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ダイズおよびトウモロコシ抽出 DNA の精製度の検討

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Qualitative study of DNA extracted from soybean and maize

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Abstract

The quality and yield of DNA extracted from soybean and maize samples were compared using two commercial DNA extraction kits, the DNeasy Plant Mini kit (Mini kit) and the GM quicker kit. Subsequent quantification of the extracted DNA samples by UV spectrophotometry and fluorometry revealed that the yields of soybean DNA extracted using the Mini kit were approximately three times higher when determined by UV spectrophotometry than when they were determined by fluorometry. Conversely, the yields of soybean DNA extracted using the GM quicker kit were only slightly higher when determined by UV spectrophotometry than when they were determined by fluorometry. However, the relative DNA yields of maize DNA samples estimated by UV spectrophotometry and fluorometry were 1.77 with the Mini kit and 1.52 with the GM quicker kit. To validate the soybean DNA yields obtained using both extraction kits, DNA samples were analyzed by agarose gel electrophoresis and real-time PCR of a reference gene. These analyses indicated that the Mini kit yields estimated by UV spectrometry were over-estimated, due to more low-intensity bands and fewer gene copies being observed, compared to DNA extracted with the GM quicker kit. Conversely, the maize DNA yields obtained using the Mini and GM quicker kits showed only slight differences between the real-time PCR and agarose gel electrophoresis analyses. The extracted DNA samples were then analyzed by size-exclusion chromatography. The results showed that the soybean DNA extracted using the Mini kit contained more low-molecular weight impurities than the DNA extracted using the GM quicker kit and maize extracts obtained with both extraction kits. Therefore, it appears that the presence of low molecular weight impurities in soybean DNA extracted with the Mini kit interferes with the UV quantification of DNA.

Keywords : DNA 抽出法、ダイズ、トウモロコシ、不純物、サイズ排除クロマトグラフィー
DNA extraction method, soybean, maize, impurity, size exclusion chromatography

I　緒言

安全性審査を受けていない遺伝子組換え（GM）食品の流通防止および安全性審査済みの GM 食品の適正な表示とその検証のため、厚生労働省および消費者庁から組換え DNA 技術応用食品の検査方法[1-2]が通知されている。GM 食品検査は各都道府県の衛生研究所および登録検査機関等で広く実施されているが、我々はこれらの検査機関における分析の信頼性の確認および向上を目的として GM 食品検査に関する外部精度管理調査を実施してきた[3-7]。

2006 年度および 2009 年度に実施したラウンドアップ・レディ・ダイズを対象とした遺伝子組換え食品検査外部精度管理調査において、定量 PCR 法により測定されたダイズ内在性遺伝子レクチンのコピー数は参加機関が採用した DNA 抽出法によって大きく異なり、GM quicker 法を使用した機関のレクチンのコピー数が、他の抽出法を採用した機関に比べ多

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