

## Phylogenetic Analysis of Vegetable-type (Edamame) and Grain-type Soybean [*Glycine max*(L.) Merr.] Cultivars Through ISSR Markers

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**Abstract:** ISSR markers are reported to be highly polymorphic and to be useful in studies on genetic diversity. Analysis of the diversity in edamame cultivars would aid breeders by making a cross choice of parents. Thirty-seven edamame cultivars/lines and 13 grain-type ones were used in ISSR analysis. Of a total of 50 ISSR primers used 11 primers showed no amplified fragments. Another 39 primers produced 132 bands, of which 81 were polymorphic, accounting for 61.4%. The number of amplified bands varied from 1 to 7, with a size range from 830 to 3530 bp. The average numbers of bands per primer and polymorphic ones were 3.3 and 2.1, respectively. A dendrogram based on UPGMA analysis grouped 50 cultivars/lines into 3 main clusters. Taikadaizu, fasciated-type soybean, appeared to be distinct from all others. Group B comprised 7 grain-type cultivars, most of them developed in China. Group C comprised all Japanese edamame cultivars/lines and 4 grain-type ones. Genotypes grouped in the group C were divided into several subgroups. Among the subgroups, cultivars grouped in the same subgroups had identical characters, for example a subgroup comprised all cultivars with brown hilum and seed coat, white flower and the pod which was not easy to open. Selected cultivars and their original cultivars showed closely relationships in the dendrogram. These results indicated that the dendrogram based on ISSR reflected the genetic relationships of edamame cultivars, and the genetic diversity existed between edamame and grain-type soybeans. The preservation of edamame germ-plasms would be useful for the better soybean project in the future.

**Key words:** Soybean; ISSR Marker; Diversity

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## 利用 ISSR 标记进行菜用大豆和普通大豆的分类

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**摘要:** ISSR 标记技术因具有很高的多态性而被广泛应用于遗传多样性研究。进行菜用大豆品种多样性研究将有助于育种家进行杂交亲本的合理选配。采用 ISSR 标记技术对 37 个菜用大豆品种(系)和 13 个普通大豆品种(系)进行了分类研究。结果表明: 50 个 ISSR 引物中有 11 个引物没有多态性, 其他 39 个引物共产生 132 条谱带, 其中 81 条多态性谱带, 占 61.4%。引物扩增的谱带数在 1~7 条之间, 大小为 830~3530 bp。每个引物平均扩增的谱带数和多态性带数分别为 3.3 和 2.1。聚类分析结果表明, 可将供试的 50 个品种划分为 3 个类组, 扁茎大豆 Taikadaizu 单独一组, 7 个普通大豆(多数为中国选育)划归为 B 组, C 组由日本菜用大豆和 4 个普通大豆组成。C 组还可以划分成若干亚组, 同一亚组的品种具有相似的性状, 比如有一亚组的品种均是褐脐、茶色种皮、白花、不易炸荚。系选的品种和原始品种在分类图中也显示出其密切的亲缘关系。基于 ISSR 标记的分类可以反映菜用大豆的遗传关系, 菜用大豆和普通大豆之间存在遗传多样性, 合理保存菜用大豆种质将有助于未来大豆品质改良计划。

**关键词:** 大豆; ISSR 标记; 多样性

## 1 Introduction

In recent years, soybean (*Glycine max*(L.) Merr.) products are more related with the life of human beings, soybean production developed very fast. Soybean could be classified into different types as the needs of consumers are different, such as grain-type soybean and vegetable-type soybean. Vegetable-type soybean (called edamame in Japan or maodou in China) are large-seeded soybean harvested as green pods at the R6 stage<sup>[1]</sup>, the seed are approximately 80% matured and with a sweet, nutty flavor. Masuda<sup>[2]</sup> reported edamame had nearly 56% more protein content than that of green peas (*Pisum sativum*). As edamame has low oil content and the relatively high protein content, it is particularly desirable to the health conscious people seeking low fat, high protein snacks<sup>[3]</sup>. Monma et al<sup>[4]</sup> found that green immature soybeans had carotenoid profiles similar to those of green vegetables.

The demand for edamame as fresh or frozen vegetable is increasing worldwide. Currently, USA imports more than 10 000 Mg of frozen edamame each year<sup>[5]</sup>. However, lack of adapted edamame varieties is one of the major factors which limit edamame commercial production in the world. Some edamame traits are waiting to be improved such as the adaptability to mechanical harvest and yield. Japanese edamame genotypes may be utilized as a source of edamame traits in breeding programs<sup>[6]</sup>. Edamame is one of Japanese traditional foods, compared with other countries, there are a lot of edamame varieties in Japan. Knowing the relationship between edamame and grain cultivars would give breeders some hints as to how to improve edamame. It is necessary to find a proper molecular technique for evaluating and identifying the genetic backgrounds of vegetable soybean, especially the genetic diversity between vegetable-type and grain-type soybean varieties or/and among the vegetable-type soybean varieties, which would benefit for the genetic improvement of edamame.

ISSR makers are highly polymorphic and are useful in studies on genetic diversity, gene tagging, phylogeny, genome mapping and evolutionary biology<sup>[7]</sup>. This technique has been used in genetics and plant breeding in a wide range of crops, for example, Joshi et al<sup>[8]</sup> eval-

uated the genetic diversity and phylogenetic relationship of the genus *Oryza* and Qian et al<sup>[9]</sup> analyzed the genetic variation within and among populations of a wild *Oryza granulata* from China. Kantety et al<sup>[10]</sup> assessed the genetic diversity in dent and popcorn (*Zea mays* L.) inbred lines. Also researchers used the technique of ISSR to evaluate some vegetable crops or fruit plants: Ajibade et al<sup>[11]</sup> studied the genetic relationships in the genus *Vigna* and McGregor<sup>[12]</sup> et al studied the tetraploid potato (*Solanum tuberosum* L.) germplasm. Moreno et al<sup>[13]</sup> and Sanker et al<sup>[14]</sup> studied the grapevine and Citrus germplasm respectively. ISSR makers are reported to be highly polymorphic and to be useful in studies on genetic diversity. The objective of this study was to demonstrate genetic diversity of edamame cultivars by ISSR markers.

## 2 Materials and Methods

This experiment included 50 cultivars, among them, 37 were vegetable-type soybeans (edamame), 13 were grain-type soybeans (see Table 1). For analysis of edamame diversity by ISSR markers, we used all of 37 edamame cultivars kept in Sato Masayuki Seed Company in Iwate prefecture of Japan, 5 popularly adapted grain-type cultivars in the north of Japan, one of fasciated-type soybean, and 7 cultivars / lines developed at Shenyang Agricultural University, China.

For seedling culture, each cultivar/line was sown in 6 pots on May 21<sup>st</sup> in paper pot (diameter 5 cm, 10 cm high), in which filled with soil. One seed was planted in each pot. All the pots were maintained in the plastic greenhouse, and watered every 3 days. All the seedlings were transferred into field when they were at 1 trifoliolate leaf stage (V1). Each cultivar/line was planted in one row, the row space was 1 m, plant distance was 0.3 m. During the soybean growth period, the leaf shape index (length/width of the matured middle leaflet at blooming stage), the days from emergence to blooming, the blooming duration, the days of blooming to maturity, maturity, and flower color were measured in the field. As the plants matured, all plants were harvested and the color of seed-coat and hilum, pod split (easy to open = over 50% pods open by itself as matured plant drying in greenhouse for a week, not easy to open = less than half of pods open by itself as ma-

tured plant drying in greenhouse for a week, tight = no pods open by itself as matured plant drying in greenhouse for a week), and grain weight per plant were measured.

Table 1 List of soybean cultivars/lines

| Type      | Number of entry | Name of cultivars/lines  | Origin           |
|-----------|-----------------|--------------------------|------------------|
| Vegetable | 1               | Banotome                 | Japan            |
|           | 2               | Ryokuryo                 | Iwate, Japan     |
|           | 3               | Hiratadadacya            | Japan            |
|           | 4               | Akita - midori           | Akita, Japan     |
|           | 5               | Kemame                   | Japan            |
|           | 6               | Misuzu kuro              | Japan            |
|           | 7               | Hihou                    | Japan            |
|           | 8               | Hatsumusume              | Japan            |
|           | 9               | Mikawashima              | Chiba, Japan     |
|           | 10              | Kinkaori                 | Japan            |
|           | 11              | Kurosaki cyamame         | Japan            |
|           | 12              | Taiwan - takao           | Taiwan           |
|           | 13              | Goyozairai               | Iwate, Japan     |
|           | 14              | Takihime                 | Japan            |
|           | 15              | Hakoirimusume            | Japan            |
|           | 16              | Akahige                  | Japan            |
|           | 17              | Kaorimame                | Japan            |
|           | 18              | Kyosanto                 | Japan            |
|           | 19              | Kitanokaori              | Japan            |
|           | 20              | Echigohani               | Japan            |
|           | 21              | Echigomame               | Japan            |
|           | 22              | Gankuimonogatari         | Japan            |
|           | 23              | Kurozokin                | Kyoto, Japan     |
|           | 24              | Akamame                  | Kyoto, Japan     |
|           | 25              | Ajiichiban               | Japan            |
|           | 26              | Hideh                    | Japan            |
|           | 27              | Hitorimusume             | Japan            |
|           | 28              | Kaorihime                | Japan            |
|           | 29              | Hashikamiwase            | Aomori, Japan    |
|           | 30              | Jobojizairai             | Iwate, Japan     |
|           | 31              | Iwate - midori           | Iwate, Japan     |
|           | 32              | Shonai 1                 | Yamakata, Japan  |
|           | 33              | Shirayamadadacya         | Yamakata, Japan  |
|           | 34              | Murasakidadacya          | Yamakata, Japan  |
|           | 35              | Banshakucyamame          | Japan            |
|           | 36              | Kurogoyou                | Fukushima, Japan |
|           | 37              | Higan - ao               | Japan            |
| Grain     | 38              | Wasesuzunari             | Akita, Japan     |
|           | 39              | Okushirome               | Akita, Japan     |
|           | 40              | Suzukari                 | Akita, Japan     |
|           | 41              | Osuzu                    | Akita, Japan     |
|           | 42              | Shennong 6               | Liaoning, China  |
|           | 43              | Shennong 7               | Liaoning, China  |
|           | 44              | Shennong 8               | Liaoning, China  |
|           | 45              | Shennong A31 Roundleaf   | Liaoning, China  |
|           | 46              | Shennong A31 Narrowleaf  | Liaoning, China  |
|           | 47              | Shennong 6059 Roundleaf  | Liaoning, China  |
|           | 48              | Shennong 6059 Narrowleaf | Liaoning, China  |
|           | 49              | Menashi                  | Iwate, Japan     |
|           | 50              | Taikadaizu               | Iwate, Japan     |

As the soybean seedlings were at about 6 trifoliate leaf stage, about 100 mg young leaf tissues were taken for each cultivar, and the DNA was isolated from the leaves using QIAGEN Dneasy Plant Mini Kit. All

isolated DNA samples were kept at -86°C for storage.

ISSR primers were from British Columbia University of Canada, the series of the primers was from UBC 801 to UBC 850 (Table 2). PCR amplification was per-

Table 2 Sequences of 50 ISSR primers and their PCR amplified bands

| Primer | Primer sequence      | Total bands | Polymorphic bands |
|--------|----------------------|-------------|-------------------|
| UBC801 | (AT) <sub>8</sub> T  | 3           | 3                 |
| UBC802 | (AT) <sub>8</sub> G  | 0           | 0                 |
| UBC803 | (AT) <sub>8</sub> C  | 0           | 0                 |
| UBC804 | (TA) <sub>8</sub> A  | 0           | 0                 |
| UBC805 | (TA) <sub>8</sub> C  | 0           | 0                 |
| UBC806 | (TA) <sub>8</sub> G  | 0           | 0                 |
| UBC807 | (AG) <sub>8</sub> T  | 5           | 5                 |
| UBC808 | (AG) <sub>8</sub> C  | 5           | 2                 |
| UBC809 | (AG) <sub>8</sub> G  | 2           | 1                 |
| UBC810 | (GA) <sub>8</sub> T  | 5           | 2                 |
| UBC811 | (GA) <sub>8</sub> C  | 6           | 4                 |
| UBC812 | (GA) <sub>8</sub> A  | 6           | 3                 |
| UBC813 | (CT) <sub>8</sub> T  | 4           | 3                 |
| UBC814 | (CT) <sub>8</sub> A  | 4           | 4                 |
| UBC815 | (CT) <sub>8</sub> G  | 2           | 2                 |
| UBC816 | (CA) <sub>8</sub> T  | 3           | 3                 |
| UBC817 | (CA) <sub>8</sub> A  | 3           | 0                 |
| UBC818 | (CA) <sub>8</sub> G  | 3           | 3                 |
| UBC819 | (GT) <sub>8</sub> A  | 2           | 0                 |
| UBC820 | (GT) <sub>8</sub> C  | 1           | 0                 |
| UBC821 | (GT) <sub>8</sub> T  | 3           | 3                 |
| UBC822 | (TC) <sub>8</sub> A  | 2           | 2                 |
| UBC823 | (TC) <sub>8</sub> C  | 7           | 6                 |
| UBC824 | (TC) <sub>8</sub> G  | 4           | 4                 |
| UBC825 | (AC) <sub>8</sub> T  | 3           | 0                 |
| UBC826 | (AC) <sub>8</sub> C  | 3           | 1                 |
| UBC827 | (AC) <sub>8</sub> G  | 2           | 1                 |
| UBC828 | (TG) <sub>8</sub> A  | 4           | 2                 |
| UBC829 | (TG) <sub>8</sub> C  | 3           | 2                 |
| UBC830 | (TG) <sub>8</sub> G  | 3           | 2                 |
| UBC831 | (AT) <sub>8</sub> YA | 0           | 0                 |
| UBC832 | (AT) <sub>8</sub> YC | 0           | 0                 |
| UBC833 | (AT) <sub>8</sub> YG | 0           | 0                 |
| UBC834 | (AG) <sub>8</sub> YT | 3           | 3                 |
| UBC835 | (AG) <sub>8</sub> YC | 3           | 2                 |
| UBC836 | (AG) <sub>8</sub> YA | 5           | 3                 |
| UBC837 | (TA) <sub>8</sub> RT | 0           | 0                 |
| UBC838 | (TA) <sub>8</sub> RC | 0           | 0                 |
| UBC839 | (TA) <sub>8</sub> RG | 0           | 0                 |
| UBC840 | (GA) <sub>8</sub> YT | 2           | 1                 |
| UBC841 | (GA) <sub>8</sub> YC | 3           | 2                 |
| UBC842 | (GA) <sub>8</sub> YG | 5           | 1                 |
| UBC843 | (CT) <sub>8</sub> RA | 1           | 0                 |
| UBC844 | (CT) <sub>8</sub> RC | 3           | 2                 |
| UBC845 | (CT) <sub>8</sub> RG | 2           | 0                 |
| UBC846 | (CA) <sub>8</sub> RT | 3           | 2                 |
| UBC847 | (CA) <sub>8</sub> RC | 1           | 0                 |
| UBC848 | (CA) <sub>8</sub> RG | 4           | 2                 |
| UBC849 | (GT) <sub>8</sub> YA | 6           | 5                 |
| UBC850 | (GT) <sub>8</sub> YC | 3           | 0                 |
| Total  |                      | 132         | 81                |

\* Y = (C, T) R = (A, G)

formed in  $20 \text{ ng} \cdot \mu\text{l}^{-1}$  genomic DNA  $7.5 \mu\text{l}$ ,  $15 \mu\text{M}$  ISSR primers  $0.26 \mu\text{l}$ ,  $10 \times$  PCR Buffer  $2.0 \mu\text{l}$ ,  $2.5 \text{ mM}$  dNTPmix  $1.6 \mu\text{l}$ , Taq DNA polymerase  $0.1 \mu\text{l}$ , sterilized water  $8.54 \mu\text{l}$ , the total solution was  $20 \mu\text{l}$ , using a TaKaRa PCR Thermal Cycler (MP TP3000). The procedure was that after 3 min at  $94^\circ\text{C}$ , 45 cycles were performed with 1 min at  $94^\circ\text{C}$ , 1 min at  $48^\circ\text{C}$ , 2 min at  $72^\circ\text{C}$ , and a final extension step of 5 min at  $72^\circ\text{C}$ , and then kept at  $4^\circ\text{C}$ . The PCR amplified products were analyzed by 1% agarose gel electrophoresis in  $1 \times$  TAE buffer under the 200V for 45 min, and then put the gels into ethidium bromide solution for 20 min. The marker was  $\lambda$ DNA/EcoR I + Hind III.

The PCR products were detected by 1% agarose-ethidium bromide system. If it had a band, we recorded it "1", if no band, it was recorded by "0". After then the data were analyzed by InforBIO software for the dendrogram based on UPGMA analysis.

### 3 Results

Of a total of 50 ISSR primers used (UBC801-UBC850), 11 primers showed no amplified fragments. Another 39 primers produced 132 bands, of which 81 (i. e., 61.4%) were polymorphic. The number of amplified bands varied from 1 to 7, with a size range from 830 to 3530 bp (see Fig. 1). The average numbers of bands per primer and polymorphic bands were 3.3 and 2.1, respectively.

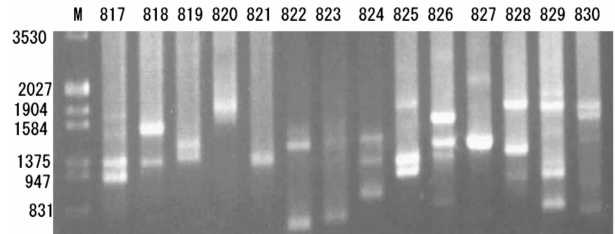


Fig. 1 Fragments amplified by ISSR analysis using various ISSR primers for Shennong 8'

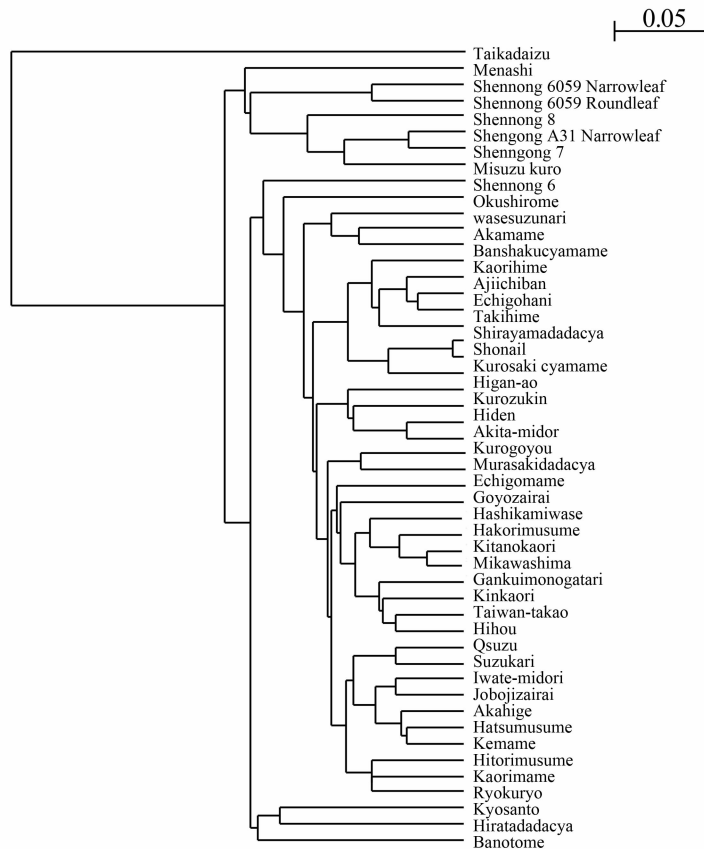


Fig. 2 Dendrogram generated using unweighted pair group method with arithmetic average analysis (UPGMA) using ISSR data, showing relationships between edamame and grain-type cultivars

After data were analyzed by InforBIO software, a dendrogram based on UPGMA analysis grouped 50 cul-

tivars/lines into 3 main clusters (Fig. 2). Taikadaizu', fasciated-type soybean, appeared to be distinct from all

others. Group B comprised 7 grain-type cultivars (Shengnong 7, Shengnong 8, Shengnong A31Roundleaf, Shengnong A31Narrowleaf, Shengnong 6059Roundleaf, Shengnong 6059Narrowleaf, and Japanese cultivar Menashi), most of them developed in China.

Group C comprised all Japanese edamame cultivars/lines and 4 grain-type ones. Genotypes grouped in the group C were divided into several subgroups. If the division genetic distance was at the 0.071, this group could be divided into 9 sub-groups (Table 3). Among

Table 3 Classification of Edamame and grain-type cultivars and their agronomic characteristics

| Group | Cultivar                  | Leaf Shape index | Emergence - blooming /day | Blooming duration /day | Blooming - maturity /day | Maturity /day | Seed coat | Hilum | Pod | Flower | Yield /g · plant <sup>-1</sup> |
|-------|---------------------------|------------------|---------------------------|------------------------|--------------------------|---------------|-----------|-------|-----|--------|--------------------------------|
| A     | Taikadaizu +              | 1.59             | 79.0                      | 12.7                   | 80.0                     | 159           | Y         | Br    | ++  | W      | 21.78                          |
| B-1   | Menashi +                 | 1.41             | 56.0                      | 24.0                   | 103.0                    | 159           | Y         | Br    | +++ | W      | 20.33                          |
| B-2   | Shennong 6059Narrowleaf + | 3.41             | 56.3                      | 18.7                   | 80.7                     | 137           | Y         | Br    | +++ | W      | 46.04                          |
|       | Shennong 6059Roundleaf +  | 1.72             | 56.4                      | 18.6                   | 80.6                     | 137           | Y         | Br    | +++ | W      | 58.39                          |
|       | Shennong 8 +              | 1.34             | 50.2                      | 20.6                   | 86.8                     | 137           | Y         | Br    | ++  | P      | 50.27                          |
|       | Shennong A31Narrowleaf +  | 2.66             | 52.0                      | 19.0                   | 85.0                     | 137           | Y         | W     | +++ | W      | 55.95                          |
|       | Shennong A31Roundleaf +   | 1.54             | 52.8                      | 18.2                   | 84.2                     | 137           | Y         | W     | +++ | W      | 49.82                          |
|       | Shennong 7 +              | 1.72             | 43.0                      | 37.0                   | 94.0                     | 137           | Y         | Br    | +++ | P      | 60.14                          |
| C-1   | Misuzu kuro               | 1.59             | 53.5                      | 23.5                   | 83.5                     | 137           | B         | B     | +   | W      | 88.30                          |
| C-2   | Shennong 6 +              | 1.47             | 52.2                      | 16.8                   | 82.8                     | 135           | Y         | W     | ++  | W      | 56.39                          |
| C-3   | Okushirome +              | 2.02             | 50.0                      | 19.0                   | 85.0                     | 135           | Y         | W     | +++ | P      | 42.73                          |
|       | Wasesuzunari +            | 1.98             | 37.3                      | 21.8                   | 66.8                     | 104           | Y         | W     | +++ | P      | 19.48                          |
|       | Akamame                   | 1.53             | 73.0                      | 25.0                   | 86.0                     | 159           | R         | B     | +   | W      | 41.27                          |
| C-4   | Banshakucyamame           | 1.57             | 55.5                      | 14.5                   | 81.5                     | 137           | Br        | Br    | ++  | W      | 11.56                          |
|       | Kaorihime                 | 1.54             | 52.7                      | 21.3                   | 76.3                     | 129           | Br        | Br    | ++  | W      | 35.02                          |
|       | Ajichiban                 | 1.68             | 51.0                      | 16.0                   | 68.0                     | 119           | Br        | Br    | ++  | W      | 31.52                          |
|       | Echigohani                | 1.38             | 54.0                      | 19.5                   | 75.0                     | 129           | Br        | Br    | ++  | W      | 13.52                          |
|       | Takihime                  | 1.61             | 41.0                      | 13.5                   | 59.0                     | 100           | Br        | Br    | ++  | W      | 18.28                          |
|       | Shirayamadadacya          | 1.52             | 52.0                      | 18.2                   | 77.0                     | 129           | Br        | Br    | ++  | W      | 23.85                          |
|       | Shonai 1                  | 1.70             | 43.5                      | 14.5                   | 85.3                     | 128           | Br        | Br    | ++  | W      | 18.12                          |
|       | Kurosakicyamame           | 1.47             | 45.3                      | 19.0                   | 83.8                     | 129           | Br        | Br    | ++  | W      | 12.93                          |
| C-5   | Higan - ao                | 1.43             | 65.8                      | 18.0                   | 85.3                     | 151           | G         | B     | +   | P      | 30.08                          |
|       | Kurozokin                 | 1.81             | 32.0                      | 18.3                   | 68.0                     | 100           | B         | B     | ++  | W      | 13.48                          |
|       | Hiden                     | 1.26             | 61.7                      | 14.0                   | 91.3                     | 153           | G         | Br    | +   | P      | 50.31                          |
|       | Akita - midori            | 1.56             | 58.3                      | 25.0                   | 90.0                     | 148           | G         | B     | +   | P      | 91.33                          |
| C-6   | Kurogoyou                 | 1.55             | 55.8                      | 14.7                   | 73.2                     | 129           | B         | B     | +   | P      | 54.08                          |
|       | Murasakidadacya           | 1.39             | 55.7                      | 20.0                   | 81.3                     | 137           | Br        | Br    | +   | P      | 32.97                          |
|       | Echigomame                | 1.55             | 68.0                      | 23.0                   | 85.0                     | 153           | G         | B     | +   | p      | 43.82                          |
|       | Goyozairai                | 1.74             | 50.0                      | 24.2                   | 99.0                     | 149           | G         | B     | +   | W      | 41.41                          |
|       | Hashikamiwase             | 1.47             | 58.4                      | 20.8                   | 77.8                     | 136           | Br        | Br    | +   | W      | 62.95                          |
|       | Hakoirimusume             | 1.58             | 58.8                      | 22.3                   | 94.3                     | 153           | G         | Br    | +   | P      | 62.24                          |
|       | Kitanokaori               | 1.76             | 34.0                      | 19.0                   | 70.0                     | 104           | G         | Br    | ++  | W      | 21.29                          |
|       | Mikawashima               | 1.76             | 42.5                      | 21.5                   | 61.5                     | 104           | Y         | W     | +   | W      | 23.21                          |
|       | Gankuimonogatari          | 1.55             | 50.5                      | 24.5                   | 101.5                    | 152           | B         | B     | +   | P      | 27.42                          |
|       | Kinkaori                  | 1.78             | 34.0                      | 17.4                   | 67.0                     | 101           | G         | Br    | +   | W      | 16.22                          |
|       | Taiwan - takao            | 1.58             | 34.0                      | 19.0                   | 66.0                     | 100           | B         | B     | ++  | W      | 17.06                          |
|       | Hihou                     | 1.56             | 64.0                      | 26.0                   | 95.0                     | 159           | G         | W     | +   | P      | 55.06                          |
|       | Osuzu +                   | 1.55             | 50.0                      | 19.0                   | 85.0                     | 135           | Y         | W     | ++  | P      | 50.36                          |
|       | Suzukari +                | 1.86             | 50.0                      | 18.5                   | 85.0                     | 135           | Y         | W     | ++  | P      | 42.47                          |
|       | Iwate - midori            | 1.74             | 59.3                      | 16.7                   | 93.7                     | 153           | G         | B     | +   | P      | 53.44                          |
|       | Jobojizairai              | 1.55             | 55.0                      | 21.0                   | 98.0                     | 153           | G         | B     | +   | P      | 53.53                          |
|       | Akahige                   | 1.45             | 56.3                      | 18.8                   | 92.8                     | 149           | G         | B     | +   | P      | 57.74                          |
|       | Hatsumusume               | 1.49             | 58.0                      | 18.3                   | 79.0                     | 137           | G         | B     | +   | P      | 62.99                          |
|       | Kemame                    | 1.48             | 52.5                      | 24.5                   | 84.5                     | 137           | G         | B     | +   | P      | 66.89                          |
|       | Hitorimusume              | 1.58             | 66.3                      | 23.7                   | 92.7                     | 159           | G         | B     | +   | P      | 35.93                          |
|       | Kaorimame                 | 1.50             | 59.3                      | 25.0                   | 93.7                     | 153           | G         | Br    | +   | P      | 46.89                          |
|       | Ryokuryo                  | 1.67             | 56.0                      | 27.5                   | 73.0                     | 129           | G         | Br    | +   | P      | 70.78                          |
| C-7   | Kyosanto                  | 1.44             | 60.7                      | 23.7                   | 88.3                     | 149           | Y         | Br    | ++  | P      | 105.33                         |
| C-8   | Hiratadadacya             | 1.36             | 56.2                      | 20.0                   | 72.8                     | 129           | Br        | Br    | ++  | P      | 48.48                          |
| C-9   | Banotome                  | 1.46             | 51.0                      | 16.3                   | 78.0                     | 129           | Br        | Br    | ++  | W      | 37.24                          |

+ grain - type cultivar;

\* Seed - coat; Y = Yellow, G = Green, B = Black, Br = Brown; \*\* Hilum; W = White, B = Black, Br = Brown;

\*\*\* Pod; + = easy to open, ++ = not easy to open, +++ = tight; \*\*\*\* Flower; W = White, P = Purple

the 9 sub-groups, in the sub-groups C-1, C-2, C-7, C-8, C-9 each subgroup had only one cultivar, which meant these cultivars had their specific characteristics. Among the subgroups, subgroup C-4 comprised all cultivars with brown hilum and seed coat, white flower, and the pod which was not easy to open (e. g. Kurosakicyamame, Shonai, Shirayamadacya, Takihime, Echigohani, Ajiichiban, Kaorihime, Bansyakucyamame). In subgroup C-6, the genetic distances of 8 edamame soybean cultivars with green seed coat were close to the grain-type cultivars Suzukari and Osuzu.

Selected cultivars and their original cultivars showed closely relationships in the dendrogram (Wasesuzunari- Okushirome, Shonai 1- Shirayamadacya). These results indicated that the dendrogram based on ISSR reflected the genetic relationships of edamame cultivars.

Among different cultivars the total amplified bands of the 50 ISSR primers ranged from 84 (Taikadaizu) to 110 (Wasesuzunari), the average band number of each primer was from 2.15 to 2.82.

Different soybean varieties had different genetic backgrounds, some varieties had specific polymorphic bands or no band. For example there were some specific bands for Taikadaizu at 1584 – 1375bp of primer 801, 831bp and < 831bp of primer 807, 1904bp and 947bp of primer 811, 1584- 1375bp of primer 823. There were no bands at 1584bp of primer 807, 1584bp of primer 815, but there were some specific bands at 2027- 1904bp and 1375bp of primer 812, 1904bp of primer 821, 1584bp of primer 829 for Menashi cultivar, a specific cultivar for tufu use in Iwate prefecture, Japan.

Compared with Okushirome, Wasesuzunari, which developed from Okushirome by radiation, had specific bands at 2027-1904bp of primer 801, 1584bp of primer 816, 3530-2027bp and 1375bp of primer 818, 1904bp and 1584-1375bp of primer 821, 947bp of primer 834, 1904bp of primer 846, but no bands at 1375bp of primer 816, 1904-1584bp of primer 823.

Some lines were from the same parents, but the leaf shape was different between the lines, one was round leaf, other was narrow leaf. Shennong

6059Roundleaf and Shennong 6059Narrowleaf was a pair, the other was Shennong A31Roundleaf and Shennong A31Narrowleaf. Different bands between Shennong 6059 Roundleaf and Shennong 6059Narrowleaf were at 947bp of primer 813, 1584bp of primer 815, 3530bp of primer 823, 947-831bp of primer 828, 2027-1904bp of primer 836, 1375bp of primer 836, 947bp of primer 842, 3530- 2027bp of primer 844. Different bands between Shennong A31Roundleaf and Shennong A31 Narrowleaf were at 2027-1907bp of primer 801, 947bp of primer 813, 1375bp of primer 816, 947bp of primer 836, 947-831bp of primer 849.

Also some cultivars had some specific common bands, for example, Hashikamiwase (29), Wasesuzunari (38) and Okushirome (39) had a common specific band at 1584-1375bp of primer 844 (see Fig. 3).

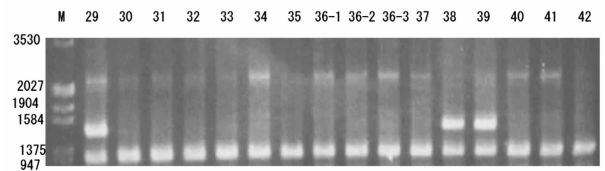


Fig. 3 Fragments amplified by ISSR analysis using ISSR primer 844 for several edamame cultivars. Cultivars of entry 29, 38 and 39 have a common band at 1584-1375bp.

\* 36-1, 36-2, 36-3 represent different plants of the same edamame cultivar Kurogoyou

## 4 Discussion

The lack of genetic diversity may limit crop breeding progress, soybean genetic diversity is important for the improvement of traits related with yield, grain quality and pest resistance<sup>[15-20]</sup>. North America soybean breeding progress has rested upon a narrow genetic base: 14 dominant ancestors introduced to North America prior in 1930. Carter et al<sup>[21]</sup> demonstrated that several Chinese soybean cultivars performed well agronomically in North America, Chinese cultivars may be an important reservoir of diversity with which to expand the genetic base of North American breeding program<sup>[21]</sup>. The physical distinctness of North American and Chinese varieties shows that introgression of Chinese soybean cultivars into North American breeding should broaden the morphological, agronomic, and biochemical

diversity of North American germplasms. Introgression may be accomplished most effectively by avoiding crossing of Chinese and North American cultivars from the same phenotypic cluster<sup>[22]</sup>.

Populations derived from biparental crosses of diverse northern parents of America were more likely to have a big genetic variance for yield than were populations derived from crosses of parents that are more related<sup>[23-24]</sup>. Studies indicate that sufficient genetic variation for yield may be obtained from some elite × elite crosses, but that limited genetic diversity between parents renders many crosses useless. As the development of edamame, avoiding matings of the cultivars from the same cluster also was of importance. This experiment data showed that genetic diversity existed between edamame and grain-type soybeans, therefore, the preservation of edamame germplasms would be useful for the better soybean project in the future.

In the soybean pedigree analyses, molecular analyses of diversity were used effectively. RFLP<sup>[25-27]</sup> and SSR<sup>[28]</sup> analyses have shown the clear separation of northern and southern (US) cultivars and the limited diversity in the southern gene pool. RAPD markers could be used for analyzing the relationship of primitive cultivars genetically isolated in relatively small geographical areas<sup>[29-30]</sup>. Also, RAPD markers could be used for exploiting the genetic diversity in the two species (*Glycine max*, *Glycine soja*) in breeding programs and in sampling and managing germplasm collections<sup>[31]</sup>. ISSR makers were reported to be highly polymorphic and be useful in studies on genetic diversity. This experiment demonstrated the genetic diversity of edamame cultivars by ISSR markers.

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## 第二届全国大豆科研生产研讨会简讯

由中国作物学会大豆专业委员会主办,安徽省农业委员会、安徽省农业科学院、阜阳市人民政府联合承办的第二届全国大豆科研生产研讨会于2008年8月28日至8月30日在安徽省阜阳市召开。共有来自全国21个省、自治区和直辖市的280多名代表参加了会议。

会议期间,举行多场报告会、专题研讨会,并邀请农业部种植业管理司曾令清处长、原中国农业科学院王连铮院长、南京农业大学盖钧镒院士、中国农业科学院常汝镇研究员、中国科学院遗传与发育生物学研究所陈受宜研究员做特约报告,会议通报了我国大豆产业的政策、形势和国内外相关领域的最新研究动态,研讨全国和黄淮海地区大豆生产发展的问题和途径,交流各地大豆科研和生产领域的最新进展。共有55名代表在大会作报告,内容涉及大豆的遗传育种、耕作栽培、加工、病虫害防治等多个方面。会议还邀请温特斯泰格公司等一些企业代表,有力促进了大豆科研与生产的结合。会议期间与会代表参观了新品种展示田和大面积高产示范田。

在大会闭幕式上,颁发了王金陵青年科教奖励基金——首届王金陵青年科教奖励基金由黑龙江省农业科学院合江分院郭泰研究员获得。中国作物学会大豆专业委员会副理事长、秘书长韩天富研究员对大会进行总结并介绍了第八届世界大豆研究大会筹备工作。最后,中国作物学会大豆专业委员会理事长盖钧镒院士就我国大豆产业形式、大豆科研及大豆科技队伍的发展等方面提出了宝贵的建议,同时邀请各位代表积极支持并参加将于2009年8月在北京举办的第八届世界大豆研究大会。

宋显军  
《大豆科学》编辑部