

## Evaluation of Diversity of Germplasm in Sweet Potato

Hiroko TAKAGI\*

### Abstract

Sweet potato, *Ipomoea batatas* (L.) Lam., plays a very important role in traditional cropping systems in the tropics and sub-tropics. It ranks fifth among food crops in terms of total production in developing countries. Despite its importance and the large number of germplasm accessions collected, useful genetic markers in sweet potato and its wild relatives are quite limited in comparison with many other crop plants due to biological characteristics such as polyploidy, vegetative propagation, self and cross-incompatibility.

In this paper, past and present status of collection and evaluation of sweet potato germplasm is outlined. Problems and needs for the further development of the germplasm activities are also discussed.

### Introduction

Sweet potato, *Ipomoea batatas* (L.) Lam., ranks seventh in total production among the world's food crops with an annual production of around 110 million metric tons. China alone accounts for 82 % of the world's production followed by Asia/ Oceania 10% and Africa 5%. In some countries, such as Solomon Islands, Tonga, Rwanda, Papua New Guinea, Uganda where sweet potato plays an essential role as a staple food, per capita production levels are higher than in China (Horton, 1989).

The crop is widely grown throughout the tropical, sub-tropical and temperate areas of the world and is used for human consumption, animal feed and industrial purposes. In the tropics and sub-tropics it plays an important role in the traditional cropping systems, mainly, as a subsistence crop because of its low input requirement, high energy production per unit area, wide adaptability to unfavorable conditions and high nutritional value (Luh and Moomow 1979). In temperate countries especially, China, Japan and Korea, sweet potato was an important material for starch and alcohol industries but has lost ground to imported corn and cassava. Recently it has been attracting more attention as a supplementary food for fresh market.

### Collection and evaluation of sweet potato germplasm

Sweet potato is an established tropical crop that originated from Central or northern South America and its centers of production and consumption are located in Asia and Oceania. There is a large number of sweet potato cultivars in the tropical and sub-tropical areas, mostly, from farmers' selections in populations resulting from natural hybridization and spontaneous mutation. On the other hand, many cultivars in the temperate region such as China, Korea, Japan, Taiwan, U. S. A. have evolved through systematic breeding using introduced germplasm and limited indigenous cultivars.

#### 1 Collection

Noteworthy collection was made by Yen (1974) for ethnological study. For breeding programs, comprehensive collections were established at the International Institute of Tropical Agriculture (IITA),

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\* Tropical Agriculture Research Center, Okinawa Branch, Japan

Ibadan, Nigeria; the Asian Vegetable Research and Development Center (AVRDC), Tainan, Taiwan; and the Centro de Agricultura de Investigacion y Ensenanza (CATIE), Turrialba, Costa Rica.

Due to the awareness of the rapid erosion of plant genetic diversity and the enormous potential value of genetic resources, collection and conservation of plant genetic resources have become an issue of common global concern. The International Board for Plant Genetic Resources (IBPGR) initiated concerted activities on sweet potato in 1980 with IITA, AVRDC and the other national institutes. At that time, a total of 6,900 germplasm accessions were listed in the IBPGR Directory of germplasm collection (IBPGR, 1980). Additional 5,000 accessions were collected from 13 countries (Burkina Faso, Burundi, Guatemala, Indonesia, Malaysia, Mexico, Papua New Guinea, Peru, Philippines, Solomon Islands, Thailand, Uganda and Zaire) during the period 1980 to 1984 (Williams and van Sloten, 1988).

Based on the recommendations of TAC (Technical Advisory Committee for CGIAR), sweet potato was recognized as a neglected crop in the CG system and the International Potato Center (CIP) was given the world mandate for research and development of this crop in 1985. Further intensive germplasm collection was initiated by CIP/IBPGR joint project with emphasis on the collection of wild *Ipomoea* species and sweet potato cultivars in Latin-America, the primary center of diversity. CIP holds a total of 3,520 accessions (Acc.) and the collections from IITA (1,202 Acc.) and AVRDC (1,439 Acc.) will be centralized at CIP (CIP, 1990). Although, an updated compiled list of the germplasm collections is not yet available, the number of collections of genetic resources throughout the world has increased especially, at the national levels. In the Asia and Pacific area alone, there are more than 12,000 accessions of sweet potato held by national programs.

## 2 Evaluation

Numerous accessions have been accumulated as a result of the recent activities, and systematic characterization and evaluation studies are in progress at the International Agriculture Centers. However, a great deal still remains to be done especially, at the various national institutes. Thus, the gap between conservation and utilization is widening. Since sweet potato germplasm is preserved in field gene banks there are the attendant financial and workload problems, which are difficult to handle for national institutes alone.

Obviously, characterization/evaluation should be given more attention and better coordination should be achieved so as to promote work in the following aspects:

- 1) Identification of duplicated accessions, 2) Rationalization of the existing germplasm, 3) Utilization of germplasm for breeding, 4) Establishment of active and core collections, 5) Substantial planning for further collection activities and 6) Varietal differentiation and phylogenetic studies.

## Evaluation of genetic diversity in sweet potato germplasm

### 1 Wild relatives as a gene pool for sweet potato

The genus *Ipomoea* section *Batatas* contains 12 species, two named hybrids, and one unnamed hybrid (Austin, 1988). All the species have chromosome numbers based on a multiple of  $x=15$ . Within the genus, *I. batatas* is the only known hexaploid species with sizable storage roots while, the other species in the section are diploids or tetraploids. There is a considerable uncertainty on the phylogenetic relationships among the species. Although, *I. trifida* (H. B. K) G. Don is considered to be the most closely related species with an hexaploid cytotype, its taxonomic identification is still being debated (Nishiyama, 1959; Kobayashi, 1984; Austin, 1988; Jones, 1967; Martin *et al.*, 1974). The potential value of utilizing *I. trifida* for sweet potato breeding has been demonstrated (Sakamoto, 1976; Iwanaga, 1989). However, wild germplasm cannot be utilized effectively yet due to the differences in ploidy levels, and lack of storage root forming ability in wild species (Iwanaga *et al.*, 1990). The practical utilization of wild relatives requires further information on specific traits and heterosis effects which do not occur in the cultivated sweet potato.

### 2 Constraints in genetic studies of sweet potato

Genetic studies of sweet potato have met with little success and useful genetic markers for evaluating

genetic diversity are quite limited due to its biological characteristics of the plant. Due to its hexaploid nature, the segregation ratios in sweet potato are complex and thus continuous variation can be expected for most traits. The inheritance appears to be quantitative, even for characters that might be controlled by one gene with two alleles in a diploid. Sweet potatoes are mostly self-incompatible and self-pollination leads to severe inbreeding depression. When cross pollinations are made, recombination occurs, and each seed represents a new unique combination. Moreover, hybridization between desirable genotypes is often hindered by the existence of cross-incompatibility groups.

### 3 Evaluation of variation in sweet potato

Due to the slow progress and the laborious procedure of recombination and accumulation of useful genes, sweet potato breeders have emphasized the need for evaluating the germplasm to identify some specific traits for better combination of important traits. The hexaploid and vegetatively propagated nature allows the species, *I. batatas*, to hold extensive variability for many morphological and important agronomic traits. However, studies at "gene level" are very limited due to the difficulties in pursuing analysis. Moreover, due to the strict quarantine regulations for transferring vegetative materials, concerted studies on genetic diversity on extensive materials from different areas have become rather difficult.

#### 1. Variation in morphological characters

In the evaluation of his worldwide collection of 580 cultivars, Yen (1974) observed homologous morphological variation throughout the collection. Although, no specific regional variation was evident, the American materials covered a wider range of variability. However, they lacked such traits as near-glabrous ovary, greater degree of dissection of leaves and presence of a mauve rather than white stigma that were found in materials from other areas such as Philippines and New Guinea.

A comprehensive list of descriptors involving 31 morphological characters for the characterization and evaluation of sweet potato germplasm has been developed (CIP/ AVRDC/ IBPGR, 1991). Many curators are using this list as universal standard and it is expected that more information on the variation of morphological traits and its geographical distribution would become available once all the data can be centralized through the IBPGR network.

#### 2. Variation in disease resistance

Although a large number of diseases occurring in sweet potato has been reported, most of them are region specific and the yield reduction caused by those diseases is negligible. Diseases and pests which have received more attention are leaf scab, black rot, fusarium wilt, scurf, soil rot and root-knot nematodes. Since low input cultivation is one of the most important requirements, the control by resistant cultivars is desirable. For these major diseases, variations in levels of resistance have been reported. However, due to the availability of resistant materials within regional collections, there have been few reports on the geographical distribution of the source of resistance.

**Leaf scab:** Leaf scab caused by *Elisimoe batatas* is one of the most common diseases in Southeast Asia and Pacific, especially under warm and humid climate and large yield reductions have been reported (Floyd, 1988). Wide variation of the resistance level has been reported and highly resistant cultivars are frequently present especially, in the germplasm that originated from Indonesia, Papua New Guinea, Philippines and Solomon Island (Guarino and Jackson, 1986; Takagi, 1987; AVRDC, 1990).

**Root-knot nematode:** Materials resistant to the root-knot nematode (*Meloidigyne incognita*) are readily available and have been systematically incorporated into the screening procedure of most of the breeding programs. Shiga and Takemata (1981) reported that 75% of 69 accessions from Papua New Guinea were highly resistant to the root-knot nematode. The frequency of the resistant clones was the highest in Papua New Guinea and it decreased with the distance from the island.

#### 3. Variation of tolerance to abiotic stresses

Tolerance to adverse environmental conditions has also been one of the important objectives of sweet potato breeding programs to ensure constant supply, reinforce the crop's traditional role as a subsistence

crop and make use of marginal land which otherwise cannot be fully utilized.

**Excess moisture:** Although, sweet potato can be grown all the year round in the tropics, ordinarily the yield is adversely affected by wet conditions (King, 1985). During the wet season, the soils are over-saturated and/ or flooded for days to weeks. Such a condition affects root formation as well as enlargement. Differences among cultivars in their ability to withstand excess moisture have been reported (Martin, 1983). In Taiwan, 230 accessions of the AVRDC collections were screened under hot wet conditions and 14 cultivars were evaluated as tolerant to excess moisture. Interestingly, eleven of them originated from the highlands of Papua New Guinea where annual rainfall is close to 2,500 mm (AVRDC, 1984).

**Cold:** Yen (1974) evaluated 293 cultivars for their reaction to cold in New Zealand. It was notable that cultivars from the Andean and New Guinea highlands consistently showed a lower cold susceptibility.

#### 4. Incompatibility

Self-incompatibility of *Ipomoea* is characterized by a sporophytic type and genetic analysis of diploid species showed that this trait is controlled by a single locus with multiple S-alleles (Kowyama *et al.*, 1980). Knowledge of the incompatibility system in a diploid makes it possible to interpret the incompatibility of sweet potato. A duplication or possibly triplication of the self-incompatibility locus is sufficient to explain even cross-incompatibility (Martin and Jones, 1986).

Different numbers of cross-incompatibility groups in sweet potato have been reported by many workers (Fujise, 1964; Wang, 1964). However standardized classification of this trait has not yet been established. Nakanishi and Kobayashi (1979), using the Japanese classification of 16 distinct groups, examined the geographical distribution of cross-incompatibility groups for more than 700 sweet potato cultivars from various countries of the world. They reported that cultivars from South America, especially the Peruvian cultivars showed a wide diversity with 10 groups while the cultivars from Asian countries contained only 3-4 groups. It was notable that the cultivars from the Philippines showed a wider variation among the Asian countries, suggesting that they had been introduced through different dispersal routes into this area.

### **Application of biochemical markers in sweet potato**

Numerous attempts have been made to develop biochemical markers which could be utilized in phylogenetic studies and varietal identification for various crops and there is growing evidence for their usefulness. There have also been some attempts to adopt these markers in genetic studies of sweet potato and its relatives.

#### **1 Isozymes and protein**

Biochemical genetic markers using isozymes and protein electrophoresis have been found to be useful for genetic diversity and phylogenetic studies of various economically important crops. However, very limited information is available for sweet potato.

Chen *et al.* (1992) reported that peroxidase zymograms were more stable than the other isozymes examined ( $\alpha$  and  $\beta$  amylase, polyphenol oxidase and phosphorylase). Although, no significant variation in the major peroxidase bands was noted, some minor variations based on variety, tissue specificity, cultural status and nutrient level did occur. Varietal differences were observed between two Taiwanese cultivars, Tainong 66 and Tainong 67, based on their peroxidase zymograms.

Stegemann *et al.* (1992) characterized three sweet potato breeding lines by their protein and esterase patterns in disc-PAGE and Poro-PAGE. The patterns were consistent, independent of environment and growing stages. The protocol developed in this study is being used to identify duplications at CIP.

#### **2 Restriction fragment length polymorphisms**

Restriction Fragment Length Polymorphisms (RFLPs) have already been found to be useful for obtaining many genetic markers with high heritability. Since the co-dominant nature of RFLP markers permits genetic analysis to be carried out in heterozygous, the evaluation of the polymorphisms in sweet po-

tato germplasm could lead to the development of a new aspects in the genetic studies of sweet potato.

### 1. Phylogenetic studies

Jarret *et al.* (1992) analyzed 13 species of the section *Batatas* and the outgroup species *I. gracilis* and *I. pes-caprae* for RFLPs by probing Southern blots of restriction enzyme-digested genomic DNA with 20 low or moderate copy number sequences isolated from an *I. batatas* cv. Georgia Red genomic library. They detected polymorphism and used it to discuss the intrasectional relationships of section *Batatas* species and the role of tetraploid related species in the evolution of the cultivated *I. batatas*.

Kowiyama *et al.* (1992) investigated interspecific and intervarietal relationships among 9 *Ipomoea* species and two reference species from the distantly related genus by the RFLPs of nuclear and cytoplasmic DNAs, and found extensive RFLP variation among the materials tested. Their results suggest that polyploidization of nuclear DNA may have played an important role in the genetic differentiation of the *Ipomoea* species throughout the evolutionary process. Also, the RFLP analysis of cytoplasmic DNA indicates that a hexaploid *I. trifida*, identified as an ancestral species of sweet potato by Nishiyama (1959), may be a feral type of cultivated sweet potato.

### 2. RFLP variation within sweet potato cultivars

The usefulness of RFLP markers is largely dependent on the degree of polymorphism and the levels of variability vary considerably among the species. Usually, outcrossing species, such as *Brassica* and maize exhibit high polymorphisms within cultivars. Regarding RFLP-analysis there is a paucity of information on the degree of the polymorphisms within cultivated sweet potato, although a high variability is anticipated due to the cross-pollinated, hexaploid and vegetatively propagated nature of the plant.

Takagi *et al.* (1993) have applied RFLP-analysis for use in varietal identification of sweet potato germplasm. Nine cultivars from different areas and 3 reference species were analysed by Southern blots of restriction enzyme-digested (Eco RI and Hind III) genomic DNA with the sequences encoding Sporamin A and  $\beta$ -amylase isolated from *I. batatas* and a cDNA sequence isolated from stigma of *I. leucantha*. Preliminary data revealed a prominent variation among the cultivars tested and for all the restriction enzyme / probe combinations, specific patterns of fragments were observed for each cultivar. Therefore, fingerprinting by RFLPs appears to be quite promising.

## Conclusion

The increase of the sweet potato germplasm collections throughout the world in recent years has resulted in the widening of the gap between conservation and utilization. Attention needs to be shifted to the characterization, evaluation and data base construction for the better use of these germplasm samples.

Due to its biological nature, genetic studies on sweet potato have been limited. The evaluation of genetic diversity of the crop still remains at the stage of germplasm screening for certain specific agronomic traits. For the facilitation of the work aimed at further development of the evaluation of sweet potato germplasm, whose international transfer is not as easy as that of seed-propagated crops, it would be useful to 1) centralize information on the evaluation from past studies and 2) compile and develop screening techniques under a standardized protocol.

For further development of germplasm activities, application of new technologies and basic genetic studies also need to be emphasized. The use of biochemical markers, especially, DNA markers based on RFLPs is considered to be suitable for providing new information in this very heterozygous crop.

To conclude, I wish to emphasize that, although, this report is only a brief overview on the present situation of sweet potato germplasm and its evaluation, the problems outlined are also quite specific to vegetatively propagated tropical crops.

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### Discussion

**Valkoun, J. (ICARDA):** You used RFLP to gain information on the DNA structure and composition of your crop, did you use other DNA techniques such as RAPD or PCR for molecular characterization and what was the correlation with the RFLP technique?

**Answer:** I did not try other techniques so far. However, due to the co-dominant nature and considerable variations of sweet potato, I believe that it is preferable to use RFLP as RAPD may show a Mendelian inheritance pattern.

**Songkran Chitrakon (Thailand):** Can the use of RFLP makers help discard duplicate samples in case no differences are revealed by this technique?

**Answer:** If the RFLP patterns are different although the morphological characters appear to be identical, the accessions are presumably genetically different. RFLP can be considered to be one of the characters. How the results obtained are interpreted and utilized for the identification of duplicates should be discussed thoroughly.

**Riley, K. (IBPGR):** Are you collaborating in your studies with the CIP regional and country offices in Indonesia, Philippines and China?. IBPGR regional office for the Asia, Pacific and Oceania region is interested in collaborating with CIP and national programs on the conservation and use of sweet potato in the region.

**Answer:** We have contacted CIP, Indonesia and received some accessions which are considered to be duplicates based on the analysis of morphological characters. The materials will be used to analyse RFLP patterns.