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IDENTIFICATION OF WHITE COMMON BEAN VARIETIES (OTEBO, *PHASEOLUS VULGARIS* L.) BY DNA POLYMORPHISM USING PCR AMPLIFICATION

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IDENTIFICATION OF WHITE COMMON BEAN VARIETIES (OTEBO, *PHASEOLUS VULGARIS* L.) BY DNA POLYMORPHISM USING PCR AMPLIFICATION

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Abstract

Tebo is a kind of white common bean and it had been a specific agricultural product in Hokkaido. But recently, the white common bean named Otebo was imported and Tebo production in Hokkaido decreased to half. For the purpose of plant variety protection, the identification method of white common bean was established by DNA polymorphism using PCR amplification. RAPDs between varieties were screened using random primers. Then the selected RAPDs were sequenced and the three specific primer sets were newly designed. By PCR amplification with the specific primers, major varieties of Tebo in Hokkaido were distinguished from imported white beans. And the imported Otebo were identified as "Yukitebo" or "Himetebo" the major varieties in Hokkaido. By this method, we could identify the variety from a single seed in one day. The identification method by DNA polymorphism is rapid and sensitive. We developed the method for plant variety protection but also it will be a useful tool for management of seed multiplication and quality control of processing food production.

Introduction

1. Hokkaido is the northern island in Japan and a highly productive land of agriculture. In Hokkaido, soybean, azuki bean and common bean are grown and Hokkaido has the largest share in Japan. Most of the bean varieties grown in Hokkaido were bred in Hokkaido Prefectural Agricultural Experiment Stations. The bean varieties bred by Hokkaido were registered and the Hokkaido government owns the breeder's rights of the varieties. Tebo is a kind of white common bean and a traditional sweet bean paste is made from it. Tebo became a specific agricultural product in Hokkaido. But at 1997, the white common bean named Otebo was begun to import from over seas and the cultivation of Tebo in Hokkaido decreased from 6,000 ha to 3,000 ha (Fig 1). For the purpose of plant variety protection, we developed the identification method of common bean and investigated the variety of imported Otebo.

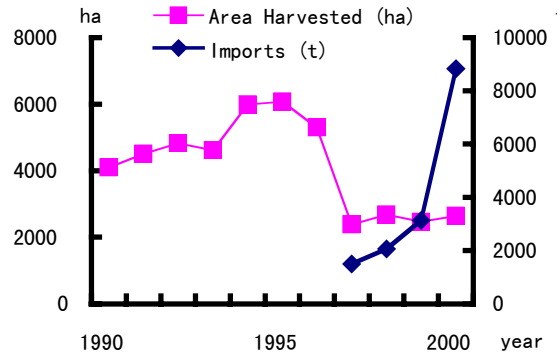

Fig. 1. Imported Otebo and Tebo production in Hokkaido

Materials and Methods

2. Three varieties of Tebo in Hokkaido ("Yukitebo", "Himetebo" and "Gintebo"), imported common bean (Great northern bean, Pea bean, Xiao-bai-yi-dou), lima bean (Baby lima bean and Butter bean), scarlet runner bean (Da-bai-yi-dou) and imported Otebo were used as samples.

3. Individual seeds were drilled in the center of cotyledon using an electric drill (drill diameter: 2.5 mm) and the powder was used for DNA extraction.

4. PCR with random primer (UBC primer, University of British Colombia, Canada) was performed in 15 µl reaction solutions containing 30 ng of DNA extract, GeneAmp PCR Buffer II (Applied Biosystems, USA), 2 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 µM primer and 0.5 units of Ampli*Taq* Gold DNA Polymerase (Applied Biosystems, USA). Amplification was carried out in GeneAmp PCR System 9700 (Applied Biosystems, USA). The amplification program was as follows: initial denaturation step at 95°C for 7 min, and 45 cycles at 94 °C for 1 min, 36 °C for 1 min, 72 °C for 2 min followed by final extension at 72 °C for 5 min. Using specific primers, $MgCl_2$ content was decreased to 1.5 mM and the amplification program was as follows: initial denaturation step at 95 °C for 7 min, and 35 cycles at 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 1 min followed by final extension at 72°C for 5 min. The amplified products were resolved by electrophoresis with 1.5% agarose gel. The gels were stained with SYBR GreenI (FMC BioProducts, USA) and photographed under ultraviolet light.

Results and Discussion

5. At first, we screened polymorphisms between Hokkaido varieties in RAPD analysis. After using 700 random primers, 8 RAPDs were selected. By PCR amplification with the selected RAPD markers, major varieties in Hokkaido were distinguished from imported white beans (Table 1). Then the selected RAPDs were sequenced and the three specific primer sets were designed. SP01 was the specific primer set which amplified the 290 bp band of DNA in "Yukitebo" and "Himetebo". SP02 was a co-dominant marker and it amplified the 390 bp band in "Yukitebo" but also the 560 bp band in "Himetebo" and "Gintebo". SP03 amplified the 450 bp band in "Yukitebo" and "Himetebo". Using the specific markers, major varieties in Hokkaido were distinguished. In Great northern bean and Pea bean, also the 800 bp band was amplified by SP02 (Table 2). By the markers, we evaluated one hundred genetic resources of white common bean in Tokachi Agricultural Experimental Station. Three strains

showed the same genotypes with “Yukitebo” in the specific markers but differed in other RAPD markers and in 100 seeds weight (Table 3).

Table 1. The selected RAPDs for variety identification

	u105 1000bp	u157 500bp	u218 1500bp	u245 500bp	u276 1300bp	u289 1900bp	u355 1200bp	u375 1200bp
Yukitebo	+	+	+	+	+	+	+	+
Himetebo	+	+	+	+	+	+	-	-
Gintebo	-	-	-	-	-	-	-	-
GN(USA)	+	+	-	-	-	-	-	-
PB(USA)	+	+	-	+	-	+	+	-
XB(CHN)	-	-	-	-	-	-	-	+
BL(USA)	-	-	-	-	+	-	-	-
BU(Myan)	-	-	-	-	+	-	-	-
DB(CHN)	-	-	-	-	+	-	-	-

GN: Great northern bean, PB: Pea bean, XB: Xiao-bai-yi-dou, BL: Baby lima bean, BU: Butter bean, DB: Dao-bai-yi-dou

6. It was able to amplify with the primer sets SP01 and SP02 at once as a multiplex PCR. The imported Otebo were identified as “Yukitebo” or “Himetebo” by multiplex PCR (Fig 2). Later, we grew the imported Otebo and observed in morphological and physiological characters and concluded that the varieties of Otebo were “Yukitebo” or “Himetebo”. “Yukitebo” is the new variety registered and protected. Then the Hokkaido government warned the traders not to import.

7. By this method, we could identify the variety from a single seed in one day. The identification method by DNA polymorphism is rapid and sensitive. We developed the method for plant variety protection but also it will be a useful tool for management of seed multiplication and quality control of processing food production.

Table 2. Specific markers for variety identification

	SP01	SP02			SP03
	290bp	390bp	560bp	800bp	450bp
Yukitebo	+	+	-	-	+
Himetebo	+	-	+	-	+
Gintebo	-	-	+	-	-
Great northern bean	-	-	+	+	-
Pea bean	-	-	+	+	+
Xiao-bai-yi-dou	-	+	-	-	-
Baby lima bean	-	-	-	-	-
Butter bean	-	-	-	-	-
Da-bai-yi-dou	-	-	-	-	-

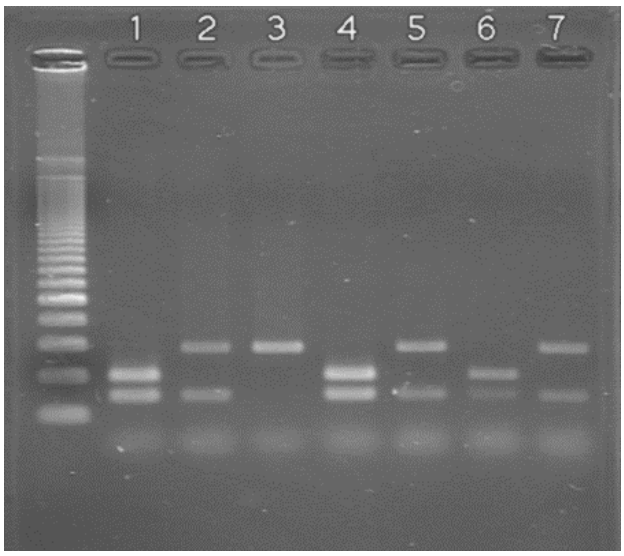

Fig. 2. Multiplex PCR (SP01 + SP02) of imported Otebo
1; Yukitebo, 2; Himetebo, 3; Gintebo, 4-7; Otebo

Table 3. Evaluation of genetic resources

	100 seeds weight (g)	SP01 290bp	SP02 390bp	SP02 560bp	SP02 800bp	SP03 450bp	u105 1000bp	u157 500bp	u245 500bp	u276 1300bp	u355 1200bp
Yukitebo	32.7	+	+	-	-	+	+	+	+	+	+
Pearl bean	17.2	+	+	-	-	+	-	-	+	+	-
Michigan pea bean	20.2	+	+	-	-	+	-	+	+	+	-
Merton	18.7	+	+	-	-	+	-	-	+	+	-
Total in 100 strains	-	15	38	55	7	64	34	64	38	20	11

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