section had most amounts of fresh wt, dry wt, length, width and high, between explants of petiole and Callus formation was started for leaf explants after a week and for explants of pedicel and pedicel. The highest shoot number (84.1) was obtained too, for petiole explants with 4mm section in explants of petiole and pedicel with different sections. The petiole explants with 2 and 0.4mm of sections did not show significant regeneration and produce any shoot. The petiole explants with most amount of thickness (4mm) after 4mm sectioned of petiole explants, produced most amount of fresh wt, dry wt, length, width and high, among treatments and 35.80 shoot .The pedicel explants with 2mm section have significantly could increase, parameters of fresh wt, dry wt, length, width and shoot number, than 0.4mm section. The petiole explants with 0.4mm section, could not induce, callus formation in explants and for result, produce any plantlet. According to Table 1, in petiole explants, with decrease of thickness from 4mm to 2 and 0.4mm, was inhibited of callus formation and regeneration of petiole explants. In pedicel explants, decrease of thickness from 4mm to 2mm was followedby decrease in amount of investigated parameters but 0.4mm of thickness induces any callus formation.

Table 1: Comparison of average of growth parameters from various sections of petiole and pedicel explants.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh weight(g)</th>
<th>Dry weight(g)</th>
<th>Length(cm)</th>
<th>Width(cm)</th>
<th>High(cm)</th>
<th>Shoot(number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.C.</td>
<td>3.784a</td>
<td>0.195a</td>
<td>3.010a</td>
<td>2.350a</td>
<td>1.460a</td>
<td>84.10a</td>
</tr>
<tr>
<td>B.C.</td>
<td>0.002d</td>
<td>0.001b</td>
<td>0.002c</td>
<td>0.001d</td>
<td>0.002d</td>
<td>0.004d</td>
</tr>
<tr>
<td>B.C.</td>
<td>0.001d</td>
<td>0.003b</td>
<td>0.003c</td>
<td>0.001d</td>
<td>0.003d</td>
<td>0.005d</td>
</tr>
<tr>
<td>B.C.</td>
<td>0.866b</td>
<td>0.055b</td>
<td>1.020b</td>
<td>0.970b</td>
<td>0.610b</td>
<td>35.80b</td>
</tr>
<tr>
<td>B.C.</td>
<td>0.653c</td>
<td>0.140a</td>
<td>0.890b</td>
<td>0.660b</td>
<td>0.450c</td>
<td>28.00b</td>
</tr>
<tr>
<td>B.C.</td>
<td>0.002d</td>
<td>0.002b</td>
<td>0.003c</td>
<td>0.003d</td>
<td>0.002d</td>
<td>0.003d</td>
</tr>
</tbody>
</table>

Values followed by the same letters are not statistically different for p=0.05 according Duncan test.

B.1: petiole explants  
B.2: pedicel explants

C.1: 4mm section  
C.2: 2mm section  
C.3: 0.4mm section

Table 2: Comparison of average of growth parameters from various sections of petiole and pedicel explants.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh weight(g)</th>
<th>Dry weight(g)</th>
<th>Length(cm)</th>
<th>Width(cm)</th>
<th>High(cm)</th>
<th>Shoot number</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.1</td>
<td>3.390a</td>
<td>0.152a</td>
<td>3.330a</td>
<td>2.340a</td>
<td>1.541a</td>
<td>179.0a</td>
</tr>
<tr>
<td>C.2</td>
<td>1.626b</td>
<td>0.100b</td>
<td>2.350b</td>
<td>1.500b</td>
<td>1.370b</td>
<td>65.20b</td>
</tr>
<tr>
<td>C.3</td>
<td>1.085c</td>
<td>0.067c</td>
<td>2.100c</td>
<td>1.650c</td>
<td>1.200c</td>
<td>49.90c</td>
</tr>
</tbody>
</table>

Values followed by the same letters are not statistically different for p=0.05 according Duncan test.

C.1: 10x10mm section  
C.2: 6x6mm section  
C.3: 3x3mm section

Discussion:

According to results the regeneration was observed to be efficient in petiole explants than pedicel explants. Previously, Bilkey et al [3] reported that African violet petiole tissue has a high in vitro regeneration capacity. A previous study on plant regeneration of African violet showed high frequency regeneration from leaves and petioles explants supplemented with PGRs [19]. Also this report is an agreement with Nhtut et al [14] in *Vietnamese ginseng* that in their study, high rate of embryogenesis, callogenesis, shoot and root formation were achieved from petiole explants. Although in this experiment, explants section of pedicel had lower potential of regeneration compared to explants section of petiole but regenerated plantlets from pedicel explants have special importance in regeneration of these cultivars because they have been kept mother plant properties similar to chimera cultivars of African violet. However, in experiments of Lineberger et al [9], were produced low number of plantlets from pedicel explants for production of chimera plantlets similar to parent plant which TLCs technique is efficient approach against these difficulties and can produce large number of plantlets from different sections of pedicel, which this is an agreement with Altamura et al [1] in *N. Tabacum* that by use of...
rtLC technique, pedicels showed the highest flowering potential. According to previous reports TCLS technique has been used successfully in the micropropagation of many plant species [2, 5, 6, 16, 22, 4, 17, 14]. Although in rTLCs technique, thin sections can increase yield of propagation generally, but according to reports of Nhut et al [13] on Begonia tuberosus, in their experiments, 3 and 4mm sections of stem had most regeneration than 1 and 2mm sections. It should be emphasized that separated cell layer as explants in this technique do not must be very small that may small explants were not have ability of proper regeneration, due to poor nutrients or damaged tissues during of explants excising, according to this, in an experiment on different size of rtLC, contains 0.5, 1, 2 and 3mm thick in *Lilium longiflorum*, were found that 1, 2 and 3mm thick rTLCs produced the highest number of shoots with survival rate of 100% and rTLCs 0.5 mm thick exhibited necrosis in 90% of explants and produced much lower shoot [15].

Conclusions:
Although leaf explants had high regeneration and could produce a lot of plantlets but this ability is important for the time which the aim is only production of non-chimera cultivars. When the aim of micropropagation is production of chimera cultivars of African violet, according to the results of Lineberger et al [9], pedicel explants is adequate selection.

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REFERENCES


