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·技术方法·

白檀离体快繁技术

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摘要 以白檀(*Symplocos paniculata*)幼嫩茎段为实验材料, 通过对启动培养、增殖、生根培养及移栽的影响因子进行研究, 初步建立了白檀的组织培养体系。结果表明: 白檀外植体最适灭菌方案为0.1%升汞3分钟, 无菌苗获得率达81%; 最适初代启动培养基为1/2MS+30 g·L^{-1}蔗糖+8 g·L^{-1}琼脂, 出芽率达86.83%; 增殖最适培养基为1/2MS+1.0 mg·L^{-1} 6-BA+0.02 mg·L^{-1} IBA+30 g·L^{-1}蔗糖+8 g·L^{-1}琼脂, 增殖系数达3.57; 最适生根培养基为WPM+0.5 mg·L^{-1} IBA+0.5 mg·L^{-1} NAA+20 g·L^{-1}蔗糖+2 g·L^{-1} AC+8 g·L^{-1}琼脂, 生根率达93%; 炼苗后, 移入园土:草炭土=1:1 (v/v)的基质中, 成活率达83%。

关键词 白檀, 外植体, 组织培养

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白檀(*Symplocos paniculata*)为山矾科山矾属落叶灌木或小乔木, 其树形优美, 花白色、芳香, 果成熟时蓝紫色(尹翔等, 2012), 是极具观赏价值的园林绿化树种; 其耐干旱瘠薄, 根系发达, 固土能力强(左长清等, 1994), 是优良并适合推广的园林树种。白檀的果实油质好且含油量高, 可作润滑油、食用油和生物柴油原料等(刘强等, 2015)。同时, 其在医疗保健和农业等领域均有着广泛的用途(Aggarwal et al., 2011; Gupta and Peshin, 2012), 可见白檀是集多种价值于一体的植物资源, 亟待开发利用。目前, 有关白檀繁殖的研究主要集中在播种和扦插, 白檀的种子具有隔年萌发的生理特性, 种仁率不足50% (刘立言等, 2016); 嫩枝扦插移栽成活率也较低(孟德悦等, 2013)。在其组织培养方面, 仅见Yang等(2016)利用白檀种胚研究了其体细胞胚胎发生技术, 而以白檀茎段为外植体进行组培快繁技术研究鲜见报道。因此, 本研究以白檀幼嫩茎段为外植体, 建立其组培苗快繁技术体系, 为进一步研究和工厂化生产奠定基础。

1 植物材料

实验材料为采集于吉林省通化市二道江区的野生白檀(*Symplocos paniculata* (Thunb.) Miq.)植株。取白檀母株健壮休眠枝条, 去除细弱的部分, 将枝条剪成约13–15 cm长的茎段, 放入玻璃瓶内催芽, 温度控制在25–27°C, 第5天左右芽陆续萌发, 待芽长成3–4 cm嫩茎时剪下作外植体。

2 培养基成分与培养条件

2.1 外植体消毒

用0.1%氯化汞溶液浸泡嫩茎, 时间分别为1、2、3、4和5分钟, 再用无菌水冲洗5–6次, 用无菌纸吸干其表面多余水分, 切成单芽小茎段后接入启动培养基中。

2.2 培养基配方

启动培养基为WPM、Read、MS、1/2MS、1/4MS、1/8MS、mMS (在MS基础上, 添加50 mg·L^{-1} Na_2SO_4, $Zn_2SO_4·7H_2O$减至8 mg·L^{-1} (Yang et al., 2016))及1/2mMS, 以上培养基均添加30 g·L^{-1}蔗糖。增殖培养基为1/2MS+30 g·L^{-1}蔗糖, 分别加入0.06–1.0 mg·L^{-1} 6-BA和0.0–1.0 mg·L^{-1} IBA组合的培养基(表3)。生根培养基为WPM (通过预实验选用WPM作为基本培养基进行生根培养)+20 g·L^{-1}蔗糖+2 g·L^{-1}活性炭, 分别添加不同浓度的IBA和NAA (表4)。以上培养基均添加8 g·L^{-1}琼脂, pH5.8–6.0, 在121°C下高

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压灭菌20分钟。

2.3 切割方法

启动培养: 将单芽小茎段接种在启动培养基上, 30天后比较不同培养基对白檀初代培养中芽萌动及其生长的影响。增殖培养: 从启动培养基中的无菌苗上取下侧芽, 接种于增殖培养基上, 35天后比较不同激素处理对白檀组培苗增殖的影响。生根培养: 将长度为1.5–2.0 cm的双节茎段接入生根培养基, 45天后比较不同浓度生长素对白檀组培苗生长及生根的影响。

2.4 培养条件

培养室温度(25±1)°C, 光照强度为18.75 μmol·m^{-2}·s^{-1}, 光周期为每天12小时光照/12小时黑暗。每个处理包括20个外植体, 重复3次。

2.5 炼苗与移栽

将装有根长为3–4 cm再生苗的瓶子开盖炼苗2天, 在自来水下冲去组培苗根部培养基, 分别移栽至园土:细沙=1:1 (v/v)、园土:草炭土=1:1 (v/v)和草炭土:细沙=1:1 (v/v) 3种栽培基质中, 置于温室下, 保持温室内温度为15–25°C, 相对湿度为60%–70%, 及时浇水, 勤加管理。

2.6 数据统计分析

利用以下公式进行数据统计。

污染率=(污染苗数/接种苗数)×100%;

无菌苗获得率=(获得无菌苗数/接种苗数)×100%;

出芽率=(出芽外植体数/接种外植体数)×100%;

增殖系数=形成的有效芽数/接种苗数;

生根率=(生根外植体数/接种外植体数)×100%;

移栽成活率=(移栽成活株数/移栽株数)×100%。

采用SPSS 20.0软件中的LSD和Duncan方法对数据进行方差分析和多重比较。

3 结果与讨论

3.1 白檀外植体消毒条件的优化

由表1结果可知, 白檀单芽小茎段经0.1%升汞消毒处理, 随着处理时间的延长, 污染率逐渐下降, 无菌苗获得率在1–3分钟时不断提高, 当灭菌时间为4分钟时, 无菌苗获得率有所下降。故白檀嫩茎在浓度为0.1%升汞中消毒3分钟时, 得到的无菌苗最多, 污染率也较低。

表1 0.1%升汞溶液不同时间梯度处理对白檀无菌培养体系建立的影响

Table 1 Effects of different time gradient of 0.1% mercuric chloride on establishment of sterilized seedling culture system of *Symplocos paniculata*

Sterilization time (min)	1	2	3	4	5
Acquisition rate (%)	46.0	70.0	81.0	45.0	10.8
Pollution rate (%)	50.0	25.0	18.2	16.7	14.0

3.2 不同启动培养基对白檀芽萌动及生长的影响

外植体在接入供试的8种启动培养基之后, 第7天至14天均开始萌芽, 到第30天, 不同启动培养基上白檀芽的萌发率达14.13%–86.83%, 最低与最高萌发率之间相差6倍(表2), 表明不同启动培养基对白檀外植体萌芽率的影响较大。其中, 1/2MS培养基上白檀外植体的出芽率最高, 且茎段长势良好, 新生芽长势健壮, 叶片浓绿(图1A)。

3.3 不同浓度6-BA和IBA组合对白檀组培苗增殖的影响

将白檀新芽接种于添加不同浓度6-BA和IBA的1/2MS培养基中进行增殖培养, 接种后约15天开始萌芽, 22天左右芽长至2–4 cm (图1B)。从表3可以看出, 增殖培养35天后, 随着6-BA浓度的升高, 增殖系数呈上升趋势; 在6-BA浓度不变的条件下, 随着IBA浓度的升高, 增殖系数呈先上升后降低趋势。当6-BA浓度为1.0 mg·L^{-1}、IBA质量浓度为0.8 mg·L^{-1}时, 增殖系数最高, 但与0.6 mg·L^{-1} 6-BA+0.05 mg·L^{-1} IBA及1.0 mg·L^{-1} 6-BA+0.02 mg·L^{-1} IBA、1.0 mg·L^{-1} 6-BA+0.08 mg·L^{-1} IBA培养基的平均增殖系数非常接近, 无极显著差异, 即这4种培养基与其它培养基相比均达极显著差异。但综合分析萌芽早晚、基部愈伤组织多少、侧芽状态以及成本、激素积累对植物生长的不利影响等多种因素, 确定添加1.0 mg·L^{-1} 6-BA+0.02 mg·L^{-1} IBA的1/2MS培养基为白檀的最适增殖培养基。

3.4 不同浓度生长素对白檀组培苗生根的影响

当生长素IBA和NAA浓度在0–0.5 mg·L^{-1}范围内时,

表2 不同启动培养基对白檀初代培养芽萌发及生长的影响

Table 2 Effects of initiation medium on bud germination and growth of *Symplocos paniculata*

Culture medium	Average germination rate (%)	State of growth
WPM	43.30±5.03 eE	Sprouts grew generally, leaves were normal shape and color
Read	56.37±4.05 dD	Sprouts grew generally, leaves were normal shape but yellow
MS	14.13±5.00 fF	Sprouts grew poorly, leaves were narrow, curly, green
mMS	16.00±5.00 fF	Sprouts grew poorly, leaves were curly, green
1/2MS	86.83±5.53 aA	Sprouts grew well, leaves were flat and dark green
1/2mMS	54.33±4.51 dD	Sprouts grew well, leaves were curly, dark green
1/4MS	74.13±3.61 bB	Sprouts grew generally, leaves were curly, green
1/8MS	66.33±3.21 cC	Sprouts grew generally, leaves were curly and yellow

平均出芽率数据为平均值±标准差。同列不同小写字母表示差异显著(P<0.05), 同列不同大写字母表示差异极显著(P<0.01)。

Average germination rate indicated means±SE. Different lowercase letters in the same column indicate significant differences at P<0.05, different uppercase letters in the same column indicate significant differences at P<0.01.

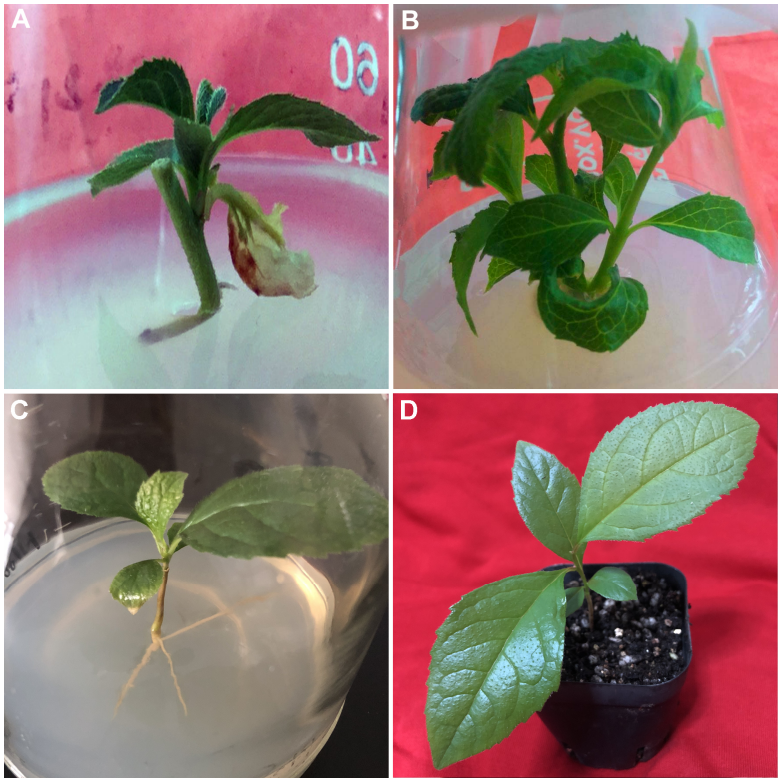

图1 白檀启动培养、增殖培养、生根及植株移栽情况

(A) 在1/2MS培养基中启动培养30天的生长情况; **(B)** 在添加1.0 mg·L^{-1} 6-BA+0.02 mg·L^{-1} IBA培养基中增殖培养22天的生长情况; **(C)** 在添加0.5 mg·L^{-1} IBA+0.5 mg·L^{-1} NAA增殖培养基中培养30天的生长情况; **(D)** 在园土:草炭土=1:1 (v/v)栽培基质中生长30天的情况

Figure 1 Status of initiation culture, proliferation, rooting culture and transplantation of *Symplocos paniculata*

(A) Status of *Symplocos paniculata* initiation cultured on 1/2MS medium after 30 days; **(B)** Status of *S. paniculata* regeneration buds cultured for 22 days on the medium with 1.0 mg·L^{-1} 6-BA+0.02 mg·L^{-1} IBA during proliferation; **(C)** Status of *S. paniculata* regeneration buds cultured for 30 days on the medium with 0.5 mg·L^{-1} IBA+0.5 mg·L^{-1} NAA during rooting; **(D)** Status of *S. paniculata* regeneration buds 30 days after transplanting in garden soil:peat soil=1:1 (v/v) cultivation medium

表3 不同浓度6-BA和IBA组合对白檀组培苗增殖的影响

Table 3 Effects of combination of different concentration of 6-BA and IBA on proliferation of *Symplocos paniculata* plantlets

6-BA ($mg·L^{-1}$)	IBA ($mg·L^{-1}$)	Average proliferation coefficient	State of growth
0.06	0	1.33±0.21 kJ	Sprouted within 15 days in average, base of seedlings had few calli, lateral buds were weak
0.06	0.02	1.40±0.00 klJ	Sprouted within 15 days in average, base of seedlings had very few calli, lateral buds were weak
0.06	0.05	1.47±0.15 klJ	Sprouted within 15 days in average, base of seedlings had very few calli, lateral buds were weak
0.06	0.08	1.67±0.21 ijkHIJ	Sprouted within 16 days in average, base of seedlings had many calli, lateral buds were weak
0.3	0	1.63±0.23 jkHIJ	Sprouted within 15 days in average, base of seedlings had few calli, lateral buds were slender
0.3	0.02	1.57±0.12 jkHIJ	Sprouted within 22 days in average, base of seedlings had few calli, lateral buds were thick
0.3	0.05	1.57±0.29 jkHIJ	Sprouted within 15 days in average, base of seedlings had few calli, lateral buds were thick
0.3	0.08	1.90±0.20 hijGHI	Sprouted within 15 days in average, base of seedlings had very few calli, lateral buds were thick
0.3	0.2	2.43±0.12 fgDEF	Sprouted within 9 days in average, base of seedlings had few calli, lateral buds were brawny
0.3	0.5	1.70±0.17 ijkHIJ	Sprouted within 15 days in average, base of seedlings had many calli, lateral buds were thick
0.6	0	2.77±0.25 defCD	Sprouted within 13 days in average, base of seedlings had few calli, lateral buds were slender
0.6	0.02	2.03±0.06 hiFGH	Sprouted within 45 days in average, base of seedlings had many calli, lateral buds were thick
0.6	0.05	3.50±0.26 abAB	Sprouted within 13 days in average, base of seedlings had many calli, lateral buds were thick
0.6	0.08	2.93±0.15 cdCD	Sprouted within 16 days in average, base of seedlings had many calli, lateral buds were thick
0.6	0.2	2.60±0.20 defDE	Sprouted within 15 days in average, base of seedlings had very few calli, lateral buds were thick
0.6	0.5	3.17±0.29 bcBC	Sprouted within 15 days in average, base of seedlings had few calli, lateral buds were brawny
0.6	0.8	2.20±0.27 ghEFG	Sprouted within 13 days in average, base of seedlings had many calli, lateral buds were brawny
1.0	0	2.70±0.20 defCD	Sprouted within 12 days in average, base of seedlings had many calli, lateral buds were brawny
1.0	0.02	3.57±0.32 aAB	Sprouted within 5 days in average, base of seedlings had few calli, lateral buds were brawny
1.0	0.05	1.67±0.12 ijkHIJ	Sprouted within 5 days in average, base of seedlings had few calli, lateral buds were brawny
1.0	0.08	3.50±0.20 abAB	Sprouted within 14 days in average, base of seedlings had few calli, lateral buds were brawny
1.0	0.2	2.53±0.25 defgDE	Sprouted within 8 days in average, base of seedlings had very few calli, lateral buds were brawny
1.0	0.5	2.90±0.17 cdeCD	Sprouted within 9 days in average, base of seedlings had few calli, lateral buds were brawny
1.0	0.8	3.87±0.06 aA	Sprouted within 5 days in average, base of seedlings had few calli, lateral buds were brawny
1.0	1.1	2.93±0.32 cdCD	Sprouted within 4 days in average, base of seedlings had a great many calli, lateral buds were brawny

平均增殖系数数据为平均值±标准差。同列不同小写字母表示差异显著(P<0.05)，同列不同大写字母表示差异极显著(P<0.01)。

Average proliferation coefficient indicated means±SE. Different lowercase letters in the same column indicate significant differences at P<0.05, different uppercase letters in the same column indicate significant differences at P<0.01.

芽的生根率随着生长素浓度的升高而增加，即生长素的添加对白檀组培苗生根影响显著；当生长素浓度超过0.5 $mg·L^{-1}$时，生根率呈下降趋势(表4)。生长素浓度为0.5 $mg·L^{-1}$时，组培苗较早出根尖，主根数量为

表4 不同浓度生长素对白檀组培苗生根的影响

Table 4 Effects of plant growth hormone concentration on rooting of *Symplocos paniculata* plantlets

IBA ($mg·L^{-1}$)	NAA ($mg·L^{-1}$)	Average rooting rate (%)	State of roots growth
0	0	2.86±0.60 hH	Rooted within 50 days in average, the number of taproots were 1–2, root length reached 1–2 cm
0.02	0.02	10.01±0.10 gG	Rooted within 41 days in average, the number of taproots were 1–2, root length reached 1–4 cm
0.05	0.05	25.13±0.23 fF	Rooted within 35 days in average, the number of taproots were 1–2, root length reached 1–3 cm
0.08	0.08	33.17±0.15 eE	Rooted within 25 days in average, the number of taproots were 3–4, root length reached 1.5–3 cm
0.2	0.2	88.00±1.73 dD	Rooted within 21 days in average, the number of taproots were 3–4, root length reached 1.5–3 cm
0.5	0.5	93.00±0.00 aA	Rooted within 21 days in average, the number of taproots were 6–7, root length reached 2.5–3 cm
0.8	0.8	92.03±0.06 bB	Rooted within 38 days in average, the number of taproots were 5–6, root length reached 1–2 cm
1.5	1.5	90.17±0.15 cC	Rooted within 60 days in average, the number of taproots were 3–4, root length reached 1–2 cm

平均生根率数据为平均值±标准差。同列不同小写字母表示差异显著(P<0.05), 同列不同大写字母表示差异极显著(P<0.01)。

Average rooting rate indicated means±SE. Different lowercase letters in the same column indicate significant differences at P<0.05, different uppercase letters in the same column indicate significant differences at P<0.01.

表5 不同栽培基质对白檀组培苗移栽成活率的影响

Table 5 Effects of cultivation medium on transplanting of *Symplocos paniculata* plantlets

Cultivation medium	Transplant number (individual plants)	Surviving number (individual plants)	Survival rate (%)
Garden soil:sand=1:1 (v/v)	30	23	77
Garden soil:peat soil=1:1 (v/v)	30	25	83
Peat soil:sand=1:1 (v/v)	30	21	70

6–7根, 根较长(图1C)。因此WPM+0.5 $mg·L^{-1}$ IBA+0.5 $mg·L^{-1}$ NAA+20 $g·L^{-1}$蔗糖+2 $g·L^{-1}$ AC+8 $g·L^{-1}$琼脂为最适生根培养基。

3.5 不同栽培基质对白檀组培苗移栽成活率的影响

经过炼苗, 白檀幼苗的茎变粗壮, 叶片面积变大, 叶色更加浓绿、有光泽, 根系更加发达。移栽到3种基质30天后, 均长出新的叶片和根系(图1D)。从表5可以看出, 白檀组培苗在园土:草炭土=1:1 (v/v)的基质中成活率最高(83%), 其次是园土:细沙=1:1 (v/v) (77%), 在草炭土:细沙=1:1 (v/v)中成活率最低(70%)。故园土:草炭土=1:1 (v/v)为白檀组培苗的最适移栽基质。

3.6 讨论

外植体木质化程度对植物启动培养产生影响。随着外植体木质化程度的增加, 其组培过程中的污染率不断上升, 而死亡率和芽诱导率却不断降低(周婧等, 2011)。但水培催出的芽比较幼嫩, 能否成苗易受杀菌时间的影响(孙清荣等, 2015)。本研究采用白檀休眠枝条水培催芽生长的嫩茎段作外植体, 可以有效控制污染率。灭菌时间控制在3分钟, 可使茎段受0.1%升汞溶液伤害较小, 从而不影响其正常生长发育。

培养基的基本成分和激素是影响植物组织培养的重要因素(张存旭等, 2005)。本研究中启动培养平均出芽率由高到低依次为1/2MS>1/4MS>1/8MS>Read>1/2mMS>WPM>mMS>MS, 可见低盐培养基

(1/2MS、1/4MS、1/8MS、Read、1/2mMS和WPM)相对于高盐培养基(mMS和MS)更适用于白檀嫩茎的启动培养。培养基中加入细胞分裂素和生长素对组培苗侧芽萌发有促进作用，可使增殖周期缩短，从而提高繁殖效率(彭少兵, 2012)。本研究表明，1.0 mg·L^{-1} 6-BA与0.02 mg·L^{-1} IBA激素组合最适合白檀增殖。

诱导生根是组培过程中难度较大且比较重要的环节之一，生根率及不定根形成的好坏直接影响繁殖系数的大小和组培苗移栽后的成活率(何承忠等, 2009)。在植物组织培养过程中，常采用添加一定浓度的生长素促进生根。但因植物种类不同，对生长素的种类及浓度要求也不尽相同(孙姿, 2015)。Yang等(2016)研究表明，不用添加激素也可诱导芽生根(1/2改良MS+1.5%蔗糖)。本研究结果表明，空白WPM培养基可诱导根的形成，但生根率低，添加0.5 mg·L^{-1} IBA、0.5 mg·L^{-1} NAA和2 g·L^{-1}活性炭对白檀组培苗生根有积极影响，但激素浓度过高会导致苗基部愈伤组织增多，抑制组培苗生长及生根。培养基中的活性炭具有无选择性的吸附作用，高浓度的活性炭必然会吸附培养基内的其它营养物质，对培养基产生副作用(孙姿, 2015)。因此，今后可进一步探讨活性炭浓度对白檀组培苗生根的影响。

在适宜的环境条件下，组培苗移栽基质的透气性和含水量对其移栽成活率影响较大，且对诱发新根和移栽成活率发挥关键作用(尹淑莲等, 2005)。本研究表明，含有细沙的基质相对通气透水，但保肥力差，易干旱且移栽成活率低；园土:草炭土=1:1 (v/v)配方中的园土物理性状结构疏松，透气、保肥、保水且排水效果好，草炭土质地松软，持水能力强，有机质含量高，二者结合使用移栽效果最佳。这与张洁茹(2014)对萱草(*Hemerocallis* spp.)离体快繁最适栽培基质研究及王玉娇等(2014)在兴安白头翁(*Pulsatilla dahurica*)组培苗移栽研究中得到的结果一致。

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Rapid Propagation of *Symplocos paniculata In Vitro*

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Abstract With tender stem segments of *Symplocos paniculata* as the experiment material, the tissue culture system of *S. paniculata* was preliminarily established to research the key factors influencing initiation culture, proliferation, rooting culture and transplantation. Treatment with 0.1% $HgCl_2$ for 3 min was the optimum scheme for explant sterilization of *S. paniculata*; survival rate of sterile seedlings was 81%. The rate of germination of initiation culture was 86.83% in the medium with 1/2MS+30 g·L^{-1} sucrose+8 g·L^{-1} agar. The proliferation coefficient was 3.57 in optimum culture medium with 1/2MS+1.0 mg·L^{-1} 6-BA+0.02 mg·L^{-1} IBA+30 g·L^{-1} sucrose+8 g·L^{-1} agar. The suitable rooting medium was WPM+0.5 mg·L^{-1} IBA+0.5 mg·L^{-1} NAA+20 g·L^{-1} sucrose+2 g·L^{-1} AC+8 g·L^{-1} agar, and rooting rate reached 93%. After being acclimatized, the regenerated plants were transplanted into substrate of garden soil and peat soil (v:v=1:1), with survival rate of regenerated plants of 83%.

Key words *Symplocos paniculata*, explant, tissue culture

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