E

BMT/7/12
ORIGINAL: English
DATE: November 6, 2001

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA-PROFILING IN PARTICULAR

Seventh Session
Hanover, Germany, November 21 to 23, 2001

DISTINCTNESS AND UNIFORMITY BASED ON DNA MARKERS IN SOYBEAN VARIETIES

prepared by experts from Japan

Distinctness and uniformity based on DNA markers in soybean varieties

M. Osono, T. Kimura and Y. Ban
National Center for Seeds and Seedlings, Ibaraki 305-0852, Japan

I. Introduction

In Japan, soybean is an important field crop for food and as a material of Japanese traditional seasonings, such as a soy sauce, *Tofu*, etc.

Based on a variation in growth habit, soybean is divided into three ecological types (Natsudaizu gata, Cyukan gata, Akidaizu gata) in Japan and is sensitive to photoperiod. Some soybean varieties belonging to the Akidaizu gata do not produce mature seeds at latitude 36° where soybean phenotypic characters are affected by environmental condition.

So difficulties are encountered when the Distinctness, Uniformity and Stability (DUS)-test for soybean is performed according to established procedures, e.g., comparison of morphological characters.

Currently biochemical and molecular techniques are well developed. These techniques have the potential of overcoming difficulties involved in the morphological comparison.

The research section of National Center for Seeds and Seedlings, Japan, has been investigating the effectiveness of DNA based markers in the field of DUS-test for several years. During this period, we have been trying to discriminate mainly some vegetatively propagated crops, vegetables, flowers and fruits, such as potato, strawberry, carnation and pear. RFLP, RAPD, AFLP, SSR primer analyses have been used to estimate the genetic distances (or similarities) among these. Almost all materials belonging to various kinds of plant groups could be discriminated. Thus DNA based markers have a high power of discriminating plant varieties of different species.

In this study we present results using DNA markers to test for genetic uniformity. The results on both distinctness between varieties and uniformity within varieties in soybean, based on DNA markers generated by PCR with SSR primer pairs, are presented.

II. Materials and Methods

Nine varieties were used for investigating distinctness, including two applicant varieties and 7 commercial varieties, that were named from A to I respectively. The number of individuals of each variety used for analyzing uniformity ranged from 8 to 30.

DNA was extracted from fully expanded fresh leaves of field-grown soybean plants using the extraction protocol of McDonald *et al.* (1994) with some modifications. PCR was conducted using the following conditions: Reaction mixture (20 µl) contained 2 µl 10×reaction buffer (10 mM Tris-HCl, pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 200 µM of each dATP, dTTP, dGTP and dCTP, 0.5 µM of each SSR primer , approximately 20 ng genomic DNA and 0.5 U of Taq polymerase (AmpliTaq Gold). The SSR primers used for amplification were developed by USDA-Agriculture Research Service (ARS) and sequence data of these primer pairs were

obtained from Soybase Internet Homepage. Twenty-three primer pairs were analyzed. Amplification was performed in 0.5 ml tube placed in thermal cycler (PERKIN ELMER PJ-2000). Amplification condition consists of 9 min activation of AmpliTaq Gold and denaturation step at 95°C and followed by 40 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C ending with a 7 min extension at 72°C. After thermal cycling, amplified products (3 to 5 µl) were separated by electrophoresis in 1.8% MetaPhor agarose gel (BMA products) and run in 0.5×TBE buffer at 15 V/cm for 1 h. The gel was stained with ethidium bromide (0.5 µg/l in distilled water) for 20 min and was destained for 15 min with distilled water.

The gel was photographed under UV light using a Charge Coupled Device (CCD) camera. The profiles were compared visually.

III. Results and Discussion

Inter varietal variation

Of 23 SSR primer pairs tested, 12 primer pairs showed polymorphism between varieties. Some of them showed highly polymorphic profiles and two of them grouped nine varieties into two groups (Fig. 1). Based on data generated by the three primer pairs, seven varieties could be discriminated (Table 1) but one pair of varieties (C and G) could not be discriminated with 12 primer pairs. These two varieties showed clear morphological differences (e.g., seed coat color).

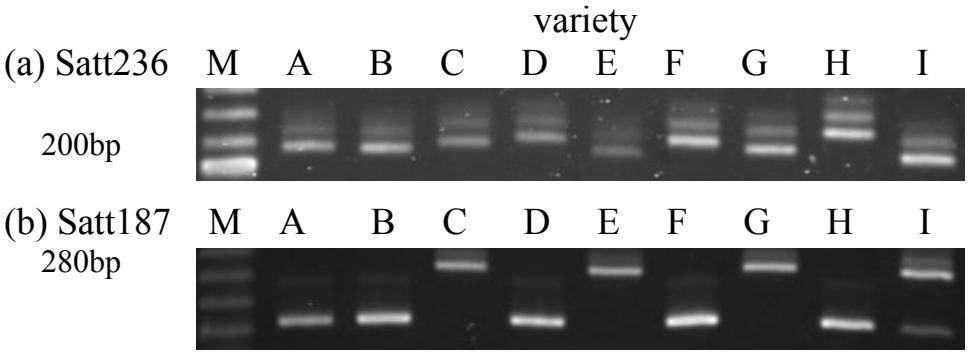

Figure 1. Polymorphisms detected among soybean varieties with SSR primer pairs (Satt236, Satt187)
(a) Nine varieties were divided into four groups
(b) Nine varieties were divided into two groups
M: size makers (20bp ladder markers)

Table 1. Discrimination of nine soybean varieties. Seven varieties could be discriminated with these DNA markers generated by three SSR primer pairs

Satt	Variety								
Markers	A	B	C	D	E	F	G	H	I
236 - 1	0*	0	0	0	0	0	0	1**	0
- 2	0	0	0	1	0	1	0	0	0
- 3	0	0	1	0	0	0	1	0	0
- 4	1	1	0	0	1	0	0	0	1
187 - 1	0	0	1	0	1	0	1	0	1
- 2	1	1	0	1	0	1	0	1	0
453 - 1	1	0	0	0	1	1	0	0	0
- 2	0	0	1	0	0	0	1	0	1
- 3	0	1	0	1	0	0	0	1	0

(C and G could not be discriminated)
*: band is absent. **: band is present

Intra varietal variation

We analyzed 188 individuals. Eight of nine varieties showed no intra varietal polymorphism. However within variety E, 3 out of 28 individuals analyzed showed three types of polymorphism. One showed polymorphism with primer pair, Satt079 (identified on No. 1), one showed polymorphism with Satt187 (No. 2), the third showed polymorphism with two primer pairs, Satt079 and Satt453 (No. 3) (Fig. 2). The other 14 individuals out of 28 showed the same profiles with Satt187 and Satt453 respectively.

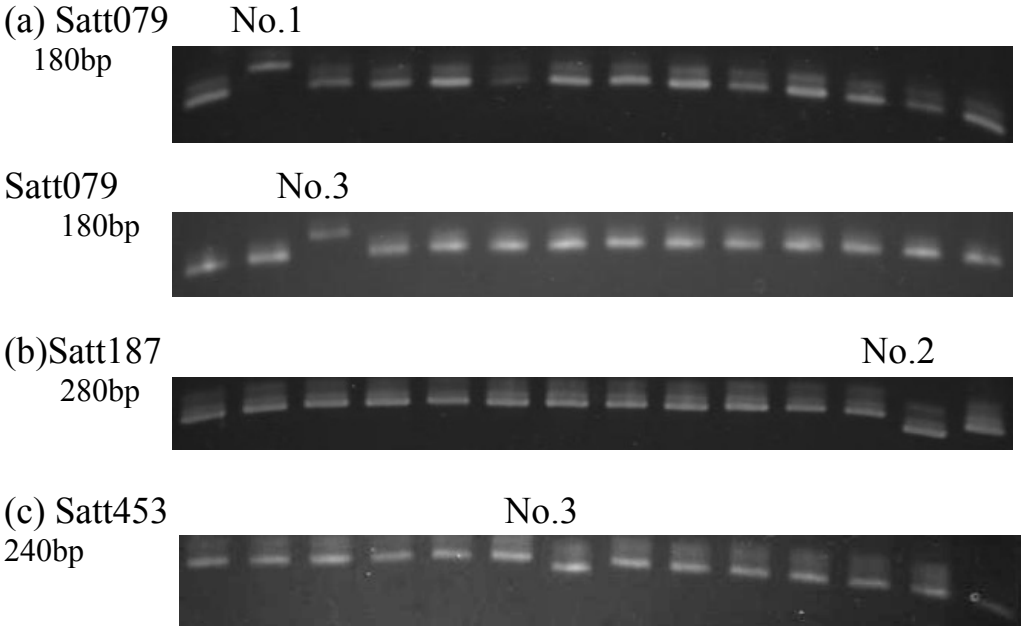

Figure 2. Polymorphisms detected within soybean variety E with three SSR primer pairs (Satt079, Satt187, Satt453). Three out of twenty-eight individuals analyzed showed polymorphisms. (a) and (b) No. 1 and No. 2 showed polymorphism with only one primer pair, Satt079 and Satt187 respectively. (a) and (c) No. 3 showed polymorphisms with two primer pairs, Satt079 and Satt453.

The possibility of seed contamination was checked.

No. 1 and No. 3 generated the same profile as variety F and I with Satt079, but was discriminated from F with Satt236 and from I with Satt277. With respect to No. 2, it could be discriminated from the other eight varieties with Satt187 (Fig. 3).

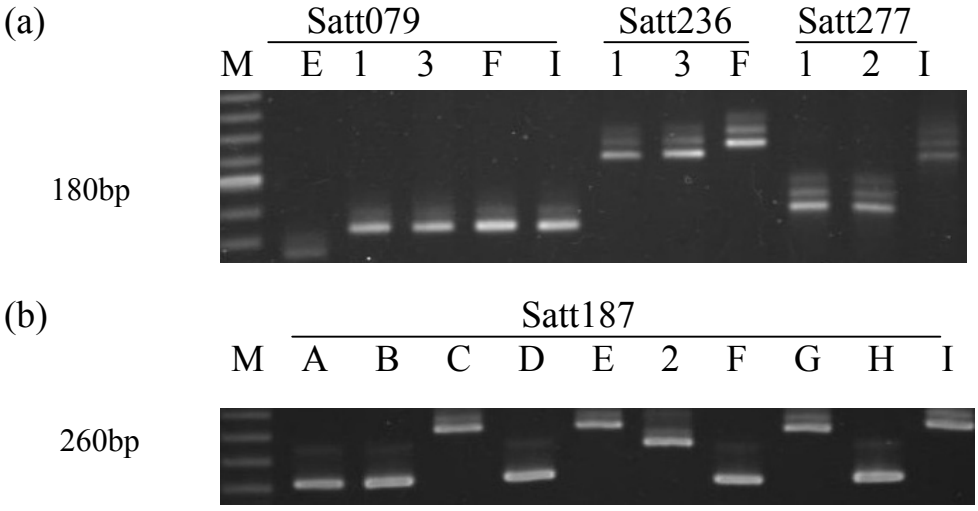

Figure 3. Seed contamination was checked. (a) No. 1 and No. 3 generated the same profile as F and I with primer pair, Satt079. But they could be discriminated from F with Satt236 and from I with Satt277. (b) No. 2 could be discriminated from the other eight varieties with Satt187. M: size markers (20bp ladder markers)

In addition to this result, observation of morphological characters indicated that there were no differences among all field-grown individuals of variety E (included No. 1, No. 2 and No. 3). These results indicate that the possibility of seed contamination was low.

We are now carrying out further analyses for genetic variation within variety E (another seed lot) and a few varieties related to variety E.

The present study revealed intra varietal genetic variation in an autogamous crop, the soybean. Phenotypic differences were not apparent within variety E, despite detection of genetic variation by means of DNA based markers. In this case DNA based marker analysis is a powerful tool for analyzing genetic variation as compared with morphological observation. As discussed in BMT sessions, in order to discriminate exactly between varieties, it is necessary to clarify the genetic variation both within and between varieties. Studies to determine the experimental conditions such as analysis techniques, sort of primer, number of primers and number of samples are currently in progress.

IV. References

McDonald, M. B., Elliot, L. J. and Sweeney, P. M. (1994). DNA extraction from dry seeds for RAPD analysis in varietal identification studies. Seed Sci. & Technol. 22:171-176.

Sakamoto, S., J. Abe, A. Kanazawa and Y, Shimamoto (2001). Analysis and discussion of origin and dissemination in cultivated soybean based on SSR markers. Breeding Research. Vol. 3: Supplement No. 2: p. 296.

Suzuki, Y., D. H. Xu, J. Abe and Y. Shimamoto (2001). Genetic differentiation of soybean revealed nuclear SSRs. Breeding Research. Vol. 3: Supplement No. 2: p. 297.

[End of document]