![SHIMADZU]

Application News

Microchip Electrophoresis   No.B32

Identification of Common Bean Cultivars by RAPD-STS with MCE-202 "MultiNA"

In Japan, when a new variety of plant is breeded and its variety registration is accepted, the registered breeder of that variety is granted rights (breeder's right) to that specific plant cultivar according to Japan's Plant Variety Protection and Seed Act. For example, kidney (common) beans are cultivated all over the world, and many varieties are in circulation. A domestic variety of the kidney bean (tebo) is used as the chief ingredient in sweet white bean paste. However, infringe problems against the breeder's right occur. Without the permission of the breeder for the registered variety, the variety is exported and cultivated in overseas or the resultant unlicensed plant is re-imported to Japan.

In conjunction with the visual inspection of the fruiting body, qualitative Polymerase Chain Reaction (PCR) genetic testing is effective for distinguishing among the common bean cultivars; the RAPD-STS (Random Amplified Polymorphic DNA Sequence Tagged Sites) method has been developed to accomplish this. Here we introduce cultivar identification of common beans by RAPD-STS method using the MultiNA.
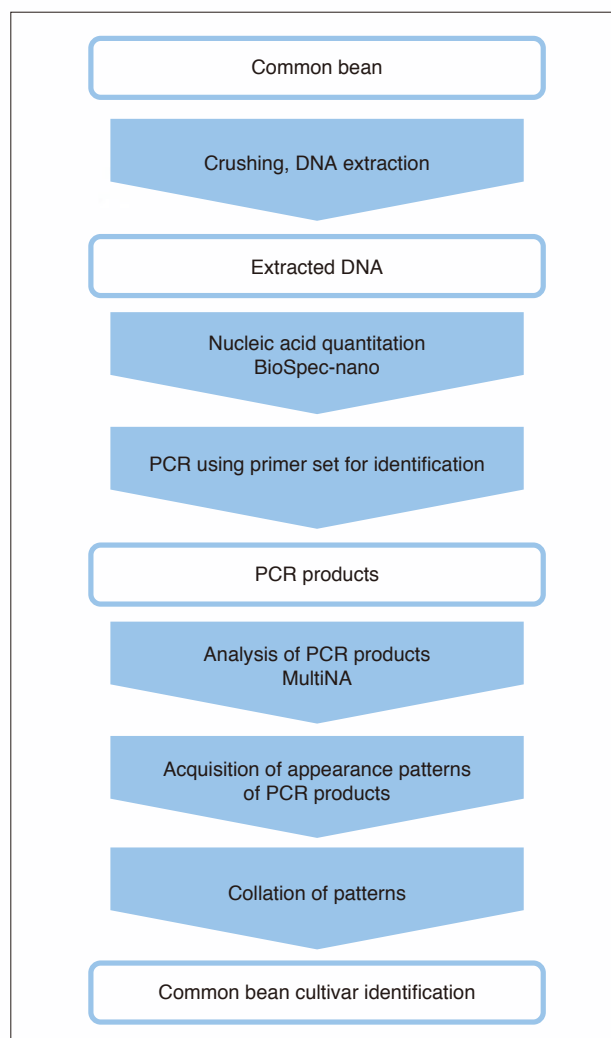
■ Analytical Procedure

For the samples, we used two varieties of commercially available domestic common beans (Yukitebo and Gintebo), and two overseas varieties (pea bean and great northern bean). Cultivar identification was conducted according to the RAPD-STS method (Japanese Patent No. 2004-16008). With the RAPD method, random primers are used. However, a feature of the RAPD-STS method is that the primers are selected based on the sequence information of the obtained PCR products. The above-mentioned four varieties of common beans were pulverized and the DNA was extracted using the DNeasy Plant Mini Kit. The extracted DNA solutions were measured using the BioSpec-nano life science UV-VIS spectrophotometer, to determine the DNA concentrations. Then, DNA extract (10 ng) from each of the bean cultivars was used as a template, and three types of primers (SP01, SP02, SP03) were used for conducting the respective PCR amplifications. The PCR products were analyzed using the MultiNA, their appearance patterns were then determined (Fig. 1).

■ Reagents / Kits

- DNeasy Plant Mini Kit (50)
  (QIAGEN) 69104
- DNA-1000 Kit
  (Shimadzu) P/N 292-27911-91
- SYBR® Gold nucleic acid gel stain
  (invitrogen) S11494
- 100 bp Ladder
  (Takara Bio) 3407A

■ Analytical Conditions for PCR Products

Analytical Instrument : MultiNA
Analysis Mode   : DNA-1000 on-chip mode

Fig. 1 Procedure for Identification of Common Bean Cultivars

■ Results

After conducting PCR using DNA templates extracted from four varieties of common bean (Yukitebo, Gintebo, pea bean, and great northern bean), the resultant PCR products were analyzed using the MultiNA, analysis results are shown in Fig. 2. The PCR product identification patterns of the various cultivars acquired through RAPD-STS method are shown in Table 1. The PCR product patterns obtained by this analysis are consistent with the identification patterns.

In agarose gel electrophoresis, acquiring the appearance patters of PCR products often accompanies difficulty due to indistinct or widened bands, non-perpendicular migration, etc. The MultiNA solves these problems and enables the smooth acquisition of PCR product appearance patterns.
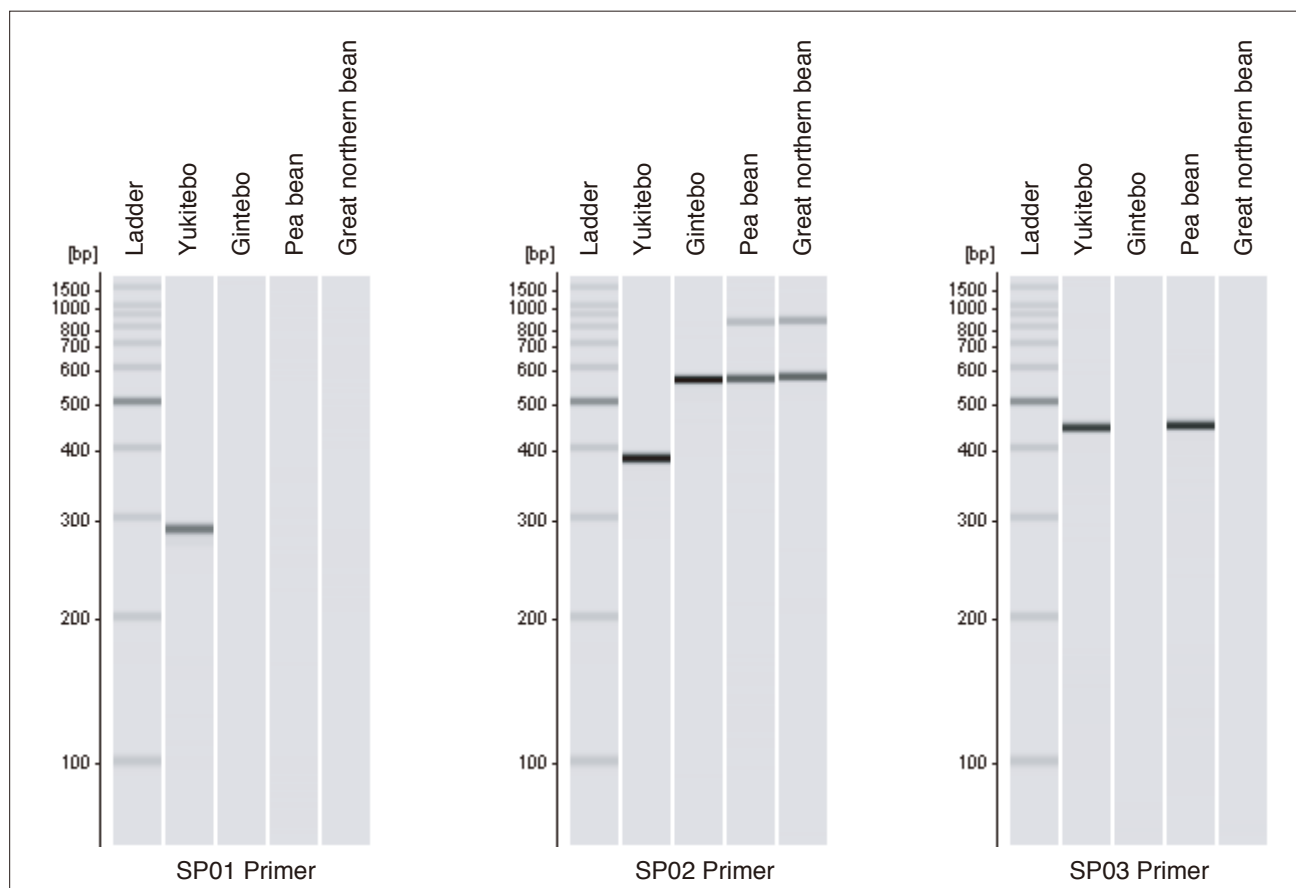

Fig. 2 Analytical Results (Gel Images) of PCR Products of Extracted DNA from Common Beans with MultiNA

Table 1 Identification Patterns of PCR Products from Common Beans

SP01 Primer					SP02 Primer					SP03 Primer				
Cultivar	Yukitebo	Gintebo	Pea bean	Great northern bean	Cultivar	Yukitebo	Gintebo	Pea bean	Great northern bean	Cultivar	Yukitebo	Gintebo	Pea bean	Great northern bean
290 bp	+	−	−	−	800 bp	−	−	+	+	450 bp	+	−	+	−
					560 bp	−	+	+	+					
					390 bp	+	−	−	−					