黑松下胚轴和针叶胚性愈伤组织的诱导

代静[1] 郭辰昊[2] 刘静[3] 孟鹏*

（青岛农业大学园林与林学院 青岛 266109）

摘 要:【目的】以黑松下胚轴为外植体，进行体细胞胚性愈伤组织的诱导研究。找出影响愈伤组织诱导的关键因素，并探讨诱导过程中培养基种类、生长素 2,4-D 和细胞分裂素 6-BA 浓度对愈伤组织的影响，为黑松营养器官体细胞胚胎发生体系的建立奠定基础。【方法】采用三因素四水平正交试验设计，分别以 MS、WPM、B5、DCR 作为基本培养基，添加不同浓度的 2,4-D（0.5 mg • L-1，1.5 mg • L-1，2.0 mg • L-1，2.5 mg • L-1）和 6-BA（0.5 mg • L-1，1.0 mg • L-1，1.5 mg • L-1，2.0 mg • L-1），附加 0.2mg • L-1 PVP 、6 g • L-1 琼脂和 20g • L-1 蔗糖，诱导愈伤组织。以统计学方法探索各因素间的互作作用、最佳因素组合及最适激素浓度比。挑取愈伤组织在体视显微镜下进行形态学观察，采用醋酸洋红与伊文思蓝双染色法在光学显微镜下进行细胞学观察，辨别是否为胚性愈伤组织。通过测定不同增殖培养的愈伤组织中多酚氧化酶（PPO）的含量，来检验诱导的愈伤组织能否维持胚性。【结果】在试验设定的因素和水平范围内，最优组合为 DCR + 2.5 mg • L-1 2,4-D + 1.0 mg • L-1 6-BA，该方案的愈伤组织诱导率可达 98.7%（±1.9%）。采用该方法，也能成功诱导出针叶外植体的愈伤组织，但诱导速度较慢。显微观察可以看到明显的胚性愈伤组织结构，以及长条形细胞组成的胚性胚柄团。由方差分析可知，2,4-D 浓度会对愈伤组织的诱导率产生显著性影响（$p<0.05$），而培养基的种类与 6-BA 的浓度对于实验的结果没有显著影响。尽管如此，交互作用分析显示，2,4-D 与 6-BA 结合使用时存在显著互作作用（$p<0.05$）。愈伤组织生长 3 周后 PPO 活力显著提高，第一次和第二次增殖培养后愈伤组织 PPO 活力下降。【结论】2,4-D 在黑松下胚轴胚性愈伤组织诱导的过程中起重要作用，虽然 6-BA 浓度本身对愈伤组织诱导率没有显著影响，但 6-BA 与 2,4-D 在信号传导途径中可能存在复杂的联系。

关键词：体胚发生；胚性愈伤组织；黑松；下胚轴；针叶

Induction of embryonic callus from hypocotyl and needle of *Pinus thunbergii* Parl.

Jing Dai[1] Chenhao Guo[2] Jing Liu[3] Peng Meng*

（College of Landscape architecture and Forestry, Qingdao Agricultural University Qingdao 266109）

Abstract: 【Objective】 To lay the foundation for the establishment of somatic embryogenesis system in the vegetative organs of *Pinus thunbergii* Parl, the induction of somatic embryogenic callus (EC) was studied using hypocotyls as explants to find out the key factors affecting EC induction, and investigate the effects of medium type, concentration of auxin (2,4-D) and cytokinin (6-BA) on the EC induction. 【Method】Orthogonal experiment design with three factors and four levels was adopted. MS, WPM, B_5 and DCR were used as the basic medium, respectively, with different concentrations of 2,4-D (1.0 mg • L^{-1}, 1.5 mg • L^{-1}, 2.0 mg • L^{-1}, 2.5 mg • L^{-1}) and 6-BA (0.5 mg • L^{-1}, 1.0 mg • L^{-1}, 1.5 mg • L^{-1}, 2.0 mg • L^{-1}), with additional 0.2 mg • L^{-1} polyvinylpyrrolidone (PVP), 6 g • L^{-1} agar and 20 g • L^{-1} sucrose to induce callus. The interactions between the factors, the optimal combination and the best hormone concentration ratio were investigated by statistical methods. Callus were sampled for morphological observation under a stereoscopic microscope, followed by cytological observation under a biological microscope using a double staining method of acetate magenta and Evans blue to identify whether they were embryonic callus. The polyphenol oxidase (PPO) contents in the callus of different succession cultures were measured to test whether

embryonic stage of the induced callus could be maintained. 【Result】 The results showed that the optimal combination was DCR + 2.5 mg • L^{-1} 2,4-D + 1.0 mg • L^{-1} 6-BA, and this protocol resulted in 98.7% (±1.9%) induction of callus. Using this protocol, callus of needle explants was also successfully induced, but the callus induction was slower. Microscopic observation reveals a distinct embryonic callus structure, an embryonic head with several suspensor cells. The analysis of variance showed that among the three factors, 2,4-D concentration significantly affected the callus induction rate ($p<0.05$), while the medium type and the 6-BA concentration had nonsignificant effect. Even so, the analysis of interaction showed that there was a significant interaction between the combination of 2,4-D and 6-BA ($p<0.05$). The PPO viability was significantly higher in the callus grown for 3 weeks, while it decreased after first and second succession. 【Conclusion】The above results inferred that 2,4-D plays an important role in embryotic callus induction process in the hypocotyl of *P. thunbergii* Parl. Although 6-BA concentration level by itself had no significant effect on callus induction rate, there may be complex links between the 6-BA and 2,4-D in the signal transduction pathways.

Key words: Somatic embryogenesis; Embryogenic callus; *Pinus thunbergii* Parl; Hypocotyl; Needle