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研究与进展

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## 陆地棉花药蛋白质组分析中双向电泳技术体系的建立与优化

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### Development of Two-Dimensional Electrophoresis for Anther Proteome Analysis of Upland Cotton (*Gossypium hirsutum* L.)

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摘要

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**摘要** 建立了适用于陆地棉花药蛋白质组研究的双向电泳技术。以陆地棉花药为材料, 采用液氮研磨的方法破碎组织, 然后用三氯乙酸(TCA)/丙酮沉淀法和苯酚抽提结合甲醇/醋酸铵沉淀法提取蛋白质。采取载体两性电解质pH梯度等电聚焦/SDS-PAGE和固相pH梯度等电聚焦/SDS-PAGE双向凝胶电泳, 对陆地棉花药总蛋白质进行了分离。通过对样品制备方法、等电聚焦方法、样品上样量等关键技术进行比较和优化, 发现苯酚抽提结合甲醇/醋酸铵沉淀的方法较适合陆地棉花药蛋白质的提取, 采用载体两性电解质pH梯度聚焦方法较pH梯度等电聚焦方法的效果好, 用硝酸银染色时, 上样量为500 μg, 得到的电泳图谱分辨率高、重复性好。

**关键词:** 蛋白质组 双向电泳 陆地棉 花药

**Abstract:** This study has established two-dimensional electrophoresis for anther proteomics of upland cotton. We used cotton anther as the material, using TCA/acetone precipitation and phenol extraction-methanol/ammonium acetate precipitation, then proteins were separated by two-dimensional gel electrophoresis. We compared and optimized the key steps, such as sample preparation, isoelectric focusing electrophoresis and the volume of the sample. It is expected that phenol extraction-methanol/ammonium acetate precipitation method could be one of the options for protein extraction from cotton anthers, and the isoelectric-Dalton-isoelectric focusing (ISO-DALT-IEF) is better than immobilized pH gradient-Dalton-isoelectric focusing (IPG-DALT-IEF). The high-resolution and well-reproducible patterns of proteins were observed when the silver staining is taken and 500 μg protein sample were added in electrophoresis.

**Keywords:** proteome two-dimensional electrophoresis upland Cotton anther

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