

褪黑素在霞多丽葡萄种子体细胞胚诱导发生中的作用

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摘要:【目的】探究褪黑素在酿酒葡萄种子体细胞胚发生及循环诱导中的作用, 建立以种子为外植体的体细胞胚诱导体系, 为缩短葡萄属植物体胚诱导周期提供依据。【方法】以花后 60、70、80 d 的欧洲酿酒葡萄品种霞多丽、马瑟兰与美乐种子为外植体, 研究不同切种和放置方法下, 5 种不同质量浓度 (0.2、0.4、0.6、0.8、1.0 mg·L⁻¹) 褪黑素对其初生子叶胚萌发及二次胚循环诱导的作用。【结果】花后 80 d 种子诱导率为 18.33%, 明显高于 70 d 和 60 d, 采用纵切法正放接种外植体 30 d 时, 初生子叶胚萌发率达到最高, 为 33.07%, 纵切倒放、横切正放和横切倒放的萌发率依次降低, 为 10.98%、5.71% 和 3.24%。褪黑素诱导 3 个酿酒葡萄品种萌发初生子叶胚的作用均强于 2,4-D, 其中, 霞多丽种子在 MS + 0.6 mg·L⁻¹ 褪黑素 + 2.0 mg·L⁻¹ 6-BA + 30 g·L⁻¹ 蔗糖 + 3 g·L⁻¹ 植物凝胶 + 0.5 g·L⁻¹ 活性炭的培养基诱导 30 d 时, 其初生子叶胚诱导率最高, 为 18.33%。利用霞多丽初生子叶胚切段循环诱导二次体细胞胚 60 d 时, 开始出现二次体细胞胚, 进一步诱导形成球形胚、心形胚、鱼雷胚和子叶胚。诱导至 90 d 时, 在添加 0.6 mg·L⁻¹ 褪黑素的培养基上诱导率最高, 为 14.40%, 0.4 mg·L⁻¹ 次之, 为 3.13%, 0.2 mg·L⁻¹ 诱导率为 1.89%, 而 0.8、1.0 mg·L⁻¹ 褪黑素培养基和 2,4-D 诱导下无次生胚的形成。【结论】种子成熟度与初生子叶胚的萌发率呈正相关; 种子纵切正放接种有利于初生子叶胚萌发; 低质量浓度褪黑素对霞多丽种子萌发初生子叶胚及二次胚发生具有缩短诱导时间的作用。

关键词: 酿酒葡萄; 褪黑素; 种子; 接种方式; 体细胞胚胎发生

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Role of melatonin in the induction of somatic embryogenesis from seeds of *Vitis vinifera* 'Chardonnay'

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Abstract:【Objective】The role of melatonin in the induction of somatic embryogenesis and induced circulation establishment from wine grape seeds was investigated with the aim to establish a somatic embryo induction system based on seed induction, and to provide a theoretical basis for shortening the cycle of grapevine somatic embryo induction.【Methods】Seeds from European wine grape varieties Chardonnay, Marselan and Merlot were used as explants, which were collected at 60 d, 70 d and 80 d after flowering, respectively. The seeds were sterilized with 70% ethanol and 1.5% NaClO, and then cut and placed in four ways, namely, transversely cut and flatwise, transversely cut and anti-put, longitudinally cut and flatwise, and longitudinally cut and anti-put, to investigate the effect of different cutting and placing patterns on the germination of primary cotyledon embryos. In addition, five different concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg·L⁻¹) of melatonin were explored on their primary cotyledon embryos ger-

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mination, and the germinated primary cotyledon embryos were further cut to 0.5 cm sections to investigate the cyclic induction of their secondary somatic embryos by five different concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg · L⁻¹) of melatonin. **【Results】**Seeds of the three wine grape varieties and different seed maturity levels differed significantly in the induction rate of primary cotyledon embryos on the medium supplemented with different concentrations of melatonin. Among them, the cotyledon embryo germination rates were all proportional to seed maturity on MC, MEL0, MEL1, MEL2, MEL3, MEL4 and MEL5 medium, with induction rates ranging from 0.9 to 16.56% at 60 d after flowering, 1.34% to 17.3% at 70 d, and up to 18.33% at 80 d. Among the three test varieties, Chardonnay had the highest germination rate of 18.33% at 80 d after flowering induced by 0.6 mg · L⁻¹ melatonin, while the control 2,4-D induced germination with the worst effect of 2.98%. The difference in germination rate between varieties may be related to genotype. The seeds of Chardonnay were inoculated into the medium in different ways, and the seed coat started to discolor at 3 d. The germination of cotyledon embryos started at 10 d. The germination rate of primary cotyledon embryos reached the highest rate of 33.07% at 30 d when the explants were inoculated by the longitudinally cut and flatwise, and the germination rates decreased in longitudinally cut and anti-put, transversely cut and flatwise, transversely cut and anti-put, with 10.98%, 5.71%, and 3.24%. The results showed that longitudinal cut and flatwise was the most suitable inoculation method, which helped to improve the germination rate of primary cotyledon embryos. Melatonin-induced germination of primary cotyledon embryos was stronger than 2,4-D in all three wine grape varieties, among which seeds from Chardonnay could occur the highest primary cotyledon embryos induction rate of 18.33% in MS + 0.6 mg · L⁻¹ melatonin + 2.0 mg · L⁻¹ 6-BA + 30 g · L⁻¹ sucrose + 3 g · L⁻¹ phytoigel + 0.5 g · L⁻¹ activated charcoal for 30 d. The secondary somatic embryos were induced by using Chardonnay primary cotyledon embryo cuttings in a cyclic manner, and callus tissues were formed in different media at 14 d. At 60 d, secondary somatic embryos began to appear, and further induction resulted in the formation of spherical embryos, heart-shaped embryos, torpedo embryos and cotyledon embryos. The highest induction rate of 14.4% was induced at 90 d with MS + 0.6 mg · L⁻¹ melatonin + 2.0 mg · L⁻¹ 6-BA + 30 g · L⁻¹ sucrose + 3 g · L⁻¹ phytoigel + 0.5 g · L⁻¹ activated carbon, followed by 0.4 mg · L⁻¹ melatonin with the induction rate of 3.13%, 0.2 mg · L⁻¹ occurred the induction rate of 1.89%, while no secondary embryos were formed either on medium supplemented with 0.8, and 1.0 mg · L⁻¹ melatonin or on medium supplemented with 2,4-D. The plant materials induced by 2,4-D gradually browned and died during the succession process. The induction of Chardonnay seeds with melatonin resulted in the formation of primary cotyledon embryos, and after cyclic induction, primary embryo clusters were produced and secondary embryos were formed, and seedlings could be established after transplanting. **【Conclusion】**The role of melatonin in the induction of grapevine somatic embryos was investigated with wine grape seeds as explant material. The results showed seed maturity was positively correlated with the germination rate of primary cotyledon embryos. The longitudinally cut and flatwise was beneficial to the germination of primary cotyledon embryos. Low concentration of melatonin had the effect of shortening the induction time for germination of primary cotyledon embryos and secondary embryogenesis in Chardonnay seeds. The primary cotyledon embryos from Chardonnay could induce the production of primary embryo clusters and somatic embryos, which could help to increase the numbers of embryo materials and provide experimental materials for grapevine genetic transformation.

Key words: Wine grape; Melatonin; Seed; inoculated pattern; Somatic embryogenesis

葡萄(*Vitis L.*)为葡萄科葡萄属多年生木质藤本,是世界上最古老的果树类经济作物之一,因其经济效益高而被广泛种植^[1-2]。然而,由于葡萄遗传背景相对复杂、基因组高度杂合,新品种的培育受到季节、地域等因素的限制,致使传统育种方式年限较长^[3]。基于高效遗传转化体系的基因工程改良可对葡萄进行精准育种^[4-5],且能够缩短育种周期。目前,葡萄遗传转化体系的建立可通过器官再生与体细胞再生途径实现。由于器官发生途径再生效率较低且易形成嵌合体,而体细胞胚再生途径具有遗传物质相对稳定、繁殖快速高效等特点^[5-7],因此成为建立葡萄属植物遗传转化体系的首选途径。

葡萄体细胞胚经脱分化诱导形成胚性愈伤组织,继而再分化为原胚团,再经诱导发育成胚^[8]。处于旺盛分裂期的组织或细胞有助于外源基因的整合^[9],从而成为植物遗传转化的最佳受体材料。Dhekney等^[5]对圆叶葡萄胚胎培养物转化和植株再生的研究表明,体细胞胚具有较好的转化和再生能力。建立高效的葡萄体细胞胚再生体系受众多因素的影响,包括基因型的差异、外植体类型、培养基的组分以及培养环境等^[10-12],从而在较大程度上限制了葡萄生物技术育种的应用。据报道,多数葡萄品种仍无法通过诱导获得体细胞胚^[13]。在欧亚种葡萄品种体细胞胚诱导中,研究者们^[12, 14-17]先后以雌蕊、雄蕊、子房以及花蕾等为外植体诱导体细胞胚发生,但往往存在接种工作量大、操作复杂、成熟期难确定等问题。同时,葡萄花期短,不易于长期保存,受季节影响较大。研究表明,外源植物生长调节剂在胚性愈伤组织和体细胞胚诱导发生中发挥重要调控作用。在无核白和火焰无核葡萄体细胞诱导中发现,2,4-D有利于胚性愈伤组织的诱导发生^[8, 17]。也有研究表明,长期使用2,4-D会导致愈伤组织褐化、干枯等问题^[18]。

褪黑素(N-乙酰-5-甲氧基色胺, melatonin, MT)是脊椎动物脑垂体分泌的一种吲哚胺类激素,1958年首次被发现存在于牛松果体中^[19],故又称松果体素。褪黑素广泛参与机体的一系列的生理活动,如调节昼夜节律、改善睡眠、治疗神经衰弱与抗氧化^[20]。褪黑素长期以来被认为是动物体内专有,因而在植物中的研究进展相对缓慢。1995年首次报道褪黑素存在于植物体内^[21],进一步研究发现其与生长素结构及作用类似,是一种低分子质量的吲哚胺类物质;且两者的前体物质均为色氨酸,可促进

植物生长发育,被认为是一种新型的植物生长调节剂^[22-24]。近年研究发现,褪黑素可改善光周期、提高光合效率^[25]、保护叶绿素、促进果实成熟^[26]、延迟衰老^[27-29],具有类似生长素的功能。此外,褪黑素还具有耐高温^[30]、低温^[31]、紫外线、重金属^[32]和化学污染等^[33-34]生物和非生物胁迫的作用。有研究表明,褪黑素作用于红球甘蓝、黄瓜、玉米种子可提高萌发率^[33, 35],褪黑素对单子叶植物大麦(*Hordeum vulgare*)、小麦(*Triticum aestivum*)、燕麦(*Avena sativa*)和金丝雀草(*Phalaris canariensis*)均有促进生长的作用^[36]。低浓度褪黑素作用于奶油生菜、羽扇豆,可促进侧根的形成^[37-38]。在滇黄芩愈伤组织的诱导中发现,添加0.1、1.0、10.0 $\mu\text{mol}\cdot\text{L}^{-1}$ 褪黑素可促进愈伤组织增殖与不定芽分化^[39]。1 $\mu\text{mol}\cdot\text{L}^{-1}$ 褪黑素对甘蓝胚状体发育具有促进作用,既能增加胚状体再生植株数量,也能增强再生植株的生长势^[40-41]。

目前,以花药、子房和花蕾为外植体建立葡萄体细胞胚再生体系均有成功报道,但以葡萄种子为外植体建立体细胞胚再生体系的研究报道较少。在以葡萄未成熟种子为外植体、剥取合子胚诱导体细胞胚胎的研究中,康拜尔早生的未熟合子胚的胚状体诱导率为47.6%^[42];6个不同酿酒葡萄品种的未成熟合子胚的体细胞胚诱导率为10.5%~37.5%^[43];酿酒葡萄神索花后50 d幼胚的体细胞胚诱导率为37.5%^[44],安芸皇后与巨峰杂交后代42、47、52 d未成熟幼胚体细胞胚诱导率为0.0%~11.1%^[45]。

鉴于上述材料在诱导体细胞胚过程中存在的众多问题,如受花期限制较大,接种工作繁琐、诱导周期较长以及诱导率低等,笔者拟以3种不同成熟度(花后60、70和80 d)酿酒葡萄品种霞多丽(*Vitis vinifera* 'Chardonnay')、马瑟兰(*V. vinifera* 'Marselan')和美乐(*V. vinifera* 'Merlot')的种子为外植体,研究不同质量浓度褪黑素对其种子体细胞胚诱导发生的作用,从初生子叶胚的萌发、二次胚形成所用的时间,不同成熟度种子和切种方式对诱导率的影响等方面进行观察,优化葡萄体细胞发生成苗体系,以期建立稳定高效遗传转化体系奠定基础,也为褪黑素在葡萄组织培养中的生理功能研究提供依据。

1 材料和方法

1.1 材料及其预处理

供试材料为欧洲酿酒葡萄品种霞多丽、马瑟兰、

美乐不同成熟时期种子。于花后 60、70 和 80 d (2020 年 7 月 24 日、8 月 3 日和 8 月 13 日) 10:00 左右,从宁夏青铜峡西鸽葡萄园采集浆果,置于冰盒中,带回实验室。搓洗去葡萄果皮及果肉,将葡萄种子洗净晾干,在超净工作台用 70%乙醇对种子表面消毒 30 s,无菌水冲洗 3 次,经有效氯为 1.5%的次氯酸钠(NaClO)消毒 15 min,无菌水洗涤 5 次,置于无菌三角瓶中,保存于 4 °C 备用。

1.2 初生子叶胚的萌发

表面消毒后的葡萄种子,参照孙兴民等^[45]横切幼胚的方法,增加设置横切、纵切 2 种方法。将种子切开表皮至露出胚乳,分别正放、倒放接种于以 NN69 为基本培养基添加 0.5 mg·L⁻¹NOA+0.55 mg·L⁻¹2,4-D+1.24 mg·L⁻¹4-CPPU 的培养基(对照)或以 MS 为基本培养基添加 0(对照)、0.2、0.4、0.6、0.8、1.0 mg·L⁻¹褪黑素的培养基(表 1)上。凝固剂均使用 3.0 g·L⁻¹植物凝胶,用 NaOH 及 HCl 调节 pH 至 5.8,121 °C 灭菌 25 min。灭菌后的培养基倒入直径 9 cm 无菌培养皿(25~30 mL),冷却凝固后接种,转至 26 °C 黑暗条件下培养,每 4 周继代 1 次。接种 7 d 后,观察并统计愈伤组织诱导率或初生子叶胚萌发率。愈伤组织诱导率/%=愈伤组织个数/接种外植体总数×100;初生子叶胚萌发率/%=初生子叶胚萌发个数/接种外植体总数×100。

表 1 不同质量浓度褪黑素培养基方案
Table 1 Different concentrations of melatonin medium regimen

培养基 Medium	$\rho(2,4-D)$ (mg·L ⁻¹)	$\rho(\text{NOA})$ (mg·L ⁻¹)	$\rho(4\text{-CPPU})$ (mg·L ⁻¹)	$\rho(\text{Melatonin})$ (mg·L ⁻¹)	$\rho(6\text{-BA})$ (mg·L ⁻¹)
MC(对照 Control)	0.55	0.5	1.24	-	-
MEL0 (对照 Control)	1.00	-	-	0	2.0
MEL1	-	-	-	0.2	2.0
MEL2	-	-	-	0.4	2.0
MEL3	-	-	-	0.6	2.0
MEL4	-	-	-	0.8	2.0
MEL5	-	-	-	1.0	2.0

注:MC 基本培养基为 NN69 + 2.0 mg·L⁻¹ 6-BA + 0.5 g·L⁻¹ 活性炭 + 30 g·L⁻¹ 蔗糖;MEL 基本培养基为 MS + 2.0 mg·L⁻¹ 6-BA + 0.5 g·L⁻¹ 活性炭 + 30 g·L⁻¹ 蔗糖。

Note: The basic medium of MC was NN69 + 2.0 mg·L⁻¹ 6-BA + 0.5 g·L⁻¹ activated carbon + 30 g·L⁻¹ sucrose; the basic medium of MEL was MS + 2.0 mg·L⁻¹ 6-BA + 0.5 g·L⁻¹ activated carbon + 30 g·L⁻¹ sucrose.

1.3 体细胞胚的循环诱导

将萌发的初生子叶胚切段至 0.5 cm 后,继续置于原培养基中培养,进行循环诱导至次生愈伤组织的形成;诱导形成的次生胚性愈伤组织转接至 X6 体细胞胚萌发培养基(MS + 60 g·L⁻¹蔗糖 + 0.5 g·L⁻¹活性炭 + 3.0 g·L⁻¹植物凝胶)中,添加 3.033 g·L⁻¹ KNO₃和 0.364 g·L⁻¹ NH₄Cl 以补充培养基中缺少的甘氨酸^[8]。26 °C 黑暗培养,每 4 周继代 1 次,直至次生体胚(somatic embryo, SE)形成。培养 2 个月后,观察并统计次生体细胞胚出现的时间与统计成胚率,成胚率/%=出现体细胞胚数/外植体接种数×100。

1.4 体细胞胚的成苗

将诱导形成的子叶胚进一步转移至成苗培养基 MS + 0.2 mg·L⁻¹ 6-BA + 0.1 mg·L⁻¹ NOA + 30 g·L⁻¹ 蔗糖 + 0.5 g·L⁻¹ 活性炭 + 3.0 g·L⁻¹ 植物凝胶,于 26 °C、16 h 光照/8 h 黑暗环境下培养。继代培养 1 个月后,体细胞胚生根并成苗。

1.5 数据统计分析

所有试验均 3 次重复,每次接种 100 个重复。采用 SPSS 24.0 软件中 One-way ANOVA 进行单因素方差分析与 Duncan's 多重比较分析,差异显著水平为 $p < 0.05$ 。试验结果数据采用平均值±标准差表示。

2 结果与分析

2.1 3 个酿酒葡萄品种不同成熟度种子愈伤组织及子叶胚的诱导

分别以 3 个酿酒葡萄品种霞多丽、马瑟兰与美乐花后 60、70 以及 80 d 采集的不同成熟度种子为外植体,表面消毒后,切开种皮至露出白色胚乳,并置于诱导培养基(表 1)上,在 26 °C 暗培养条件下,进行愈伤组织与初生子叶胚诱导。结果表明,在培养 14 d 后,3 个酿酒葡萄品种不同采收期的种子均开始出现愈伤组织或初生子叶胚。

30 d 后统计结果(图 1)表明,3 个酿酒葡萄品种的种子在添加不同质量浓度褪黑素的培养基以及不同成熟度种子的初生子叶胚诱导率差异较大。在 MC、MEL0、MEL1、MEL2、MEL3、MEL4 与 MEL5 培养基上,子叶胚萌发率均与种子成熟度呈正比,花后 60 d 诱导率为 0.90%~16.56%,70 d 诱导率为 1.34%~17.30%,80 d 时诱导率最高,达 18.33%。在 3 个供试品种中,霞多丽花后 80 d 种子在 0.6 mg·L⁻¹褪黑素诱导下萌发率最高,为 18.33%,对照 2,4-D 诱导

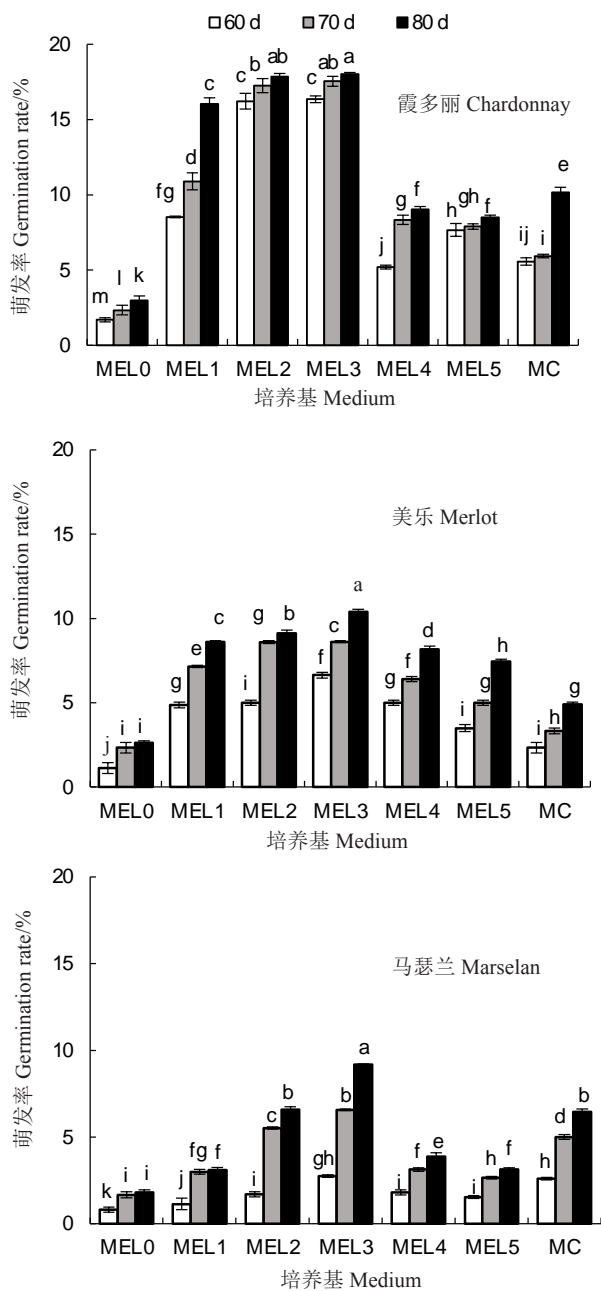


图1 霞多丽、美乐、马瑟兰不同成熟度种子萌发率

Fig. 1 Germination rate of different maturity seeds of Chardonnay, Merlot and Marselan

萌发效果最差,仅为2.98%。

2.2 不同切种与接种方式对霞多丽种子萌发的影响

为探究不同切种与接种方式对初生子叶胚萌发的影响,以花后80 d霞多丽葡萄种子为试验材料(图2-A~D),分别采取横切倒放、横切正放、纵切倒放和纵切正放4种方法(图2-E~H)接种于MEL3培养基(表1)上,在26℃暗培养条件下诱导子叶胚萌发。3 d时种皮开始褪色,10 d时陆续有子叶胚萌发,30 d

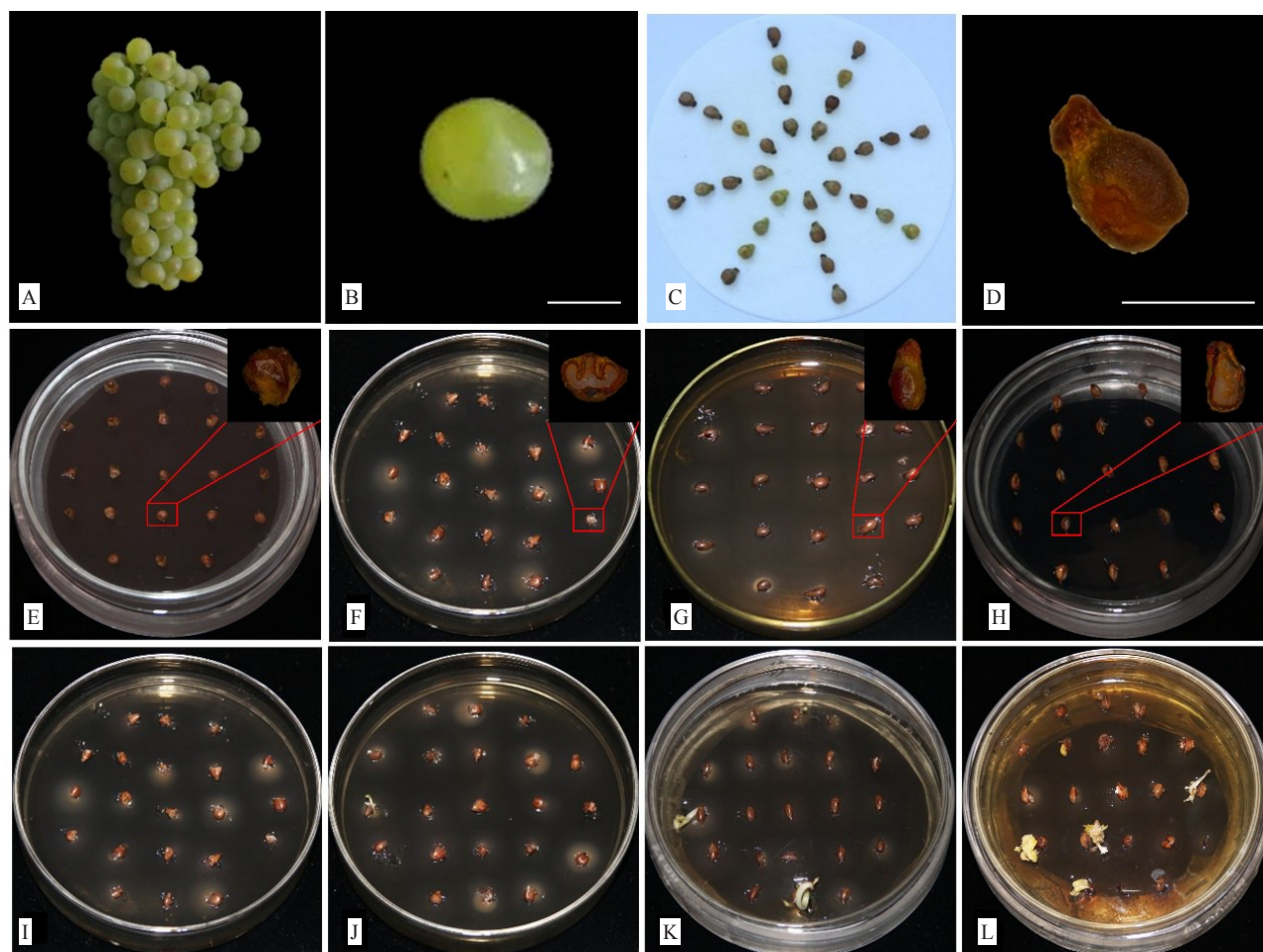
时,纵切的子叶萌发率明显高于横切,且正放高于倒放(表2),纵切正放30 d时的诱导率最高,可达33.07%,横切倒放的诱导率仅为3.24%。

2.3 霞多丽二次体细胞胚的循环诱导

由于霞多丽种子诱导初生子叶胚的萌发效率相对较高且数量相对较多,因此,继续研究了不同质量浓度褪黑素对其二次胚循环诱导形成的作用。霞多丽种子(花后80 d采集)纵切正放接种于 $0.6 \text{ mg} \cdot \text{L}^{-1}$ 褪黑素培养基(图3-A),接种10 d时子叶胚开始萌发(图3-B),30 d时子叶胚伸长(图3-C)。将萌发的霞多丽初生子叶胚切段至0.5 cm后,接种于添加不同质量浓度褪黑素MS培养基中(0.2 、 0.4 、 0.6 、 0.8 、 $1.0 \text{ mg} \cdot \text{L}^{-1}$),以 $1.0 \text{ mg} \cdot \text{L}^{-1}$ 2,4-D为对照。在接种14 d时,4种质量浓度(0.2 、 0.4 、 0.6 、 $0.8 \text{ mg} \cdot \text{L}^{-1}$)褪黑素对愈伤组织的诱导率分别为23.42%、39.67%、45.86%、30.25%,效果明显优于2,4-D处理(17.80%);而 $1.0 \text{ mg} \cdot \text{L}^{-1}$ 褪黑素诱导率为15.81%。不同质量浓度褪黑素培养基均诱导出愈伤组织(图3-D),60 d后有白色体细胞胚形成(图3-E、F), 0.2 、 0.4 和 $0.6 \text{ mg} \cdot \text{L}^{-1}$ 褪黑素处理下的初生子叶胚均产生二次体胚, 0.8 和 $1.0 \text{ mg} \cdot \text{L}^{-1}$ 褪黑素处理未产生二次体胚。结果表明,本研究中设置的5种质量浓度的褪黑素中,仅 0.2 ~ $0.6 \text{ mg} \cdot \text{L}^{-1}$ 对二次胚萌发形成具有促进作用。

对霞多丽二次体细胞胚诱导率进行统计(表3)表明,褪黑素质量浓度为 $0.6 \text{ mg} \cdot \text{L}^{-1}$ 时,诱导率最高,在初生子叶胚循环诱导90 d后,体细胞胚诱导率为14.40%。然而,添加 $0.8 \text{ mg} \cdot \text{L}^{-1}$ 与 $1.0 \text{ mg} \cdot \text{L}^{-1}$ 褪黑素的培养基中,并未观察到二次胚的形成。对照2,4-D($1.0 \text{ mg} \cdot \text{L}^{-1}$)在诱导90 d时,也无体细胞胚形成,且在继代过程中逐渐褐化死亡。

霞多丽初生子叶胚可诱导原胚团和体细胞胚的产生,有助于增加数量和保持扩繁,为葡萄遗传转化提供试验材料。本研究中建立了以霞多丽种子(图4-A)为外植体的体细胞胚诱导体系。种子诱导萌发可形成初生子叶胚(图4-B),切段后接种至褪黑素诱导培养基上(图4-C),30 d形成原胚团(图4-D),60 d出现二次胚的萌发,并可进一步诱导出现球形胚、心形胚、鱼雷胚和子叶胚(图4-E),子叶胚的胚轴伸长,至子叶变绿后,移栽于生根培养基中7 d开始生根,伴随有真叶长出(图4-F)。结果表明,利用褪黑素诱导霞多丽种子,可形成初生子叶胚,经过



A~D. 接种前采样图片;E. 横切倒放;F. 横切正放;G. 纵切倒放;H. 纵切正放;I-L. 30 d 时萌发情况。标尺为 0.5 cm。

A-D. The samples before inoculation; E. Transverse inverted; F. Transverse upright; G. Longitudinal inverted; H. Longitudinal upright; I-L. 30 days after germination. Bar=0.5 cm.

图 2 不同接种方式对子叶萌发的诱导效果

Fig. 2 Effects of different seed cutting methods on the germination rate of *Vitis vinifera* 'Chardonnay'

表 2 不同切种方式对霞多丽种子萌发率的影响

Table 2 Effects of different cutting methods on the germination rate of Chardonnay seeds

培养基 Medium	接种数 Number of inoculation	接种后时间 Time after inoculation/d			%
		10	20	30	
横切倒放 Crosscut and anti-put	210	1.39±0.04 k	2.33±0.05 j	3.24±0.49 h	
横切正放 Crosscut and flatwise	315	2.86±0.06 i	4.77±0.02 g	5.71±0.02 f	
纵切倒放 Slitting and anti-put	247	4.84±0.05 g	7.60±0.02 e	10.98±0.06 c	
纵切正放 Slitting and flatwise	378	8.92±0.06 d	13.52±0.03 b	33.07±0.04 a	

注:统计值为平均值±标准差,不同小写字母表示差异显著($p < 0.05$)。下同。

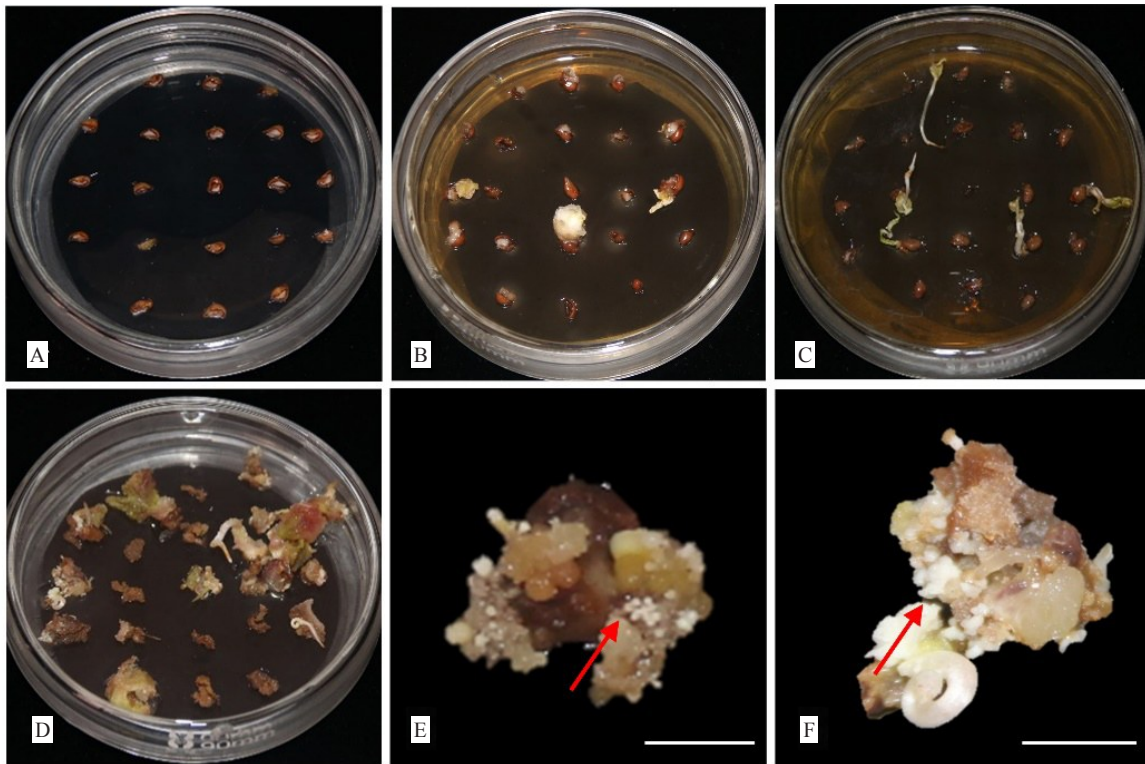
Note: The statistical value was ± standard deviation with significant difference in small letters ($p < 0.05$). The same below.

循环诱导,可产生原胚团并形成二次胚,且移栽后可成苗。

3 讨论

外植体选取的时期对植物体细胞胚诱导至关重要^[17]。前人^[8]在建立以花药、子房和花蕾为外植体的

葡萄体细胞胚诱导体系中,强调单核靠边期是诱导胚性愈伤的最佳时期,然而,葡萄花器官的取材时间严重受到花期的限制,且实验步骤繁琐、耗时耗力。笔者利用霞多丽种子为外植体,建立体细胞胚诱导体系,弥补了花期取样时期的限制。然而,本研究也发现,不同的种子成熟度也会严重影响体细胞胚的



A. 接种;B~C. 初生子叶胚萌发;D. 切段诱导;E、F. 二次胚的萌发。标尺为 1 cm。

A. Inoculation; B-C. The germination of primary cotyledon embryos; D. Segmentation induction; E, F. The germination of secondary embryos. Bar=1 cm.

图 3 由霞多丽葡萄种子萌发循环诱导体胚的再生过程

Fig. 3 Regeneration of somatic embryos induced by seed germination cycle in Chardonnay grape

表 3 霞多丽二次体细胞胚诱导率统计

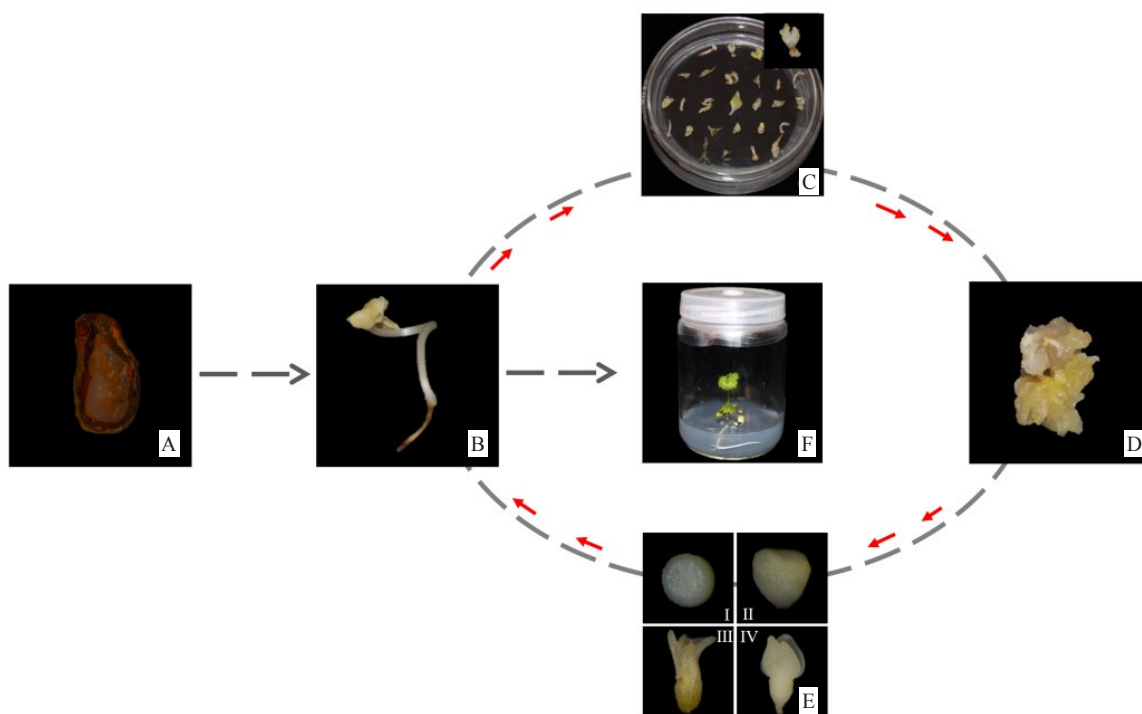
Table 3 Statistics of induction rate of secondary somatic embryos of Chardonnay %

培养基 Medium	接种数 Number of inoculation	诱导时间 Time of induction/d	
		60	90
MEL0(对照 Control)	46	0.00±0.00 i	0.00±0.00 i
MEL1	252	1.76±0.05 h	1.89±0.04 g
MEL2	283	2.09±0.04 f	3.13±0.05 e
MEL3	306	4.80±0.05 d	14.40±0.17 a
MEL4	100	0.00±0.00 i	0.00±0.00 i
MEL5	79	0.00±0.00 i	0.00±0.00 i

诱导发生。随着种子成熟度的增加,诱导形成初生子叶胚的萌发率也呈现上升的趋势,表明成熟度高的葡萄种子较适宜作为供试材料。本研究结合成熟期酿酒葡萄的采收(花后 80 d)收集种子,因而未收集到更高成熟度种子进行其影响诱导效率的探讨。此外,本研究表明,种子纵切正放有利于初生子叶胚萌发,推测可能是由于纵切时胚乳裸露面积大、正放接种不受种子本身重力影响;同时,纵切正放方式可能与空气充分接触,进而增强呼吸作用等有关^[43]。

多项研究表明,植物生长调节剂 2,4-D 在茴香、玉米等^[46-48]植物愈伤组织诱导及体细胞形成中必不可少。Evans 等^[49]也发现,在植物体胚发生诱导阶段中,57.7%的双子叶植物和所有单子叶植物均使用了 2,4-D。有关花生与烟草体细胞胚诱导的研究表明,最适 2,4-D 质量浓度均为 10 mg·L⁻¹^[50-51],而本研究中对照组应用的 2,4-D 质量浓度仅为 1 mg·L⁻¹,推测可能是由于过低的质量浓度影响了初生子叶胚的萌发,同时也不排除物种差异造成的影响。目前,有关褪黑素在其他植物体细胞胚诱导发生的研究报道较少。笔者课题组近期报道了褪黑素对无核白葡萄体细胞胚的诱导作用^[52],在此基础上,笔者探讨了褪黑素在霞多丽种子体细胞胚诱导发生中的作用。

葡萄种子的休眠机制是阻碍种子萌发的关键因素^[53],葡萄种子由于种壳和外围组织引起的透性不良等原因难以打破休眠^[54],甘阳英等^[55]以物理方法去除种皮、剥取胚乳,打破了种皮束缚,促进了葡萄种子萌发。笔者在本研究中以酿酒葡萄霞多丽、马瑟兰、美乐种子为外植体,切种后进行体细胞胚诱



A. 种子纵切后接种; B. 萌发形成的初生子叶胚; C. 切段后置于褪黑素培养基上培养; D. 诱导原胚团产生; E. 进一步诱导体细胞胚的形成 (I 球形胚、II 心形胚、III 鱼雷胚、IV 子叶胚); F. 待子叶胚变绿, 进行生根成苗培养。

A. Seeds were cut longitudinally for inoculation; B. Primary cotyledon embryos formed by germination; C. Primary cotyledon embryos were cut and cultured on melatonin medium; D. Production of proembryos after induction; E. Formation of somatic embryos (I Globular embryo, II Somatic embryo of heart shape stage, III Torpedo embryo, IV Hypocotyl elongated somatic embryos); F. after the cotyledon embryo turns green, which can be used for rooting and seedling culture.

图4 霞多丽葡萄体胚循环诱导

Fig. 4 Induction of somatic embryogenesis in Chardonnay grape

导,操作简单,打破了外植体采收的时间限制,供试材料充足且易于长期储存,可提高试验的重复性。通过比较2,4-D与不同质量浓度(0.2、0.4、0.6、0.8、1.0 $\text{mg} \cdot \text{L}^{-1}$)褪黑素对初生子叶胚萌发的诱导效果,表明褪黑素的诱导作用明显优于2,4-D,褪黑素诱导14 d时,种子开始萌发初生子叶胚,0.6 $\text{mg} \cdot \text{L}^{-1}$ 为最佳萌发质量浓度。切段后诱导二次胚,60 d开始萌发二次胚,继而可产生4种典型的胚——球形胚、心形胚、鱼雷胚以及子叶胚,自首次接种至获得二次体细胞胚共90 d,这一结果较笔者课题组前期通过小花蕾在褪黑素培养基上120 d诱导出无核白葡萄体细胞胚^[52]缩短了30 d。褪黑素诱导初生子叶胚的萌发率显著高于2,4-D,其中,0.6 $\text{mg} \cdot \text{L}^{-1}$ 褪黑素为最适质量浓度,诱导萌发率可达18.33%;但褪黑素诱导下畸形胚占总萌发子叶的52.83%,高于2,4-D(32.41%)。在0.6 $\text{mg} \cdot \text{L}^{-1}$ 褪黑素质量浓度下,二次胚诱导率最高,为14.40%。然而,2,4-D诱导下葡萄种

子仅有初生子叶胚的萌发,并未诱导产生胚性愈伤组织和体细胞胚。0.8与1.0 $\text{mg} \cdot \text{L}^{-1}$ 褪黑素诱导处理未观察到体细胞胚的发生,这与无核白葡萄愈伤组织的结果^[52]及滇黄芩愈伤组织的诱导^[39]结果相似。本研究表明,对比初生子叶和次生胚,外植体接种10 d时初生子叶开始萌发;单粒种子仅能萌发1个子叶,胚轴伸长较快,子叶多为绿色;而次生胚以簇状萌发,数量为初生子叶的4~6倍,次生胚生长缓慢,接种60 d后子叶展开,且颜色偏黄,推测可能是由于细胞活力差异及分化能力的不同所致^[56]。

葡萄体细胞胚诱导发生受众多因素的影响,主要涉及基因型^[15]、外植体和培养基类型^[57]。本研究表明,在以酿酒葡萄种子为外植体建立体细胞胚的诱导体系中,种子成熟度与接种方式均会影响体细胞胚的诱导率,花后80 d采集种子的萌发率最高,为18.33%,表明种子成熟度与初生子叶胚的萌发率呈正相关。以往研究表明,种子在成熟后期,干物质累

积合成^[58],自由水含量降低^[59],休眠机制增强^[60],因此,推测花后 80 d 后(浆果成熟采收期)采样可能与其延迟采收后种子的诱导结果相似。本研究结果进一步表明,种子纵切正放为最适接种方式。在不同质量浓度褪黑素诱导作用的比较中还发现,低质量浓度褪黑素诱导,可以缩短体胚萌发时间。同时,有关葡萄种子作为外植体与母株遗传一致性的研究报告中,杨晓明等^[44]对酿酒葡萄神索未成熟合子胚诱导体细胞胚再生的研究发现,经体细胞胚诱导的再生植株染色体及 DNA 含量与母株一致。也有研究表明,葡萄的胚在遗传上杂合,已不能代表母体性状^[45],然而,胚的遗传变异是新品种选育的前提,因此利用未成熟合子胚诱导体细胞胚,进行遗传转化与胚胎早期形态建成研究都较为有利^[61]。

此外,畸形胚的高频发生和愈伤组织褐化是当前体细胞胚诱导过程中广泛存在并亟需解决的问题。本研究在诱导初生子叶胚的过程中也出现了大量畸形胚(52.83%),其中包括 43.56%胚轴伸长胚、6.8%玻璃化胚、2.47%子叶融合胚,推测可能与褪黑素和分裂素的质量浓度配比、种子生理状态有很大关系。有研究指出,菠萝畸形胚的高频发生率与高质量浓度外源植物生长调节剂的添加密切相关^[62]。研究报告表明,无核葡萄离体胚珠发育过程中,愈伤组织褐化与继代时间、蔗糖质量浓度密切相关,60 g·L⁻¹为最适蔗糖质量浓度,当质量浓度过高时也会出现严重褐化现象^[63]。本研究初步建立了以酿酒葡萄霞多丽种子为外植体的体细胞胚诱导体系,但还有众多亟待解决的问题,如诱导形成的胚性愈伤增殖、长期保持与快速分化、防止愈伤的褐化等。

4 结 论

以酿酒葡萄种子为外植体材料,探讨了褪黑素在葡萄体细胞胚诱导中的作用,发现酿酒葡萄种子成熟度与初生子叶胚的萌发率呈正相关;纵切正放的接种方式有利于初生子叶胚萌发;筛选出适宜种子初生子叶胚萌发的褪黑素质量浓度为 0.6 mg·L⁻¹;经诱导产生的初生子叶胚切段可循环诱导出二次体细胞胚,且可移栽成活,为研究葡萄胚胎发育提供了试验材料及体系,也为深入开展葡萄定向遗传改良研究及抗逆与品质转基因育种奠定了基础。

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