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### 农业生物技术科学

# 树莓试管苗茎尖包埋玻璃化法超低温保存

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摘要: 插要: 本试验对树莓茎尖包埋玻璃化法种质资源保存进行了研究,以期建立简单、高效的树莓种质资源超低温保存体系。试验以树莓试管苗茎尖为材料,探讨了不同因素对包埋玻璃化法超低温保存后成活率的影响。结果表明: 长约1mm的树莓试管苗茎尖用海藻酸钙包埋后,采用分步预培养的方法,最后终止蔗糖浓度为0.9mol/L的MS培养基中进行预培养3d,再用含2M甘油和0.9M蔗糖的装载液处理90min,在0℃下用PVS2处理180min后投入液氮中保存1h,在40℃下化冻3min,用含1M蔗糖的MS液体培养基洗涤20min,最后转到恢复培养基上,30d后在没有形成愈伤组织的情况下形成新的植株。以上的保存程序应用于6个树莓品种,成活率达87%。该结果为树莓种质资源的长期保存提供了理论依据。

关键词: 关键词: 树莓 茎尖 包埋玻璃化法

Cryopreservation of in vitro-grown shoot tips of raspberry by encapsulation vitrification

Abstract: Abstract: This study was aimed to establish efficient raspberry (Rubus idaeus L.) cryopreservation procedure based on encapsulation-vitrification. In vitro-grown shoot tips of raspberry (Rubus idaeus L.) as materal, the effect of different factor relate to survival were explored. The result was as followed: Excised shoot tips were encapsulated into alginated-gel beads. Then alginate-coated shoot tips were precultured on MS basal medium supplemented with sucrose at 0.9mol/L in a stepwise way. After preculture, encapsulated shoot tips were loaded with a mixture of 2 mol/L glycerol plus 0.9mol/L sucrose for 90 min. Following loading, encapsulated shoot tips were dehydrated with vitrification solution (PVS2 solution for 180min at 0°C), and then plunged directly into liquid nitrogen for 1h. After rapid warming in water for about 3 min at 40°C, the shoot tips were rinsed with MS medium containing 1.0mol/L sucrose for 20 min, and then cultured on the resumed medium in dark prior to exposure to the light. Successfully vitrified shoot tips resumed growth and developed shoots within 30d without intermediary callus formation. This simple protocol was successfully applied to the 6 cultivars. The average rate of survival was 87%. The result provided a feasible proof of raspberry conservation through cryopreservation of in vitro shoot-tips by vitrification for long term.

Keywords: Key words: raspberry shoot tips encapsulation-vitrification

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