

· 技术方法 ·

黑莓APF-190T茎段离体再生体系的建立

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摘要 为建立黑莓(*Rubus fruticosus*) APF-190T体外再生体系, 以其带芽茎段为外植体, 探讨了不同消毒处理、基本培养基类型、植物生长调节剂种类及浓度对初代培养、增殖和生根的影响, 进一步分析不同基质对组培苗生长的影响。结果表明, 用75%乙醇消毒30秒配合2%次氯酸钠消毒7分钟为黑莓茎段外植体最佳消毒方案; 初代培养最适培养基为MS+1.0 mg·L⁻¹ 6-BA+0.2 mg·L⁻¹ NAA, 芽诱导率达100%; 最佳增殖培养基为MS+0.8 mg·L⁻¹ 6-BA+0.1 mg·L⁻¹ NAA; 最佳生根培养基为1/2MS+1.0 mg·L⁻¹ NAA; 最佳移栽基质为草炭土:蛭石=2:1 (v/v)。该研究建立了稳定的黑莓APF-190T再生体系, 为规模化生产黑莓优质脱毒苗及种质资源创新奠定了基础。

关键词 黑莓, 外植体, 组织培养, 增殖培养

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黑莓(*Rubus fruticosus*)隶属蔷薇科悬钩子属, 其植株多为直立、半直立或匍匐灌木, 果实可鲜食, 亦可加工成果酱、糖浆、果酒、果茶、果汁、浓缩汁及果泥等(薛陈心, 2013; 刘晓微, 2018)。黑莓果实富含纤维素、维生素及酚类代谢物, 具有保健功效(Xu et al., 2023)。APF-190T是阿肯色大学通过人工授粉杂交选育的黑莓品种, 该品种植株生长茂盛, 茎直立无刺, 早熟, 果实较大, 果肉酸甜适口, 风味浓郁, 且耐储存(Clark, 2017)。中国是亚洲黑莓的主产国, 自1987年引入后, 产业规模不断扩大, 仅2010–2020年, 黑莓栽培面积就从1 550 hm²扩大到6 700 hm²(李维林等, 2012; 杨媛, 2024)。在黑莓种植面积持续增加的同时, 良种苗木需求量也呈现上升趋势(赵雨佳和郜影卓, 2022)。黑莓苗木繁育方法主要有种子、压条、扦插及根蘖繁育(Bray et al., 2003; Thompson et al., 2004; 王丽玲, 2004; Dönmez et al., 2024)。压条和根蘖繁育存在出苗不整齐、易致病及操作不方便等问题(刘晓微, 2018)。种子繁育则周期较长, 发芽率偏低, 且会产生杂合后代, 导致原有的优良性状丢

失(Aguilera-Arango et al., 2019)。扦插苗木管护要求高, 费工费时, 易传播内源性病害, 影响黑莓的产量和品质(Debner et al., 2019; 王丽金和苏上, 2023)。利用组培快繁技术对母本的优良性状进行保存、提纯和复壮, 可突破季节限制, 显著提高增殖系数, 加快良种苗木繁育进程(Gichaba, 2019; Roşca et al., 2022)。目前, 已对黑莓品种Triple Crown、Arapaho和Thornfree的组培快繁体系开展研究, 但现有的黑莓组织培养技术尚不成熟, 繁殖系数较低, 不能满足生产需求(Donnelly et al., 1986; 李海燕等, 2011; 薛陈心, 2013; 刘晓微, 2018)。本研究在前人研究的基础上, 对APF-190T黑莓无菌苗培养及增殖和生根进行研究, 构建了快繁技术体系, 旨在为其工厂化育苗提供技术支持, 并为黑莓的遗传转化和基因编辑等搭建技术平台, 为种质资源创新奠定基础。

1 植物材料

2022年8月, 从青州市彭氏农产品专业合作社的

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(118°33'2"E, 36°41'45"N)温室大棚剪取黑莓(*Rubus fruticosus* L.) APF-190T一年生新枝, 作为外植体材料。

2 培养基成分与培养条件

2.1 取样及处理

选择连续3天以上的晴天, 取黑莓生长健壮且无病虫害的一年生新枝。将枝条剪成长5–8 cm的小段, 去掉叶片, 用洗洁精稀释溶液浸泡清洗, 自来水冲洗30分钟。将枝条剪成1.5–2.0 cm的单腋芽茎段, 置于超净工作台上, 用75%乙醇表面杀菌30秒, 无菌水冲洗3次, 然后用2% NaClO溶液分别振荡浸泡4、7和11分钟, 无菌水冲洗5次, 最后用无菌纸吸干水分。将消毒后的单腋芽茎段切成长1 cm的小块, 接种至MS培养基中。统计芽污染率、褐化率和存活率。

2.2 初代培养

2.2.1 基本培养基筛选

以MS、1/2MS和WPM为基本培养基, 添加30 g·L⁻¹蔗糖和7 g·L⁻¹琼脂, 调pH值至5.8–6.0, 高压灭菌后备用。将消毒后的腋芽茎段接种到不同基本培养基上。每处理接种30个外植体, 重复3次。培养30天后观察茎段生长状况, 统计芽诱导率和单株茎长。

2.2.2 植物生长调节剂优化

在MS基本培养基中添加不同浓度的6-BA (0.5、1.0和2.0 mg·L⁻¹)和NAA (0.1、0.2和0.3 mg·L⁻¹), 进行植物生长调节剂筛选。每处理接种30个外植体, 重复3次。培养30天后观察茎段生长状况, 统计芽诱导率。

2.3 增殖培养

挑选生长健壮的初代组培苗, 接种在添加不同浓度6-BA (0.5、0.8和1.0 mg·L⁻¹)和NAA (0.05、0.10和0.15 mg·L⁻¹)的MS培养基上进行增殖培养。每处理接种20个外植体, 重复3次。培养30天后观察茎段生长状况, 统计不定芽增殖系数。

2.4 生根培养

以茎长≥1.0 cm的健壮单芽为材料, 将增殖的芽切分后转接至1/2MS培养基中, 添加不同浓度的植物生长调节剂NAA (0.5、1.0和1.5 mg·L⁻¹)。每处理接种30

个外植体, 重复3次。培养30天后观察茎段生根情况, 统计生根率。

2.5 炼苗移栽

将生根良好的组培苗在培养室中敞口炼苗3天, 取出组培苗, 洗净根部培养基, 移栽至不同基质中, 各基质的比例为草炭土:蛭石=1:1; 2:1; 3:1 (v/v)。移栽之后前2周保持空气相对湿度在90%以上, 温度为(25±2)°C, 适当遮荫。2周后逐渐降低湿度, 增加光照。移栽4周后统计成活率。

2.6 数据处理

实验数据用Excel 2020软件处理。采用SPSS 20.0软件进行数据分析。利用Duncan法检验差异显著性 ($P \leq 0.05$)。利用以下公式进行计算。

污染率=(污染外植体数/接种外植体总数)×100%;

褐化率=(褐化外植体数/接种外植体总数)×100%;

存活率=(存活外植体数/接种外植体总数)×100%;

芽诱导率=(萌发腋芽的外植体数/接种外植体总数)×100%;

增殖系数=总增殖芽数/总接种芽数;

生根率=(生根芽数/总接种芽数)×100%。

3 结果与讨论

3.1 不同消毒时间对外植体消毒效果的影响

外植体用2% NaClO溶液消毒。不同消毒时间对比实验表明, 消毒7分钟, 外植体污染率最低(为20.00%), 褐化率相对较低(为6.67%), 存活率最高(为73.33%) (图1B)。若消毒时间过短, 污染率显著升高, 消毒4分钟, 外植体污染率最高(为90.00%) (图1A); 若消毒时间过长, 污染率降低, 褐化现象加剧, 消毒11分钟, 外植体污染率为33.33%, 但褐化率最高(达28.89%), 外植体活力明显下降, 严重影响后续培养效果(图1C)。存活的外植体培养15天, 可见明显生长, 叶片鲜绿, 后续无褐化或污染(图1D)。综合考虑外植体污染率、褐化率和存活率(表1), 确定使用75%乙醇消毒30秒配合2% NaClO溶液消毒7分钟为最佳消毒方式。

3.2 不同基本培养基对初代培养的影响

不同基本培养基对黑莓初代培养的影响不同(表2)。在MS培养基中,芽诱导率最高(为63.33%),茎段生长态势最好,萌发芽数较多且芽长最长,平均芽长达1.41 cm。WPM培养基上的茎段长势次之,芽诱导率

(为52.22%)比MS培养基低,平均芽长为1.13 cm。在1/2MS培养基上,芽诱导率最低(为30.00%),茎段生长迟缓,叶片发黄,平均芽长仅有0.86 cm。综合各项指标,确定MS培养基是黑莓茎段培养的最优基本培养基。

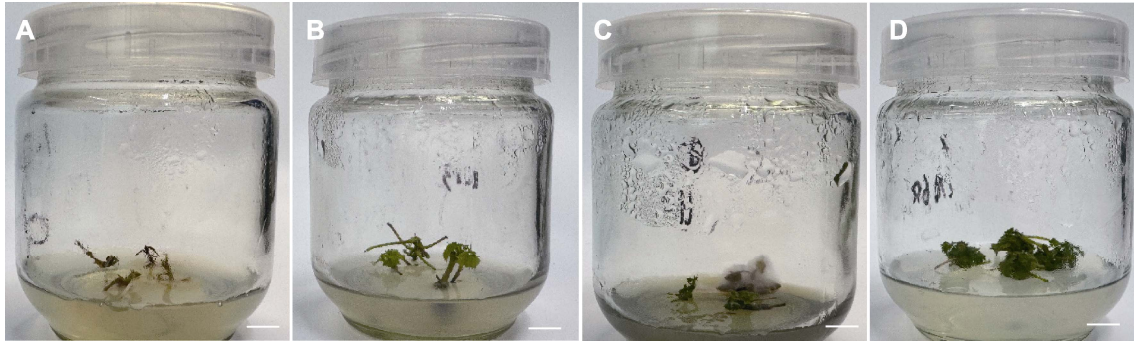


图1 黑莓外植体消毒

(A)–(C) 外植体接种5天((A) 2% NaClO消毒4分钟; (B) 2% NaClO消毒7分钟; (C) 2% NaClO消毒11分钟); (D) 外植体接种15天。Bars=1 cm

Figure 1 Disinfection of explants in blackberry

(A)–(C) Status of explants 5 days after inoculation ((A) Disinfection with 2% NaClO solution for 4 minutes; (B) Disinfection with 2% NaClO solution for 7 minutes; (C) Disinfection with 2% NaClO solution for 11 minutes); (D) Status of explants 15 days after inoculation. Bars=1 cm

表1 NaClO消毒时间对黑莓外植体污染率和褐化率的影响

Table 1 Effects of the duration of NaClO sterilization on the contamination rate and browning rate of explants in blackberry

No.	2% NaClO (min)	Number of explants	Contamination rate (%)	Browning rate (%)	Survival rate (%)
1	4	90	90.00±0.10 a	3.33±0.06 c	6.67±0.05 c
2	7	90	20.00±0.10 c	6.67±0.16 b	73.33±0.29 a
3	11	90	33.33±0.12 b	28.89±0.02 a	37.78±0.08 b

数值为3次重复的平均值±标准误; 同列不同小写字母表示用邓肯检验法差异显著($P \leq 0.05$)。

The values are means±SE from 3 replicates; different lowercase letters in the same column indicate significant differences based on Duncan's test ($P \leq 0.05$).

表2 不同基本培养基对黑莓初代培养的影响

Table 2 Effects of different basic media on the primary culture of blackberry

Basic media	Number of explants	Induction rate (%)	Shoot length (cm)	Growth condition
WPM	90	52.22±1.61 b	1.13±0.21 ab	++
MS	90	63.33±1.05 a	1.41±0.38 a	++++
1/2MS	90	30.00±2.79 c	0.86±0.27 b	+

加号(+)数量越多表示长势越好; 反之长势越弱。数值为3次重复的平均值±标准误; 同列不同小写字母表示用邓肯检验法差异显著($P \leq 0.05$)。

The more the number of plus signs (+), the better the growth, and vice versa, the weaker the growth. The values are means±SE from 3 replicates; different lowercase letters in the same column indicate significant differences based on Duncan's test ($P \leq 0.05$).

3.3 不同植物生长调节剂对初代培养的影响

不同浓度6-BA和NAA组合对黑莓初代培养影响不同(表3; 图2A)。不同浓度6-BA和NAA组合对芽诱导率有明显影响。在9组处理中, 处理5和处理7平均芽诱导率最高(均为100%); 而处理1平均芽诱导率最低(为84.67%)。处理5生长状况最好, 组培苗生长健壮, 叶片浓绿(图2B)。综合各项指标, 确定处理5 (1.0 mg·L⁻¹ 6-BA+0.2 mg·L⁻¹ NAA)是黑莓茎段初代培养的最优培养基。

3.4 不同植物生长调节剂对增殖培养的影响

不同浓度6-BA和NAA组合下黑莓增殖培养结果表明, 处理5不定芽增殖系数最高(达12.51), 芽平均高度最高(为3.83 cm), 增殖后不定芽生长旺盛(图2C); 处理9增殖系数最低(为2.39), 不定芽细弱, 基部生成大量愈伤组织。综合分析确定最适合黑莓增殖培养的调节剂组合为0.8 mg·L⁻¹ 6-BA+0.1 mg·L⁻¹ NAA (表4)。

3.5 不同植物生长调节剂对生根培养的影响

不同植物生长调节剂组合对黑莓组培苗生根影响结果表明, 当NAA浓度为1.0 mg·L⁻¹时生根率最高

表3 不同植物生长调节剂对黑莓初代培养的影响

Table 3 Effects of different plant growth regulators on primary culture of blackberry

No.	6-BA (mg·L ⁻¹)	NAA (mg·L ⁻¹)	Induction rate (%)	Growth status
1	0.5	0.1	84.67±7.02 c	+
2	1.0	0.1	86.67±6.71 c	+
3	2.0	0.1	92.22±6.36 b	+
4	0.5	0.2	98.89±1.92 a	++
5	1.0	0.2	100±0.00 a	++++
6	2.0	0.2	96.67±3.21 ab	+++
7	0.5	0.3	100±0.00 a	++
8	1.0	0.3	93.33±1.91 b	++
9	2.0	0.3	94.33±3.22 b	+

加号(+)数量越多表示长势越好; 反之长势越弱。数值为3次重复的平均值±标准误; 同列不同小写字母表示用邓肯检验法差异显著($P \leq 0.05$)。

The more the number of plus signs (+), the better the growth, and vice versa, the weaker the growth. The values are means±SE from 3 replicates; different lowercase letters in the same column indicate significant differences based on Duncan's test ($P \leq 0.05$).

(达97.78%), 平均根数为5.11条, 根系粗壮, 侧根发达, 毛细根最多(图2D, E); NAA浓度为0.5 mg·L⁻¹时, 生根率为52.22%, 平均根数为2.44条; NAA浓度为1.5 mg·L⁻¹时, 生根率为96.67%, 根系易出现愈伤组织化, 影响组培苗移栽成活率。因此, 确定最佳生根培养基为1/2MS+1.0 mg·L⁻¹ NAA (表5)。

3.6 不同基质对移栽的影响

将生根的不定芽进行炼苗移栽, 不同移栽基质下黑莓组培苗成活率(图2F, H)统计结果显示, 当移栽基质草炭土:蛭石=2:1 (v/v)时, 组培苗成活率最高(达95.56%), 植株生长健壮, 叶片舒展, 新根持续生长(图2G, I); 草炭土:蛭石=1:1 (v/v)时, 组培苗成活率最低(为33.44%), 植株生长缓慢, 叶片发黄; 草炭土:蛭石=3:1 (v/v)时, 组培苗成活率为83.33%, 植株生长较慢, 有烂根(表6)。综合比较, 确定黑莓组培苗最佳移栽基质为草炭土:蛭石=2:1 (v/v)。

3.7 讨论

黑莓的外植体来源广泛, 包括茎尖、茎段、叶片和叶柄等(刘晓微, 2018)。带腋芽茎段比叶片和叶柄诱导周期短, 且不经愈伤组织阶段, 通过腋芽萌发现快速繁殖。研究表明, 将带腋芽茎段切成1–2 cm小段, 每个茎段带有1–2个腋芽, 在适宜的培养基上, 腋芽萌发率可达80%以上(李海燕等, 2011)。有研究表明Prime-Ark45黑莓腋芽的诱导率优于茎尖(Baghdady et al., 2021)。因此, 本研究选择黑莓APF-190T茎段作为外植体。由于外植体携带微生物, 消毒是确保后续培养成功的关键步骤, 常用的消毒剂有次氯酸钠、乙醇和升汞等(Kefayeti et al., 2019)。升汞毒性较大, 次氯酸钠具有较强的杀菌能力, 并且对植物材料的伤害相对较小。在黑莓外植体消毒中, 使用2%–10%次氯酸钠溶液配合乙醇, 灭菌效果显著(Dönmez et al., 2024)。本研究表明, 消毒过程中需注意控制消毒剂的浓度和时间。消毒剂浓度过高或时间过长均会导致外植体褐化率升高; 消毒剂浓度过低或时间过短, 则无法有效灭菌, 易造成污染。研究表明, 用75%乙醇消毒30秒配合2%次氯酸钠溶液消毒7分钟效果最佳。

初代培养常用的基本培养基有MS、WPM和

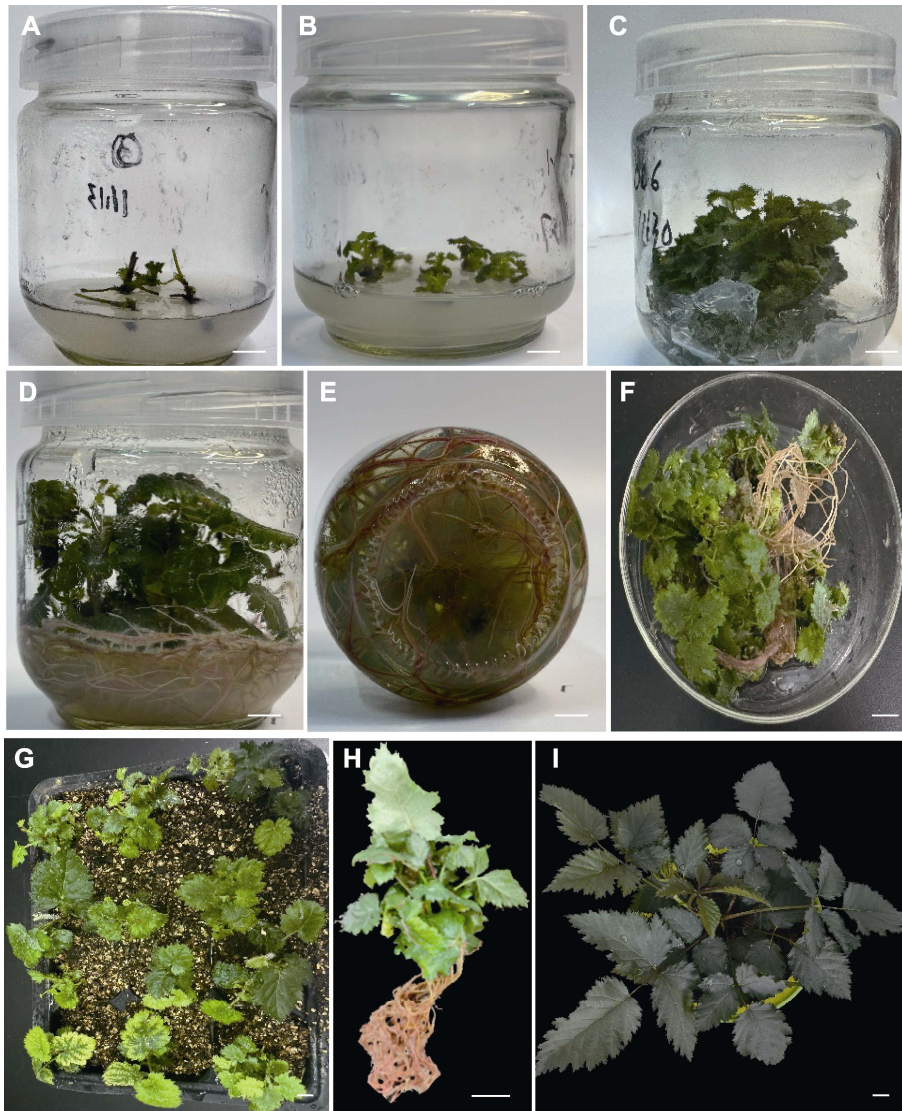


图2 黑莓组培快繁体系的建立

(A) 茎段外植体; (B) 腋芽诱导; (C) 继代苗; (D), (E) 不定根诱导; (F), (H) 再生植株; (G), (I) 炼苗。Bars=1 cm

Figure 2 Establishment of the tissue culture and rapid propagation system for blackberry

(A) Stem segment explant; (B) Axillary bud induction; (C) Subculture plantlets; (D), (E) Adventitious root induction; (F), (H) Regenerated plants; (G), (I) Acclimatization of plantlets. Bars=1 cm

1/2MS等(Fathy et al., 2018; Lapiz-Culqui et al., 2024; Clapa et al., 2024)。本研究表明,以黑莓茎段为外植体,初代培养时,MS、WPM和1/2MS培养基均可诱导出芽,MS培养基上的芽更为健壮,芽的数量也较多,这与Fathy等(2018)的研究结果一致。MS培养基中的硝酸盐浓度显著高于WPM和1/2MS培养基,硝酸盐与丰富的微量元素组合,能加速细胞周期启动,提高黑莓茎段分生组织的分化效率。

植物生长调节剂在组织培养中起关键作用。植物

组织培养离体快繁体系已在黑莓中广泛建立,但不同黑莓品种,甚至相同品种不同外植体所需植物生长调节剂的种类和浓度并不兼用(李维林等, 2009)。一方面,不同品种的基因组复杂程度不同;另一方面,相同品种不同器官再生能力有差异,影响其离体培养中细胞脱分化与再分化能力,导致对植物生长调节剂的响应有差异。6-BA是常用的细胞分裂素类物质,能够促进细胞分裂和芽分化(曾浩等, 2024)。李海燕等(2011)研究表明,在Triple Crown黑莓初代培养时仅

表4 不同植物生长调节剂对黑莓增殖培养的影响**Table 4** Effects of different plant growth regulators on proliferation culture of blackberry

No.	6-BA (mg·L ⁻¹)	NAA (mg·L ⁻¹)	Number of explants	Multiplication factor	Stem length (cm)	Growth status
1	0.5	0.05	60	4.75±0.71 d	1.08±0.36 d	+++
2	0.8	0.05	60	6.50±1.60 c	1.85±0.64 c	++++
3	1.0	0.05	60	7.38±1.51 b	1.81±0.48 c	++++
4	0.5	0.10	60	7.50±1.31 b	2.45±0.29 b	+++
5	0.8	0.10	60	12.51±2.33 a	3.83±0.45 a	+++++
6	1.0	0.10	60	7.75±1.67 b	2.78±0.33 b	+++
7	0.5	0.15	60	5.25±1.28 cd	2.85±0.34 b	++
8	0.8	0.15	60	5.13±1.24 cd	2.90±0.65 b	++
9	1.0	0.15	60	2.39±0.91 e	1.55±0.45 c	+

加号(+)数量越多表示长势越好; 反之长势越弱。数值为3次重复的平均值±标准误; 同列不同小写字母表示用邓肯检验法差异显著 ($P\leq 0.05$)。

The more the number of plus signs (+), the better the growth, and vice versa, the weaker the growth. The values are means±SE from 3 replicates; different lowercase letters in the same column indicate significant differences based on Duncan's test ($P\leq 0.05$).

表5 不同植物生长调节剂对黑莓生根培养的影响**Table 5** Effects of different plant growth regulators on rooting culture of blackberry

No.	NAA (mg·L ⁻¹)	Rooting rate (%)	Rooting number	Growth status
1	0.5	52.22±5.09 b	2.44±0.52 c	++
2	1.0	97.78±3.84 a	5.11±0.66 a	++++
3	1.5	96.67±3.33 a	3.88±1.16 b	+++

加号(+)数量越多表示长势越好; 反之长势越弱。数值为3次重复的平均值±标准误; 同列不同小写字母表示用邓肯检验法差异显著 ($P\leq 0.05$)。

The more the number of plus signs (+), the better the growth, and vice versa, the weaker the growth. The values are means±SE from 3 replicates; different lowercase letters in the same column indicate significant differences based on Duncan's test ($P\leq 0.05$).

表6 不同基质对黑莓移栽成活率的影响**Table 6** Effects of different substrates on the survival rate after transplantation of blackberry

No.	Turfy soil:vermiculite (v/v)	Survival rate (%)	Growth status
1	1:1	33.44±8.31 c	++
2	2:1	95.56±3.84 a	++++
3	3:1	83.33±3.33 b	+++

加号(+)数量越多表示长势越好; 反之长势越弱。数值为3次重复的平均值±标准误; 同列不同小写字母表示用邓肯检验法差异显著 ($P\leq 0.05$)。

The more the number of plus signs (+), the better the growth, and vice versa, the weaker the growth. The values are means±SE from 3 replicates; different lowercase letters in the same column indicate significant differences based on Duncan's test ($P\leq 0.05$).

添加0.5 mg·L⁻¹ 6-BA即可满足外植体萌发和生长需要。NAA属于生长素类物质, 可促进细胞伸长和生根。Baghdady等(2021)研究表明, 在MS培养基中添加1 mg·L⁻¹ 6-BA和0.1 mg·L⁻¹ NAA对Prime-Ark45黑莓茎段芽诱导效果最佳。增殖培养是在初代培养的基础上, 将诱导出的芽或愈伤组织进行多次转接培养, 以实现快速繁殖。蒋小满等(2007)发现MS+1.0 mg·L⁻¹ 6-BA+0.05–0.2 mg·L⁻¹ NAA适合黑莓Karvar试管苗的增殖, 增殖系数为8。Kefayeti等(2019)对Chester thornless黑莓进行了增殖培养, 结果表明, 在含有2 mg·L⁻¹ 6-BA和0.2 mg·L⁻¹ IBA的培养基上增殖率最高, 平均出芽9.66个, 添加GA₃会降低黑莓增殖率。Gichaba (2019)研究发现, 在增殖培养中适当降低培养基中6-BA浓度可避免组培苗过度生长。本研究表

明,在APF-190T增殖培养中,6-BA浓度需要降低至 $0.8\text{ mg}\cdot\text{L}^{-1}$ 以避免愈伤化,MS+ $0.8\text{ mg}\cdot\text{L}^{-1}$ 6-BA+ $0.1\text{ mg}\cdot\text{L}^{-1}$ NAA适合黑莓APF-190T增殖,增殖系数可达12.51,芽长势好且健壮。我们推测激素敏感性在不同品种基因型之间有差异,导致不同品种黑莓所需植物生长调节剂的种类和浓度不同,需通过转录组分析进一步验证。

生根培养是黑莓组培快繁的关键环节,良好的根系是移栽成活的前提。王鲁北等(2012)发现在含 $0.2\text{ mg}\cdot\text{L}^{-1}$ IBA的MS培养基中,不定芽生根率达100%,且根数多、长势好。NAA处理同样获得较好的生根效果,有研究表明在黑莓生根培养中添加 $0.5\text{ mg}\cdot\text{L}^{-1}$ NAA,生根率达100% (李海燕等, 2011)。Kefayeti等(2019)用不同浓度的IBA和NAA对Chester thornless黑莓进行生根培养,结果显示 $0.4\text{ mg}\cdot\text{L}^{-1}$ NAA处理产生的根数最多且根长最长。在培养基中添加 $2\text{ g}\cdot\text{L}^{-1}$ 活性炭可提高黑莓的生根率(Fathy et al., 2018)。不同品种黑莓所需生根条件不完全相同,在黑莓组培快繁实践中,需根据具体条件对生长素类物质进行精准优化与合理调整,以实现最佳生根诱导效果,确保组培快繁技术的高效性与稳定性。

炼苗与移栽是将组培苗从实验室环境过渡到自然环境的重要步骤,直接关系到组培苗的成活和后续生长。李海燕等(2011)研究表明, Triple Crown黑莓在1/2MS培养基中生根率高达100%,但生根苗在泥炭土:珍珠岩:沙=4:3:2 (v/v/v)的移栽基质中生长,成活率仅为73.33%。将生根后的黑莓植株转移至含灭菌泥炭:珍珠岩=1:1 (v/v)的塑料容器中,存活率为98% (Kefayeti et al., 2019)。移栽到蛭石:泥炭土:珍珠岩:园土=1:1:1:2 (v/v/v/v)的混合基质中,黑莓组培苗的成活率可达97%以上(王小敏等, 2014)。Topçu (2022)使用珍珠岩:泥炭=3:1 (v/v)的混合基质,黑莓组培苗的移栽成活率达100%。基质配比需在保水性与透气性之间取得平衡。本研究表明,草炭土:蛭石=2:1 (v/v)时组培苗的成活率最高,达95.56%,此基质配比可减少根系胁迫,为后续规模化移栽提供参考。

本研究以APF-190T黑莓带腋芽茎段为外植体,建立并且优化了组织培养体系,确定了黑莓组培快繁最适培养基成分,增殖系数达12.51,移栽成活率为95.56%,为商业化育苗提供了技术支撑。未来将聚焦黑莓遗传改良与次生代谢产物合成的组培体系优化,

重点基于CRISPR基因编辑技术靶向筛选抗褐化相关基因,解析其调控通路及分子互作网络,为实现黑莓品质性状的精准改良提供科学依据与技术保障,助力黑莓种质创新与产业化发展。

作者贡献声明

冯帅帅:完成实验与分析数据,撰写论文初稿;乔峰:参与实验并分析数据;李爱花,何璇,姜婷婷,韩葳央宗,李全希,黄爱玲,刘进:参与实验设计,提供技术支持;谭德云:构思并设计实验,指导数据分析、论文写作与修改。

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Establishment of an *In Vitro* Regeneration System for Stem Segments of Blackberry APF-190T

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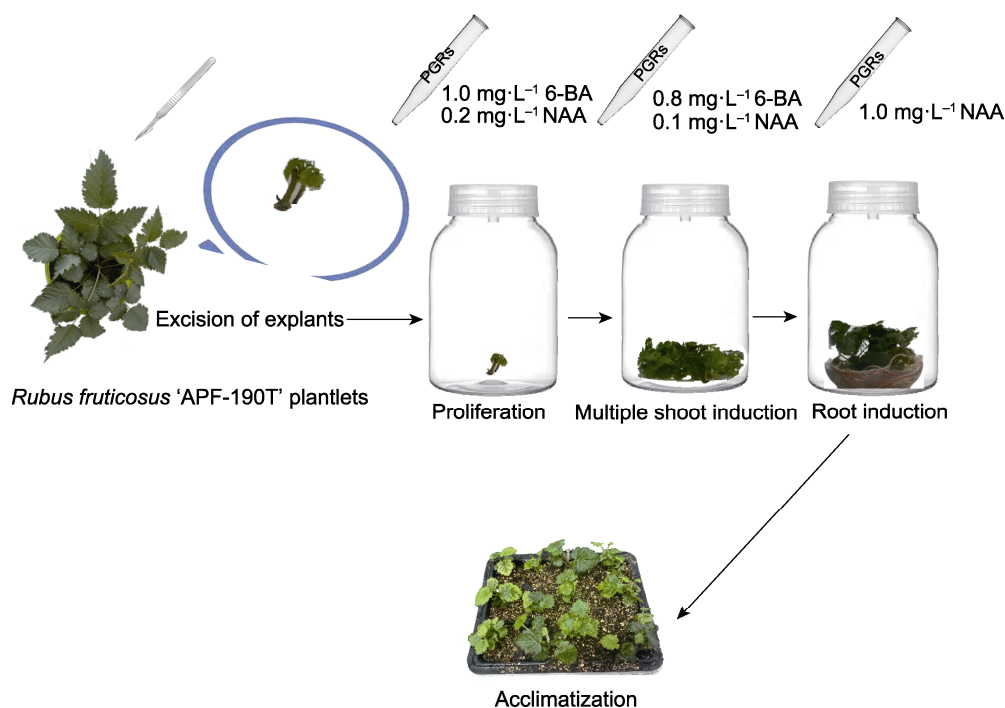
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INTRODUCTION: Blackberry (*Rubus fruticosus*) is an important economic crop whose fruits are not only rich in fiber, vitamins, and phenolic metabolites, but also have significant health benefits. Traditional blackberry propagation methods, including seed propagation, layering, cutting, and suckering, have various limitations. For example, seed propagation has a long cycle, a low germination rate, and variable offspring traits. In contrast, tissue culture-based rapid propagation technology offers distinct advantages, such as season-independent operation, a high multiplication coefficient, and preservation of superior maternal traits, making it an effective solution for blackberry plantlet propagation. Therefore, it is necessary to establish an efficient blackberry rapid propagation system, which would lay the foundation for large-scale production of high-quality virus-free plantlets and germplasm innovation.

RATIONALE: The establishment of an *in vitro* regeneration system for blackberry stem segments is based on the theory of plant cell totipotency and hormonal regulatory mechanisms, under which the meristematic cells of stem segments can regenerate into complete plants under suitable culture conditions. To establish a stable regeneration system for blackberry APF-190T, we investigated the effects of sterilization conditions, medium types, and the types and concentrations of plant growth regulators on primary culture, proliferation culture, and rooting culture. Additionally, the influence of different substrates on the growth of tissue-cultured plantlets was further analyzed.

RESULTS: Comparative experiments on disinfection durations revealed that a 7-minute treatment yielded optimal results for blackberry explants, with the lowest contamination rate of 20.00%, a relatively low browning rate of 6.67%, and the highest survival rate of 73.33%. In MS media, these conditions produced the best stem segment growth, with a maximum bud induction rate of 63.33%, characterized by abundant germinated buds reaching an average length of 1.41 cm. For primary culture, the combination of 1.0 mg·L⁻¹ 6-BA+0.2 mg·L⁻¹ NAA was the most effective, achieving a 100% bud induction rate and producing robust seedlings with dark green leaves. Subsequent proliferation culture with a combination of 0.8 mg·L⁻¹ 6-BA and 0.1 mg·L⁻¹ NAA resulted in the highest adventitious bud proliferation coefficient of 12.51 and the tallest average bud height of 3.83 cm, along with vigorous growth. The rooting efficiency peaked in 1/2MS medium supplemented with 1.0 mg·L⁻¹ NAA, attaining a 97.78% rooting rate with an average of 5.11 thick roots per plant, featuring well-developed lateral roots and abundant fine roots. Finally, transplantation success was maximized when a 2:1(v/v) peat-vermiculite substrate was used, resulting in a 95.56% survival rate with robust plant growth, expanded leaves, and continuous root development.

CONCLUSION: For *in vitro* propagation of blackberry stem explants, the optimal sterilization protocol was achieved using 75% ethanol for 30 s followed by 2% sodium hypochlorite for 7 min. For primary culture, the most suitable medium was MS+1.0 mg·L⁻¹ 6-BA+0.2 mg·L⁻¹ NAA. For shoot proliferation, the best results were obtained with MS+0.8 mg·L⁻¹ 6-BA+0.1 mg·L⁻¹ NAA, promoting optimal shoot multiplication. During the rooting stage, the highest rooting efficiency was observed in the 1/2MS+1.0 mg·L⁻¹ NAA group. For *ex vitro* acclimatization, a peat mixture proved most effective for seedling survival and growth under controlled greenhouse conditions, and this technique can potentially be applied for commercialization of the plant.



Establishment of an *in vitro* regeneration system for stem segments of blackberry APF-190T. A series of studies was carried out on sterile seedling establishment, multiplication culture, rooting culture and other aspects of the blackberry variety APF-190T.

Key words blackberry, explant, tissue culture, proliferation culture

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