Journal of Applied Horticulture, 12(2): 125-128, July-December, 2010

Genetic diversity of cultivated elephant foot yam (*Amorphophallus paeoniifolius*) in Kuningan, West Java as revealed by microsatellite markers

Edi Santosa[1]*, Yoko Mine[2]**, Miki Nakata[3], Chunlan Lian[4] and Nobuo Sugiyama[2]

*[1]Faculty of Agriculture, Bogor Agricultural University, Bogor 16680, Indonesia. [2]Facutly of Agriculture, Tokyo University of Agriculture, Funako 1737, Atsugi, Kanagawa 243-0034, Japan. [3]Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan. [4]Asian Natural Environmental Science Center, The University of Tokyo, Midoricho, Nishi-tokyo, Tokyo, 188-0002, Japan. *E-mail: editodai@yahoo.com. **E-mail: y3mine@nodai.ac.jp*

Abstract

Ten microsatellite markers were used to clarify the genetic diversity of cultivated elephant foot yams collected in 13 villages in the Kuningan District, West Java, Indonesia. Each pair of primers generated four to five alleles, with an observed heterozygosity of 0.000-1.000 and an expected heterozygosity of 0.064-0.551. These markers identified seven likely genets (clonal individuals) in the Kuningan population. Of 61 individual plants surveyed in this study, 55 plants distributed throughout the Kuningan District belonged to the same genet, while the another genet represented by a plant (ramet). These ramets were restricted to the villages located on the main road between Kuningan City and Central Java. Cluster analysis shows that the seven genets can be classified into three groups, with two groups showing a restricted distribution in the villages located on the road leading to Central Java. Elephant foot yam plants with berries were rarely observed in the Kuningan District. It is likely that a single genet has become the dominated local cultivar, possibly because of the limited genetic diversity of elephant foot yam in the Kuningan District, its reproduction by clonal propagation and the selection of a specific cultivar by farmers.

Key words: *Amorphophallus paeoniifolius*, clonal propagation, cluster analysis, genet, genetic diversity, Indonesia, SSR

Introduction

Elephant foot yams [*Amorphophallus paeoniifolius* (Dennst.) Nicolson] are distributed in many Asian countries as a local tuber crop (Jansen *et al.*, 1996). Because elephant foot yam is a shade-loving plant (Santosa *et al.*, 2006), wild forms grow predominantly under medium and deep shady conditions in forests at altitudes up to 900 m above sea level (Jansen *et al.*, 1996). Elephant foot yams are cultivated in home gardens, upland fields and at the edge of paddy fields and bamboo forests, while semi-wild (escaped) plants are often found in riverbanks and teak forests.

Although distributed widely in Sumatra, Java, Madura, Bali, Lombok and Sulawesi, as well as other islands in Indonesia, corms of elephant foot yam are only consumed occasionally, for example as appetizers at lunch in rural areas in Java (Santosa *et al.*, 2002a; Santosa *et al.*, 2003). Corms are available during the dry season, when the plants are dormant. Few genetic studies have been carried out on elephant foot yams (Widjaja and Lester, 1987; Santosa *et al.*, 2002b). Sugiyama *et al.* (2006) found that accessions of Java elephant foot yams collected in the same subdistrict clustered together in a dendogram that depicted genetic distance based on AFLP polymorphisms. Elephant foot yams are usually propagated clonally using corms or cormels, and plants that clusterd very closely may all represent a single clonal lineage (genet) that has undergone somatic mutation. The authors considered that during the introduction of elephant foot yam cultivation, several clones with desirable agronomic traits and good taste were selected and cultivated on farms, while nonpreferred ones were abandoned. However, it was unclear how many clonal individuals (genets) existed within a region and how they were disseminated.

We evaluated genetic variation in cultivated forms of elephant foot yams in Kuningan District using microsatellite (simple sequence repeat, SSR) markers. Microsatellite markers are currently the most powerful markers for identifying the genets because they are characterized by hypervariability, high reproducibility and codominance (Ouborg *et al.*, 1999).

Materials and Methods

Field observations were carried out in Kuningan District, West Java, Indonesia, in December 2002 and July 2003, and villages where at least 20 farmers cultivated more than 10 large elephant foot yams (petiole diameter larger than 3 cm) each for over three years were selected. These villages were easily accessible by the main road, which runs from the mountainside (ca. 1500 m above sea level) to the foothills (ca. 200 m) of the southeastern slope of Mount Ciremai (3078 m) (Table 1, Fig. 1). The distance between villages was more than 2 km. In this area, elephant foot yams are cultivated in home gardens and at the edge of paddy fields, fishponds and upland fields. No wild population of elephant foot yams was found in the Kuningan District. Moreover, all growers, aged 48 - 66, stated that their parents or grandparents had already cultivated elephant foot yam in their villages. Leaflet sampling was carried out in December 2003.

Three to six plants with a petiole diameter larger than three cm were selected at random from each village at distance of at least 15 m, in order to reduce the chance of sampling the same genet

Table 1. Sampling sites of cultivated *Amorphophallus paeoniifolius* in Kuningan, West Java and the number of plants sampled at each site

Site number	Name of sampling site	Code	Number of plants sampled
1	Ciherang	CIH	5
2	Jambar	JAM	4
3	Haurkoneng	HAK	5
4	Tinggar	TIN	5
5	Bayuning	BAY	4
6	Kuningan	KUN	4
7	Cibinuang	CIB	5
8	Citangtu	CIT	6
9	Kedungarum	KAR	5
10	Mekarmukti	MMU	3
11	Mekarwangi	MWA	5
12	Luragunglandeuh	LLA	5
13	Luragungtonggoh	LTO	5
Total			61

Site numbers were the same as those used in the map of Fig. 1

(Table 1). One leaflet per plant (about 5 g) was removed from the tip of the tripartite leaf, and dried in plastic bags containing 50 g of silica gel (blue when dry). When the silica gel absorbed mositure and turned purplish pink, it was replaced with the dried silica gel. In total, the leaflets of 61 plants were collected from 13 villages. A cultivar sample from Yogyakarta was added as a reference.

DNA was extracted from dry leaflets with a modified cetyl trimethyl ammonium bromide method, and stored at –30 °C until used. Nineteen microsatellite loci (Ampa 1-19 developed by Santosa *et al.*, 2007) were screened. Eight loci (Ampa 1, 2, 8, 9, 10, 13, 14 and 18) were not amplified well and one locus (Ampa 16) produced a monomorphic band, so these were excluded from further analysis. The remaining 10 primers (Ampa 3, 4, 5, 6, 7, 11, 12, 15, 17 and 19) were used in the present study.

PCR was performed as described by Santosa *et al.* (2007). The PCR solution mixture (5 µL) contained 5-20 ng of template DNA, 0.5 µM of forward primer, 0.1 µM of reverse primer tailed with U-19, 0.5 µM of U-19 primer labeled with Texas Red, 0.2 mM of each dNTP mix, 1 × PCR buffer (Mg^{2+} free), 2.5 mM $MgCl_2$ and 0.5 U of Ampli *Taq* Gold DNA polymerase (Applied Biosystems, Foster City, USA). PCR using a PCR thermal cycler (Takara PCR system, Kyoto, Japan) was performed with the following cycling profile: 9 min at 94 °C, followed by one cycle of 30 s at the locus-specific annealing temperature plus 1 min at 72 °C, and then 38 cycles of 30 s at 94 °C, 30 s at the locus-specific annealing temperature plus 1 min at 72 °C, followed by one cycle of 30 s at 94 °C, 30 s at the locus-specific annealing temperature plus 5 min at 72 °C, and ending at 4 °C. The primer annealing temperatures are shown in Table 2. After denaturation by heating at 95 °C for 5 min, PCR products were immediately placed on ice and then electrophoresed on 6% polyacrylamide gel with 0.6 × TAE buffer, using a SQ-5500E sequencer (Hitachi Co., Tokyo, Japan). Electrophoretic patterns were analyzed with FRAGLYS ver. 3 software (Hitachi Electronics Engineering Co., Tokyo, Japan).

Genotypes that matched at all microsatellite loci were presumed to represent a single genet. For each identified genet, the observed (H_O) and expected (H_E) heterozygosity and the polymorphic information content (PIC) were calculated for each locus using CERVUS ver. 3 (Marshall *et al.*, 1998). The PIC provides information of the effectiveness in differentiating among genets; loci with higher values of PIC can distinguish genets more efficiently. A dendogram was constructed using the unweighted pair group method with arithmetic averages (UPGMA) from a genetic similarity matrix using NTSYSpc (Rohlf, 2000). Bootstrapping with 1000 permutations was carried out using FreeTree software, according to Pavlicek *et al.* (1999). The inbreeding coefficient (F_{IS}), which describes the divergence of H_O from the H_E in panmixia populations, was calculated for each locus using FSTAT (Weir & Cockerham, 1984). Positive and negative F_{IS} values indicated significant excesses of homozygotes and heterozygotes, respectively.

Results and discussion

The number of alleles, heterozygosity and PIC of 10 microsatellite loci are given in Table 2. Either four or five alleles were observed (average allele number per locus was 4.4). H_O and H_E ranged from 0.000 to 1.000 and from 0.064 to 0.551, respectively. The PIC value ranged from 0.061 to 0.443 (0.262 on average). Some microsatellite markers had very low PIC, *e.g.*, Ampa 12 and Ampa 19 loci, suggesting that these markers could not discriminate genets in the Kuningan District efficiently. The average multilocus F_{IS} for all samples had a negative value (-0.702), showing an excess of heterozygotes.

Based on allelic data at 10 loci, 61 samples could be classified into seven genets in the Kuningan District. Of the seven genets, one genet (the main genet in Fig. 2) was represented by 55 sampled plants, while the other six genets were represented by one plant

Table 2. Locus name, simple sequence repeat (SSR) sequence, annealing temperature (T_a), number of alleles, observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphic information content (PIC), average inbreeding coefficient (F_{IS}) and gene diversity of microsatellite markers in cultivated *Amorphophallus paeoniifolius* from Kuningan, West Java

Locus	SSR Sequence	T_a (°C)	Number of alleles	H_O	H_E	PIC (%)	F_{IS}
Ampa 3	$(TG)_{16}$	56	4	0.984	0.504	37.5	-0.906
Ampa 4	$(CT)_7(GT)_{10}$	58	5	0.000	0.124	11.5	0.894
Ampa 5	$(TC)_{19}(TG)_{10}$	55	5	0.932	0.551	44.3	-0.657
Ampa 6	$(TG)_{18}(AG)_9$	60	5	1.000	0.528	41.0	-0.855
Ampa 7	$(TG)_{11}(AG)_{15}$	60	4	0.934	0.519	39.8	-0.761
Ampa 11	$(TC)_6(TG)_{14}$	60	4	0.951	0.511	38.6	-0.820
Ampa 12	$(TG)_{11}(AG)_{10}$	51	4	0.066	0.064	6.1	0.143
Ampa 15	$(GA)_7(GT)_{11}$	58	5	0.951	0.503	37.4	-0.847
Ampa 17	$(AG)_{12}(TG)_9(AG)_3$	57	4	0.951	0.503	37.4	-0.847
Ampa 19	$(GA)_7(GT)_8(CG)_6xx(CT)_{11}$	57	4	0.066	0.064	6.1	0.143
Overall			44				-0.702

each. Using allelic data for these seven genets, a cluster analysis was carried out using the UPGMA method. This divided the seven genets into three groups (Fig. 2). The main genet and the genets from Haurkoneng (HAK) and Luragunglandeuh (LLA) villages belonged to cluster I. The three genets from Luragungtonggoh (LTO) belonged to cluster II and the one genet from Mekarwangi (MWA) village belonged to cluster III. The four genets belonging to clusters II and III and one genet (HAK-4) belonging to different subgroup of cluster I from main genet were found only in the villages located on the road from Central Java to the centre of Kuningan City, whereas the main genet was distributed in all villages throughout the Kuningan District (Fig. 1). The elephant foot yam from Yogyakarta was genetically distant, joining with the Kuningan population at a similarity of 26%.

According to an interview with farmers in Kuningan, they usually remove inflorescences to produce big corms with good eating quality, when cultivating elephant foot yams. Removal of inflorescences of semi-wild elephant foot yams is a common practice in Kuningan as well as in other places in Java, because the inflorescences emit unpleasant odors. Plants with berries were only occasionally observed on the outskirts of Kuningan City, although evidence for berry set is common in the collection of elephant foot yams in the Herbarium Bogoriense.

In natural populations, the abundance of pollinators may affect the success of berry set. In Java, elephant foot yams can often produce inflorescences in mixed gardens where fruit trees, wood trees and medicinal plants were more extensively cultivated than in home gardens. Mixed gardens in Kuningan were usually separated by lowland paddy fields. Therefore, it is possible that only a few pollinators can carry pollen from one inflorescence to another inflorescence in elephant foot yam populations that are spatially isolated by paddy fields, leading to a scarcity of berry set in elephant foot yams in Kuningan. Although it is not clear whether the number of pollinators is large enough to ensure cross-pollination, a lack of outcrossing among plants may have restricted the expansion of genetic diversity of elephant foot yams in the present study area.

Geographically, LTO and MWA villages are located on the main road leading to Central Java (Fig. 1). There has long been the active movement of people, goods and information between Kuningan District and Central Java by this road. It is plausible that elephant foot yams have been introduced to these villages by merchants or other people from Central Java and Yogjakarta, where the cultivation of elephant foot yams has been more common (Kriswidarti, 1980; Santosa *et al.*, 2002a). A sugarcane plantation was established in LTO village in the 1800s, during the Dutch colonial period. This plantation was a powerful job creator and attracted a seasonal migration of laborers from Central Java. Sugarcane plantation laborers might have introduced several clones of elephant foot yam to Kuningan from Central Java or Yogyakarta. They could have brought either large corms of elephant foot yams as food, or small corms or cormels as planting materials. In the former case, laborers might discard or plant the skin of corms near their huts, after preparing corms for cooking.

A possible explanation for the dissemination of only one genet throughout the district and a limited distribution of other genets in villages close to the border of Central Java (*e.g.* LTO, MWA) is

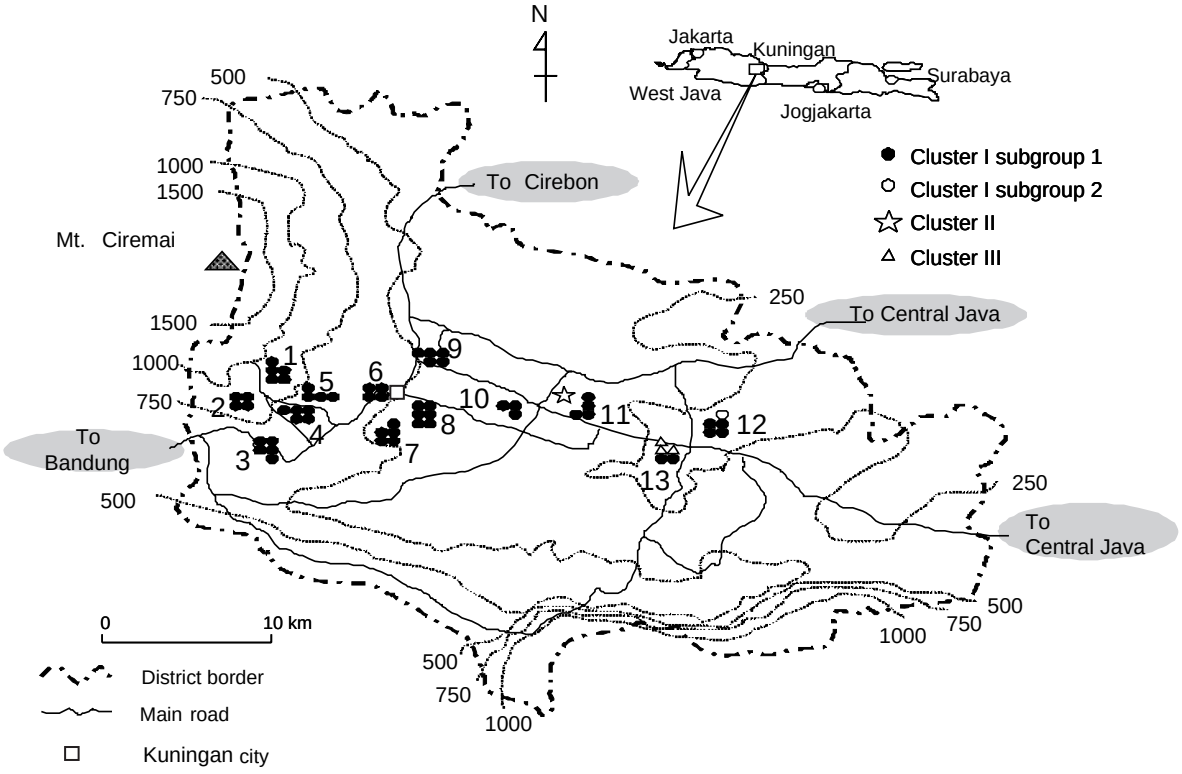

Fig.1. Distribution of elephant foot yam accessions in Kuningan District based on SSR data. Symbols represent cluster membership based on UPGMA method. Each circle or triangle represents a plant sample. Thirteen villages in Kuningan District, West Java, Indonesia for studying genetic variation of *Amorphophallus*. Main road referred to both provincial and district roads. Sampling sites were presented in Table 1.

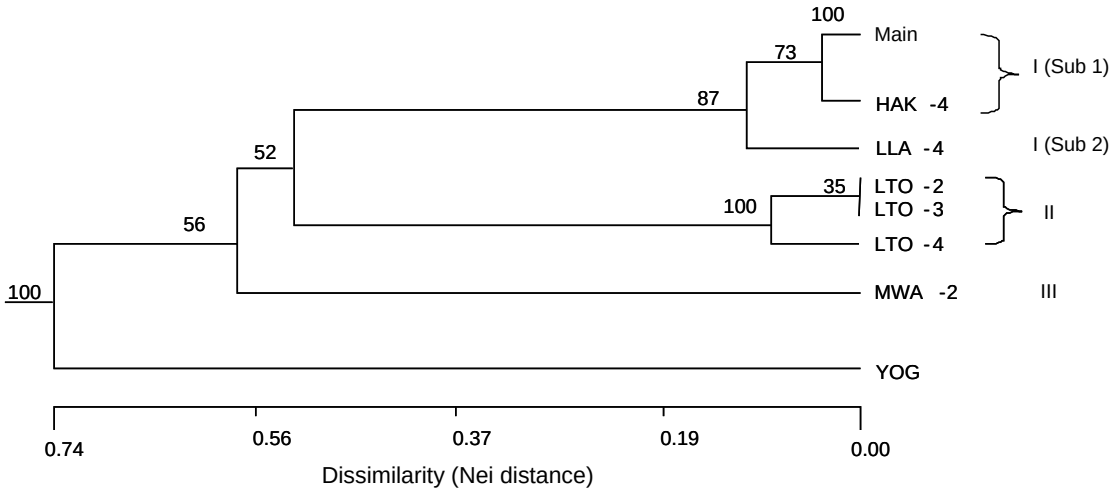

Fig. 2. UPGMA dendogram obtained from SSR markers data on 61 plants of *A. paeoniifolius* from Kuningan, West Java. Abbreviations are explained in Table 1. Main - samples other than HAK-4, LLA-4, LTO-2, LTO-3, LTO-4 and MWA-4; YOG - Yogjakarta. Bootstraping value from 1000 permutations. Roman figures are cluster numbers, and sub 1 and sub 2 in parenthesis mean the subgroup in cluster I.

that many clones (genets) were introduced to these villages from Central Java and given a evaluation by farmers based on their agronomic traits and taste. Possibly, most clones, *e.g.*, genets in clusters II and III, have been abandoned during selection, while only favoured clones might have been distributed throughout Kuningan in the form of cormels or corms. Santosa *et al.* (2003) reported that corms are usually exchanged among neighbours or relatives, and this is presumably how the corms of the cluster I genet gradually became distributed from person to person throughout the district. However, another possibility cannot be ruled out: clones (genets) belonging to clusters II and III might have been only introduced into Kuningan where the cluster I clone had already spread. Even if there is positive selection for the new clones, it must take time for farmers to adopt them. It is probable that Kuningan farmers are used to the taste of elephant foot yams which they have grown, and do not prefer new clones. However, Santosa *et al.* (2003) reported that merchants sold elephant foot yams transported from other districts at the Kuningan market. Therefore, it appears that Kuningan farmers do not necessarily prefer specific cultivars of elephant foot yams at the present time. Further study is necessary to investigatie the genetic variation in the semi-wild (escaped) plants throughout the Kuningan District, because many semi-wild clones belonging to clusters other than cluster I are expected to still remain in villages close to the border of Central Java if selection have been carried out in these villages.

Acknowledgements

This study was supported by a grant from the Japan Society for the Promotion of Science (JSPS) for Tropical Biological Resources.

References

Jansen, P.C.M., C. van der Wilk and W.L.A. Hetterscheid, 1996. *Amorphophallus* Blume ex Decaisne. In: *PROSEA 9: Plant Yielding Non-seed Carbohydrates*, M. Flach and F. Rumawas (eds.). Backhuys Publ, Leiden. p45-50.

Kriswidarti, T. 1980. Suweg (*Amorphophallus campanulatus* Bl.) family of carrion flower as carbohydrate source. *Bulletin Kebun Raya*, 4: 171-173 (in Indonesian).

Marshall, T.C., J. Slate, L. Kruuk and J.M. Pemberton, 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.*, 7: 639-655.

Ouborg, H.J., Y. Piquot and J.M. van Groenendael, 1999. Population genetics, molecular markers and the study of dispersal in plants. *J. Ecol.*, 87: 551-568.

Pavlicek, A., S. Hrda and J. Flegr, 1999. FreeTree - Freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of the genus Frenkelia. *Folia Biologica (Praha)*, 45: 97-99.

Rohlf, F.J. 2000. *NTSYSpc Numerical Taxonomy and Multivariate Analysis System ver. 2.1.* Exeter Software. Applied Biostatistics Inc., New York.

Santosa, E., N. Sugiyama, A.P. Lontoh, Sutoro, S. Hikosaka and S. Kawabata, 2002a. Cultivation of *Amorphophallus paeoniifolius* (Dennst.) Nicolson in home gardens in Java. *Jpn. J. Trop. Agric.*, 46: 94-99.

Santosa, E., N. Sugiyama, M.A. Chozin, A.P. Lontoh, S. Sudiatso, S. Kawabata, S. Hikosaka, Sutoro and A. Hidayat, 2002b. Morphological and nutritional characterization of elephant foot yam in Indonesia. *Jpn. J. Trop. Agric.*, 46: 265-271.

Santosa, E., N. Sugiyama and S. Kawabata, 2003. Reasons for farmer's decision to cultivate elephant foot yams in Kuningan District, West Java, Indonesia. *Jpn. J. Trop. Agric.*, 47: 83-89.

Santosa, E., N. Sugiyama, M. Nakata and O.N. Lee, 2006. Growth and corm production of *Amorphophallus* at different shading levels in Indonesia. *Jpn. J. Trop. Agric.*, 50: 87-91.

Santosa, E., C.L. Lian, Y. Pisooksantivatana and N. Sugiyama, 2007. Isolation and characterization of polymorphic microsatellite markers in *Amorphophallus paeoniifolius* (Dennst.) Nicolson, Araceae. *Mol. Ecol. Notes*, 7: 814-817.

Sugiyama, N., E. Santosa, O. Lee, S. Hikosaka and M. Nakata, 2006. Classification of elephant foot yam (*Amorphophallus peoniifolius*) cultivars in Java using AFLP markers. *Jpn. J. Trop. Agric.*, 50: 215-218.

Weir, B.S. and C.C. Cockerham, 1984. Estimating F-statistics for the analysis of population structure. *Evolution*, 38: 1358-1370.

Widjaja, E.A. and R.N. Lester, 1987. Morphological, anatomical and chemical analysis of *Amorphophallus paeoniifolius* and related taxa. *Reinwardtia*, 10: 271-280.

Received: May, 2010; Revised: September, 2010; Accepted: November, 2010