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棉花叶片蛋白质组双向电泳技术的优化

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Optimization of Two-Dimensional Electrophoresis for Cotton Leaf Proteomics

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摘要

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摘要 以棉花叶片为材料, 针对试材中含有大量色素、酚、醌等干扰物质的问题, 从蛋白提取方法、裂解液成分、上样量等方面对棉花叶片蛋白质组双向电泳技术进行优化。结果表明: 采用改良的TCA/丙酮法提取棉花叶片总蛋白, 含有7 mol·L⁻¹尿素、2 mol·L⁻¹硫脲、4% CHAPS、80 mmol·L⁻¹ DTT、1 mmol·L⁻¹ PMSF、0.3%载体两性电解质的裂解缓冲液, 上样量为600 μg, 采用pH 4~7、24 cm的IPG胶条进行双向电泳时, 2-DE图谱的蛋白点分布均匀、清晰且重复性好。本体系为开展棉花蛋白质组学研究奠定了技术基础。

关键词: 蛋白质组 双向电泳 棉花 叶片

Abstract: Using cotton (*Gossypium hirsutum* L.) leaves as experimental materials, the present study aimed at solving the problem of disturbance by secondary metabolites in the materials, such as chromatophores, phenols and quinones, and optimizing the protein extraction method, lysis buffer reagent, protein loading amounts and other factors. The results showed that optimal cotton leaf protein extraction was obtained by the improved TCA/acetone method, with lysis buffer containing 7 mol·L⁻¹ Urea, 2 mol·L⁻¹ Thiourea, 4% CHAPS, 80 mmol·L⁻¹ DTT, 1 mmol·L⁻¹ PMSF, and 0.3% Bio-Lyte, a protein loading amount of 600 μg, and an IPG (pH 4-7, 24 cm). Under these conditions a good protein pattern with protein spots distributed equally, of high quality and repeatability, was obtained. This system will establish a foundation for cotton proteomic research.

Keywords: proteome two-dimensional electrophoresis cotton leaf

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