

研究论文

适于双向电泳分析的苹果叶片蛋白质提取方法

曾广娟^{1,2}, 李春敏², 张新忠³, 滕云龙¹, 董文轩^{1*}

1.沈阳农业大学园艺学院, 辽宁 沈阳 110161; 2.河北省农林科学院昌黎果树研究所, 河北 昌黎 066600; 3.中国农业大学园艺植物研究所, 北京 100193

收稿日期 2009-2-11 修回日期 2009-4-10 网络版发布日期 2009-7-28 接受日期 2009-5-8

摘要 为了探索适用于双向电泳(2-DE)分析的苹果叶片蛋白质提取方法,比较了三氯乙酸(TCA)/丙酮沉淀法、二硫苏糖醇(DTT)/丙酮法、Tris-HCl提取法和改良的Tris-HCl提取法等4种蛋白质提取方法。以7 cm、pH 3~10的线性固相pH梯度(immobilized pH gradient, IPG)胶条作为第一向电泳,以十二烷基硫酸钠-聚丙烯酰胺凝胶电泳(SDS-PAGE)(12.5%的分离胶)作为第二向电泳,对提取物进行2-DE分离,采用银染显色。结果表明,上述4种方法在2-DE图谱上分别得到140, 215, 181和616个蛋白质点。其中以改良的Tris-HCl提取法得到的蛋白质点数最多,且背景清晰、图谱上没有明显的横纵条纹。为了进一步验证改良的Tris-HCl提取法的有效性,用18 cm、pH 3~10的线性IPG胶条和12.5%的分离胶对提取的苹果叶片蛋白质进行2-DE分离,考马斯亮蓝R-250染色,共检测到455个蛋白质点,其相对分子质量主要分布在14 000~66 000范围内,图谱背景清晰,再次证明应用该方法制备的样品适用于双向电泳分析,可用于苹果叶片的蛋白质组学分析。

关键词 [双向电泳](#) [蛋白质提取方法](#) [苹果叶片](#) [蛋白质组学分析](#)

Efficient protein extraction method from apple leaves for apple proteomic analysis using two-dimensional electrophoresis analysis

ZENG Guangjuan^{1,2}, LI Chunmin², ZHANG Xinzhong³, TENG Yunlong¹, DONG Wenxuan^{1*}

1.College of Horticulture, Shenyang Agricultural University, Shenyang 110161, China; 2.Changli Institute of Pomology, Hebei Academy of Agricultural and Forestry Science, Changli 066600, China; 3.Institute for Horticultural Plants, China Agricultural University, Beijing 100193, China

Abstract

In order to develop an efficient protein extraction method suitable for apple leaf proteomic analysis, four extraction methods for total protein in apple leaves were compared, including trichloroacetic acid (TCA)/acetone precipitation, dithiothreitol (DTT)/acetone method, tri(hydroxymethyl)aminomethane (Tris-HCl) method and the modified Tris-HCl method. During the two-dimensional electrophoresis (2-DE), the first dimension electrophoresis was performed on a 7 cm strip with pH 3~10 linear immobilized pH gradient (IPG) and the second one was performed on 12.5% polyacrylamide gels of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were detected by silver staining. The results showed that 140, 215, 181 and 616 protein spots were detected on 2-DE gels, respectively. The modified Tris-HCl method was the most appropriate for apple leaf proteomic analysis because of the highest resolution and no apparent vertical or horizontal streaking on the 2-DE map. In order to testify the effect of the modified Tris-HCl method on the apple leaf protein extraction, 2-DE maps were established by using 18 cm strips with linear IPG in pH range of 3~10. After 2-DE separation and Coomassie Brilliant Blue R-250 (CBB R-250) staining, about 455 spots were detected, and the relative molecular masses of most proteins were distributed in the range of 14 000~66 000 which were free of smearing or streaking. So it was once again proved that the modified Tris-HCl method can be used in apple leaf proteome analysis.

Key words [two-dimensional electrophoresis \(2-DE\)](#) [protein extraction method](#) [apple leaves](#) [proteomic analysis](#)

DOI:

通讯作者 董文轩 wxdong63@126.com

扩展功能

本文信息

- ▶ [Supporting info](#)
- ▶ [PDF\(1671KB\)](#)
- ▶ [\[HTML全文\]\(0KB\)](#)
- ▶ [参考文献](#)

服务与反馈

- ▶ [把本文推荐给朋友](#)
- ▶ [加入我的书架](#)
- ▶ [加入引用管理器](#)
- ▶ [复制索引](#)
- ▶ [Email Alert](#)

相关信息

- ▶ [本刊中 包含“双向电泳” 的相关文章](#)
- ▶ [本文作者相关文章](#)

- [曾广娟](#)
- [李春敏](#)
- [张新忠](#)
- [滕云龙](#)
- [董文轩](#)