[Short Report]

Genetic Diversity of Cambodian Rice Cultivars

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There are approximately 1270 local rice cultivars unique to Cambodia (Edwin et al., 1999). During more than three decades of civil wars, some of them were lost and studies on improving the cultivars have been started recently. However, there are a few reports studying on the genetic diversity of Cambodian rice cultivars. In order to utilize those local cultivars, studying on genetic diversity of Cambodian rice cultivars are necessary as basic information for developing new cultivars.

In this study, the authors aimed to characterize population structure and demographic history of 18 Cambodian and Japanese rice cultivars as fundamental information by employing the simple sequence repeats (SSRs). The authors crossed some of those Cambodian cultivars with Koshihikari. Thus, Koshihikari was included in the experiment.

Materials and Methods

Sixteen Cambodian indica rice cultivars (Oryza sativa L.), CAR 13, Chhma Prum, IR Kesar, Neang Minh, Phka Khgnei, Phka Mlis, Phka Rumdoul, Phka Rumchang, Popoul, Rohat, Rumpe, Santepheap 3, Sarika, Sen Pidao, Srau Sar and Torng Lahong and japonica, Koshihikari and Nipponbare, were used. Nipponbare was used as a control cultivar. Seeds of Cambodian rice cultivars were obtained from Cambodian Agricultural Research and Development Institute (CARDI).

All the cultivars were grown in a growth chamber in 2004. Leaves of young plants at the stage of 7 or 8 leaves were collected, washed by 70 % of ethanol and were scaled to 0.1 g fresh weight. DNA extraction was conducted by using a DNA extraction Kit (GE Healthcare). Concentration of each DNA solution was measured by Gene Spec III (Naka Instruments Co., Ltd.) and was adjusted to 10 ng µl⁻¹ following Bajracharya et al. (2006).

KOD Dash Kit (TOYOBO CO., LTD.) was used for PCR amplification. Primers used were RM1261, RM1272, RM1367-1, RM1880, RM3252, RM3394, RM3428, RM3509, RM3663, RM3826, RM3850, RM3872, RM4608, RM5087, RM5412, RM5479-1, RM5926, RM6364-1, RM7175, RM7389, RM8003, RM8039, which were designed on all rice chromosomes except chromosome number 8, following the instruction of Dr. Ideta, O., National Institute of Crop Science. PCR reactions were run by a PCR system 9700 (Gene Amp).

Polymorphic PCR products were pooled by 8 % of polyacrylamide gels (W90 mm × H80 mm). For checking the band size of each sample, 50 bp DNA Ladder (Invitrogen) was used. Amplified fragments were scanned using “LAS-1000UV Mini” connected with Image Reader Software Version 1.01 (Fuji Photo Film Co., LTD). Data were taken by simply giving the number 1 as for existed band, whereas 0 as for non-existed band. Nei’s genetic distance (Nei, 1972) was calculated among 18 cultivars. Clustering dendogram by UPGMA method was drawn using the program made by Aoki (2004).

Results and Discussion

In 22 primers used in the experiment, 9 primers showed polymorphism. Values of Nei’s genetic distance between all cultivars are shown in Table 1. The numbers of bands which the two cultivars have not commonly are also shown in Table 1. For instance, the value of 3 of CAR 13 and Chhma Prum means that 3 bands were different among them. No different genetic distance was found between Koshihikari and Nipponbare in this study. The genetic distance of Cambodian cultivars to Koshihikari ranged from 0.10 to 0.45 and it ranged from 0.00 to 0.32 among Cambodian cultivars. Rohat showed the closest genetic relationship with Koshihikari, of which the genetic distance was 0.10; while, Neang Minh showed the fartherest genetic distance with Koshihikari and the value was 0.45. The result of cluster analysis is shown in Fig. 1. The author roughly divided all the genotypes into three groups through their genetic relationship. Group A included CAR 13, Torng Lahong, Neang
Table 1. Nei’s genetic distance and number of different bands of 16 Cambodian rice cultivars and 2 Japanese cultivars.

| No. | Name          | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  |
|-----|---------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | CAR 13        | 0.15| 0.20| 0.32| 0.10| 0.32| 0.15| 0.10| 0.15| 0.20| 0.20| 0.20| 0.32| 0.10| 0.15| 0.20| 0.20| 0.10|
| 2   | Chhma Prum    | 3   | 0.05| 0.26| 0.15| 0.26| 0.10| 0.05| 0.10| 0.15| 0.05| 0.15| 0.15| 0.00| 0.05| 0.26| 0.15|
| 3   | IR Kesar      | 4   | 1   | 0.20| 0.20| 0.20| 0.15| 0.10| 0.15| 0.20| 0.20| 0.20| 0.32| 0.20| 0.15| 0.20| 0.20| 0.10|
| 4   | Koshihikari   | 5   | 5   | 4   | 0.45| 0.00| 0.26| 0.20| 0.26| 0.20| 0.20| 0.20| 0.26| 0.20| 0.20| 0.20| 0.20| 0.32|
| 5   | Neang Minh    | 2   | 3   | 4   | 8   | 0.45| 0.15| 0.20| 0.15| 0.20| 0.20| 0.20| 0.32| 0.20| 0.15| 0.20| 0.20| 0.10|
| 6   | Nipponbore    | 6   | 5   | 4   | 0   | 8   | 0.26| 0.20| 0.26| 0.20| 0.20| 0.20| 0.26| 0.20| 0.20| 0.20| 0.32|
| 7   | Phka Khgnei   | 3   | 2   | 3   | 5   | 3   | 5   | 0.05| 0.00| 0.05| 0.15| 0.15| 0.26| 0.15| 0.10| 0.15| 0.15| 0.05|
| 8   | Phka Mlis     | 2   | 1   | 2   | 4   | 4   | 4   | 1   | 0.05| 0.10| 0.10| 0.10| 0.20| 0.10| 0.05| 0.10| 0.20| 0.10|
| 9   | Phka Rumdoul  | 3   | 2   | 3   | 5   | 3   | 5   | 0   | 1   | 0.05| 0.15| 0.15| 0.26| 0.15| 0.10| 0.15| 0.15| 0.05|
| 10  | Phka Rumchang | 4   | 3   | 3   | 6   | 4   | 3   | 2   | 3   | 3   | 4   | 0.10| 0.10| 0.10| 0.05| 0.00| 0.20| 0.20|
| 11  | Popoul        | 4   | 1   | 0   | 4   | 4   | 4   | 1   | 2   | 1   | 0.20| 0.10| 0.20| 0.20| 0.15| 0.20| 0.10| 0.10|
| 12  | Rohat         | 4   | 3   | 2   | 2   | 6   | 2   | 3   | 3   | 2   | 2   | 0.10| 0.10| 0.15| 0.10| 0.10| 0.20| 0.20|
| 13  | Rumpe         | 6   | 3   | 2   | 4   | 6   | 4   | 5   | 4   | 5   | 4   | 2   | 2   | 0.20| 0.15| 0.10| 0.20| 0.32|
| 14  | Sarika        | 3   | 0   | 1   | 5   | 3   | 5   | 2   | 1   | 2   | 3   | 1   | 3   | 3   | 0.05| 0.26| 0.15|
| 15  | Sen Pidao     | 4   | 1   | 0   | 4   | 4   | 4   | 3   | 2   | 3   | 4   | 0   | 2   | 2   | 2   | 1   | 0.20| 0.20|
| 16  | Srau Sar      | 4   | 5   | 4   | 4   | 4   | 4   | 3   | 4   | 3   | 2   | 4   | 2   | 4   | 2   | 5   | 4   | 0.10|
| 17  | Torng Lahong  | 2   | 3   | 4   | 6   | 2   | 6   | 1   | 2   | 1   | 2   | 4   | 4   | 6   | 2   | 3   | 3   | 4   |

Right top half shows Nei’s genetic distance, and left bottom half shows the number of bands which the two cultivars have not commonly.

Fig. 1. Dendogram showing genetic relationship among Cambodian rice cultivars and Japanese cultivars.

Minh, Phka Khgnei, Phka Rumdoul, Phka Rumchang, Rohat, Sarika, Phka Mlis, IR Kesar, Popoul and Sen Pidao. Group C included Koshihikari, Nipponbore and Rumpe. All cultivars (except Rohat) in group A are traditional cultivars and group B (except Phka Mlis) includes cultivars that have the genetic relationship to IR lines (Men et al., 2001). Rumpe was placed in group C, even though, it is indica and has the genetic relationship to IR lines, and it might have a closed genetic relationship to japonica type.

Some traditional Cambodian cultivars showed farther genetic distance from Koshihikari. It is expected that crossing between those cultivars with Koshihikari may get wide genetic variation, by which genetic diversity is able to be enhanced.

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